

POSTER COMMUNICATIONS

POSTER COMMUNICATIONS – PC.003

ANTITUBERCULAR CONSTITUENTS FROM AMPALAYA (*MOMORDICA CHARANTIA* LINN.)

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An earlier study on the crude alcoholic extract of the leaves of *Momordica charantia* Linn. showed inhibition of the growth of *Mycobacterium tuberculosis* H₃₇Rv at 100 µg.ml⁻¹ (94%) and at 1000 µg.ml⁻¹ (100%). To determine if *M. charantia* would exhibit a wide range of inhibitory activities against *M. tuberculosis* H₃₇Rv with respect to plant parts, location, age, and variety, the crude alcoholic extracts were tested using the Microplate Alamar Blue Assay. The results showed that the various parts of *M. charantia* have different inhibitory activities against *M. tuberculosis* H₃₇Rv with leaves generally exhibiting the highest inhibitory activity, ranging from 48% to 100% at 100 µg.ml⁻¹.

M. charantia species from selected locations in Luzon likewise exhibited different inhibitory activities against *M. tuberculosis* H₃₇Rv with species from Bicol showing the highest percent inhibition, 100%, for the leaves, fruits and stems. The antitubercular activity of the leaves of the cultivated variety was higher (99%) compared to that of the wild variety (66%). On the other hand, the percent inhibition of the fruits of wild *M. charantia* (90%), was higher than the cultivated variety (66%). The difference between the antitubercular activity of the young (98%) and mature (99%) leaves of cultivated *M. charantia* is minimal.

Two compounds were isolated from the cultivated leaves of *M. charantia* and their structures were elucidated through spectroscopic studies. One of the compounds, is a new triterpene aldehyde (3ß,7ß,25-trihydroxycucurbita-5,23(Z)-dien-30-al) with an

antiubercular activity against *Mtb* H₃₇Rv 35 % inhibition (128 µg.ml⁻¹) and 29% inhibition (64 µg.ml⁻¹) (Figure 1); and the other one is a known phenolic compound, 2,4-bis(dimethyl benzyl) phenol, with 100 % inhibition against *Mtb* H₃₇Rv (100 µg.ml⁻¹) (Figure 2). A number of compounds were isolated and identified from the bioactive sub fractions using GC-MS analysis.

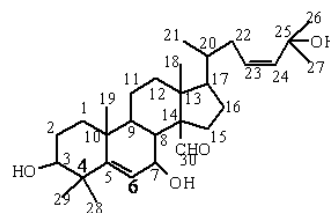


Figure 1

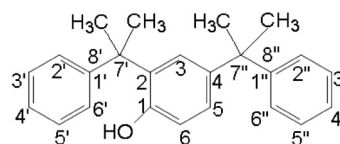


Figure 2

POSTER COMMUNICATIONS – PC.005

SYNTHESIS AND PRECLINICAL PROFILING OF NOVEL HYDROXAMIC ACIDS AS HISTONE DEACETYLASE INHIBITORS

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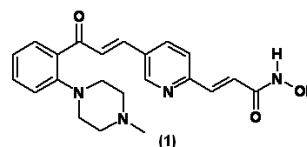
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The histone deacetylases (HDACs) are able to regulate gene expression and histone deacetylase inhibitors (HDACi) emerged as a new class of agents in the treatment of cancer as well as other human disorders such as neurodegenerative diseases.¹ SAHA (Suberoylanilide hydroxamic acid), one of the early HDAC inhibitor discovered by Breslow and colleagues,² was approved by FDA in October 2006 for the treatment of patients with cutaneous T-cell Lymphoma (CTCL).³ Dozens of other inhibitors with different structures are now in various stages of clinical trial.

In the present investigation, we describe the synthesis, the biological evaluation and preclinical profiling of compounds derived from the expansion of N-hydroxy-arylacrylamide scaffold:

- N-Hydroxy-3-phenyl-2-propenamide and N-hydroxy-3-(pyridin-2-yl)-2-propenamide as core structures containing a phenylloxopropenyl moiety, either unsubstituted or substituted by a 4-methylpiperazin-1-yl or 4-methylpiperazin-1-ylmethyl group;
- N-Hydroxyacrylamide-propenylamido derivatives.

The compounds were evaluated for their ability to inhibit nuclear extract HDACs as well as for their *in vitro* antiproliferative activity. Moreover, their metabolic stability in human and mouse microsomes and solubility was studied. Selected compounds were further characterized by *in vivo* pharmacokinetic experiments. The compounds showed a remarkable stability *in vivo*, also compared to hydroxamic acid HDAC inhibitors already entered in clinical trials. One representative compound (**1**) was submitted to an *in vivo* efficacy experiment and showed a significant antitumor activity in a human colon carcinoma xenograft model.



REFERENCES

1. Sleiman, S.F.; Basso, M.; Manishi, L.; Kozikowski, A.P.; Donhoe, M.E.; Langley, B.; Ratan, R.R. *Expert Opin Investig Drug* **2009**, *18*, 573-574
2. Breslow, R.; Marks, P. A.; Jursic, B.. WO 93/07148, April 15, 1993
3. (a) Grant, S.; Easley, C.; Kirkpatrick, P. *Nature Rev. Drug Discovery* **2007**, *6*, 21-22; (b) Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T. *Cancer Research* **2000**, *60*, 5165-5170

POSTER COMMUNICATIONS – PC.006

DISCOVERY OF NOVEL ORALLY ACTIVE MGLUR5 NEGATIVE ALLOSTERIC MODULATOR GSK2210875

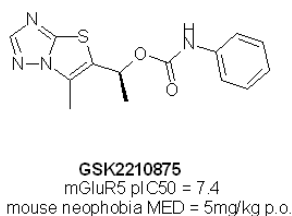
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This lecture will report the discovery of the novel orally active mGluR5 negative allosteric modulator / antagonist GSK2210875.

This novel MPEP competitive chemotype was identified by high throughput screening and demonstrates intriguing and complex structure activity relationships. Optimisation of receptor potency, oral exposure, and brain free fraction allowed identification of compounds which could achieve receptor occupancy of >90% required for activity in *in vivo* models. A focus on polar compounds (logD <2.5) of modest potency, but high free brain exposure, led to the identification of GSK2210875 which demonstrates an oral minimum effective dose (MED) of 5mg/kg in the mouse neophobia model.

The relationships between this chemotype, with its unique SAR, and other known mGluR5 negative modulators will be outlined.



POSTER COMMUNICATIONS – PC.007

STRUCTURE BASED DISCOVERY AND RATIONAL DESIGN OF FIRST-IN-CLASS INHIBITORS OF LEDGF/p75-INTEGRASE INTERACTION

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AIDS, caused by the HIV virus is a worldwide spread disease. Until today there is no cure, however with a combination of drugs to halt the replication of the virus and curing opportunistic infections, infected people can be treated. This is the so called highly active anti retroviral therapy (HAART). Due to the easily occurring mutations into the HIV genome, resistance readily arises against the currently used antiretroviral agents. This resistance calls for the development of new compounds, and more importantly, new antiretroviral strategies.

A new potential strategy is the inhibition of the integration process. During this step of the replication cycle, the viral genome is integrated into the human genome. This complex step is carried out by the viral integrase enzyme during several consecutive steps, each one of these steps being a potential pharmaceutical target. LEDGF/p75 was discovered as a cellular cofactor of the integrase enzyme^[1] and following validation, appeared to be essential for the viral replication cycle. Thus inhibition of the integrase-LEDGF/p75 interaction was proven to be a new and interesting strategy to threat HIV infected people.^[2-3]

Historically, this type of interactions (protein-protein interactions), were considered as challenging but recent successes have proven the feasibility. Therefore we initiated the search for integrase-LEDGF/p75 interaction inhibitors using computer-aided drug design methods.

An exploratory study on published small molecule protein-protein interaction inhibitors gained knowledge directly applicable in our research. Analysis of the chemical space showed that usage of 3 filter descriptors is enough to discard uninteresting molecules (molecules without the capacity to inhibit protein-protein interactions).

In a second part of the computational research we showed that small molecules, which competitively inhibit protein-protein interactions, mimic partially the protein-protein interface. Thus a pharmacophore can be constructed from the protein-protein interface for the virtual discovery of an inhibitor of this protein-protein interaction.

These insights were applied to the HIV-1 integrase - LEDGF/p75 interaction. In an initial virtual screening setup the molecules were filtered according to the protein-protein interaction inhibition capacity according to the descriptors. In the next step the molecules were selected based on a pharmacophore model which was constructed out of all available integrase X-ray structures, of which the integrase CCD - LEDGF/p75 IBD structure is the most important.^[4]

Further selection was based on the results of molecular docking simulations. The inhibitory activity of the first molecules was experimentally tested in an ALPHA-screen assay. The molecules which showed some activity were optimized, leading to the discovery of novel compounds with *in vivo* inhibitory activity. Using X-ray crystallography and biochemical assays, the binding mode and mechanism of action of the compounds was validated. Furthermore the crystal structures were used for structure based optimization of new compounds and the discovery of new classes of inhibitory compounds. Currently the most potent inhibitors show an *in vivo* IC₅₀ activity of 55nM.

We can conclude that during this collaborative research (KULeuven Laboratories for Biomolecular Modelling, for Molecular Virology and Gene therapy, for Biocrystallization and the Centre for Drug Design and Discovery (CD3)) a new antiretroviral compound class was discovered and optimized using computational and rational drug design strategies. In this study the rational modeling approach was a leading and essential part of the discovery phase.

These compounds are promising for future pharmaceutical optimizations to be used in the clinic as new antiretroviral agents.^[5]

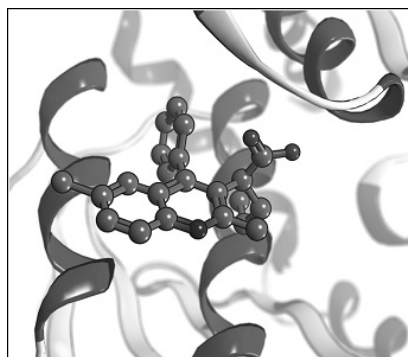


Figure: Crystallography was used to validate the binding mode of discovered inhibitors. Insights in the interactions of the ligand-protein complex allowed for rational design of optimized inhibitors.

REFERENCES

- Cherepanov P, *et al.* HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem.* 2003;278(1):372-81.
- Vandekerckhove L, *et al.* Transient and stable knockdown of the integrase cofactor LEDGF/p75 reveals its role in the replication cycle of human immunodeficiency virus. *J Virol.* 2006 Feb;80(4):1886-96.
- De Rijck J, *et al.* Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. *J Virol.* 2006 Dec;80(23):11498-509

- Cherepanov P, *et al.* Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proc Natl Acad Sci U S A.* 2005 Nov 29;102(48):17308-13
- Christ F, Voet AR, Marchand A, *et al.* Design of first-in-class inhibitors of LEDGF/p75-integrase interaction and HIV replication. *Nature Chemical Biology (accepted for publication)*

POSTER COMMUNICATIONS – PC.008

NEW N-[X-(Y-METHOXY-6,11-DIHYDROINDOLO[1,2-B]ISOQUINOLIN-11-YL)ALKYL]ALKANAMIDES: SYNTHESIS AND MELATONINERGIC POTENCY

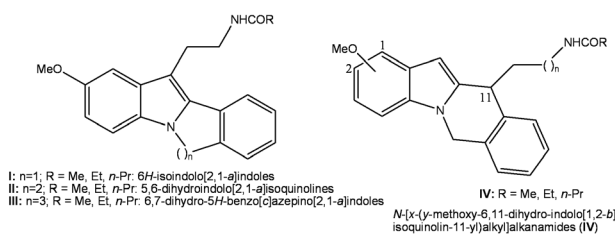
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Melatonin (*N*-acetyl 5-methoxytryptamine), a hormone synthesized and released from the pineal gland at night, has been shown to have a hypnotic action in animals and humans and there has been considerable recent interest in the therapeutic potential of melatonin analogues as hypnotics and as agents for restoring circadian rhythms disturbed by jet-lag, shiftwork and aging.¹

The physiological actions of melatonin in regulating seasonal and circadian rhythms are thought to be mediated through a family of specific, high affinity, G-protein-coupled cell membrane receptors. Two receptor subtypes (MT1 and MT2) have been cloned in mammals² which, when expressed in host cells, show the general pharmacological characteristics of native melatonin receptors.

Notwithstanding the renewed interest in melatonin and its actions in recent years, the pharmacology of melatonin is in its infancy. In a series of studies we have sought to understand how melatonin binds to and activates its receptor and to use the knowledge gained to design subtype specific receptor agonists and antagonists, which will be useful tools for defining the full physiological and pathophysiological role of this hormone. In 2000 we published on the synthesis and biology of compounds **I-III** (*vide infra*), and found that increasing the carbon chain length between *N*-1 of indole and the 2-phenyl group from *n* = 1 through *n* = 3 leads to a fairly regular decrease in the binding affinity, but, remarkably, when *n* = 3, it converts these compounds from melatonin agonists to antagonists.³



As an extension of this work, we synthesised analogues **IV**, in which the side chain of **I-III** was translocated from C3 (indole numbering) to C11 and the 2-phenyl ring moved one carbon atom further away. The evaluation of the biological action of the new analogues is currently underway.

ACKNOWLEDGMENT

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REFERENCES

- Delagrangé, P.; Guardiola-Lemaitre, B. *Clin. Neuropharmacol.* **1997**, 20, 482-5102.
- Reppert, S.M.; Godson, C.; Mahle, C.D.; Weaver, D.R.; Slangenaupt, S.A.; Gusella, J.F. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, 92, 8734-8738.
- Faust, R.; Garratt, P.J.; Jones, R.; Yeh, L.-K.; Tsotinis, A.; Panoussopoulou, M.; Calogeropoulou, T.; Teh, M.-T.; Sugden, D. *J. Med. Chem.*, **2000**, 43, 1050-1061.

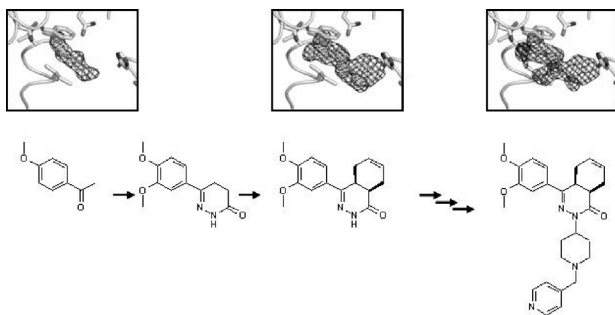
POSTER COMMUNICATIONS – PC.009

FRAGMENT-BASED DESIGN OF PDE4 INHIBITORS

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Fragment-Based Lead Generation has matured over the last couple of years into a viable methodology to generate tractable lead structures against a variety of target enzymes and receptors. Here we report on a structure-guided medicinal chemistry campaign towards PDE4 inhibitors, employing a fragment-based lead generation approach with a strong emphasis laid on a number of methodological aspects. We considered phosphodiesterase 4 (PDE4) as an ideal enzyme since it emerged as an attractive target for therapeutic intervention in inflammatory and immune disorders, such as asthma, rheumatoid arthritis, or COPD. In addition, highly active PDE4 inhibitors have been described. From a methodological point of view, we were mainly interested in probing the limits towards smallest fragments that can be identified by an in-house developed site-specific binding assay and can be successfully crystallized in complex with the target enzyme. For that purpose, smallest possible fragments based on a retro-engineering of the pyridazinone scaffold were designed and submitted to a fragment-screening and fragment crystallization campaign. Differently substituted acetophenones were identified as smallest fragments that bound site-specifically to the adenine-recognition site of PDE4. Unexpectedly, first qualitative structure-activity relationships emerged already at this initial process step.



Fragment Evolution Strategy from initial seed fragments (left) to complex lead compounds (right). Inhibitors are shown in the binding site with their electron densities as determined by protein crystallography (top).

Para-methoxy-acetophenone derivatives were successively evolved over several iterative cycles of successive design and synthesis towards a fully elaborated lead structure exhibiting sub-nanomolar PDE4 inhibitory activity. Each round of optimization was supported by structural biology, alongside with biophysical, and biochemical investigations. For optimal efficiency a synthetic route was designed that could easily be adapted for the preparation of a large variety of analogs. Special emphasis was laid on developing a detailed structural understanding of the enthalpic and entropic aspects that contribute to the total free energy of binding of the seed fragments, as well as intermediates taken from the fragment evolution strategy. We consider an in-depth analysis of thermodynamic and kinetic binding attributes as key for an efficient decision making process at the outset of and within any lead finding and optimization campaign.

POSTER COMMUNICATIONS – PC.010

NEW FLUORINE CONTAINING AMINO ACIDS AS LABELS TO STUDY ANTIMICROBIAL PEPTIDES BY SOLID STATE ^{19}F NMR

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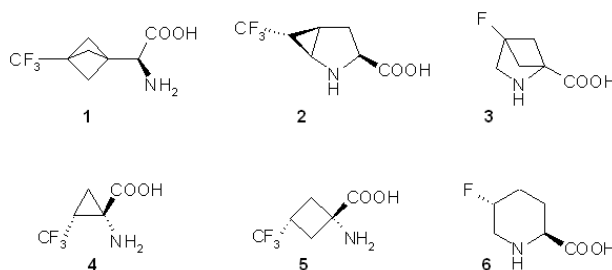
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Solid state ^{19}F -NMR is a powerful method to study membrane-active peptides, as it can reveal their conformation, orientation and dynamics when embedded in a lipid bilayer.¹ For this purpose the peptide of interest has to be selectively labeled with a suitable ^{19}F -containing amino acid at several different positions. The resulting battery of singly ^{19}F -labeled analogues then has to

be tested for full functional integrity, before they are reconstituted into lipid bilayer samples for the solid state ^{19}F -NMR analysis.

The main limitation of this approach, however, lies in the poor repertoire of suitable ^{19}F -labels, as only very few of the known ^{19}F -containing amino acid can serve as proper ^{19}F -labels. Therefore, we have rationally designed and synthesized compounds **1** and **2**, to serve as proper ^{19}F -labels for the substitution of Leu, Ile, Val, Ala and Pro, respectively, in peptides.²⁻⁶ These novel amino acids have been successfully used to study the structure and dynamic behaviour of the antimicrobial peptides GS, PGLa and cell penetrating peptide SAP in model membranes by ^{19}F -NMR.

Also, the new potential ^{19}F -labels **3-6** have been synthesized.⁷⁻⁹ Validation of their utility in peptide studies is in progress.



REFERENCES

1. A. S. Ulrich, **Prog. NMR Spectr.** 46 (2005) 1-21.
2. P. K. Mykhailiuk, S. Afonin, A. N. Chernega, E. B. Rusanov, M. Platonov, G. Dubinina, M. Berditsch, A. S. Ulrich, I. V. Komarov, **Angew. Chem.** 118 (2006) 5787-5789; **Angew. Chem. Int. Ed.** 45 (2006) 5659-5661.
3. P. K. Mykhailiuk, N. M. Voievoda, S. Afonin, A. S. Ulrich, I. V. Komarov, **J. Fluor. Chem.** 131 (2010) 217-220.
4. S. Afonin, P. K. Mykhailiuk, I. V. Komarov, A. S. Ulrich, **J. Pept. Sci.** 13 (2007) 614-623.
5. P. K. Mykhailiuk, S. Afonin, G. V. Palamarchuk, O. V. Shishkin, A. S. Ulrich, I. V. Komarov, **Angew. Chem.** 47 (2008) 5849-5851; **Angew. Chem. Int. Ed.** 47 (2008) 5765-5767.
6. P. K. Mykhailiuk, S. Afonin, A. S. Ulrich, I. V. Komarov, **Synthesis** 11 (2008) 1757-1760.
7. A. N. Tkachenko, D. S. Radchenko, P. K. Mykhailiuk, O. O. Grygorenko, I. V. Komarov, **Org. Lett.** 11 (2009) 5674-5676.
8. O. S. Artamonov, P. K. Mykhailiuk, D. M. Volochnyuk, I. V. Komarov, **Synthesis** 42 (2010) 443-446.
9. D. Radchenko, P. K. Mykhailiuk, A. Bezudnyy, I. Komarov, **Synlett** 11 (2009) 1827-1829.

POSTER COMMUNICATIONS – PC.011

THE IMPORTANCE OF DRUG-TARGET BINDING KINETICS FOR DRUG EFFICACY - WITHOUT ON YOU ARE OFF!

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Binding strength, affinity (K_D) can be broken down into kinetic constants that reflect drug-target recognition (the association rate constant, k_{on}) and the stability of the resulting complex (dissociation rate constant, k_{off}). The relationship between these properties can be described as $K_D = k_{off}/k_{on}$. The off-rate has been recognized for some time as a key parameter for understanding pharmacokinetic and dynamic (PKPD) properties of drugs¹⁻³. A slow off-rate can prolong the therapeutic effect both by tight binding and by "hiding" the drug from enzymatic degradation and clearance by keeping it out of the circulation. High off-rate selectivity can also minimize side effects caused by off-target binding.

The importance of on-rate is less recognized, however, and even (erroneously) thought to be constant (diffusion rate limited) for small molecules. In fact, the combination of available drug concentration and the on-rate is the driving force to get the drug into the binding site of the target protein. If the on-rate is too low in relation to its bioavailability, the drug will never bind to the target, and affinity/dissociation rate is then a false indication of the potency of the drug.

In a lead series, k_{on} and k_{off} can vary by several orders of magnitude even when compounds have similar affinities. It is very important, therefore, to measure and understand the binding kinetic pattern of lead series and the effect this might have on the PK/PD properties of the scaffolds. Association rate is concentration driven ($M^{-1}s^{-1}$) and can therefore be compensated by good ADME properties that give a high concentration of available drug. When the bioavailability is low, compounds with rapid on-rates might still do the job. A rapid on-rate is also preferred when a rapid effect is desired. If the on-rate is low in relation to free concentration, then an increase in affinity driven by improved complex stability (even by several orders of magnitude) does not increase the number of blocked target binding sites. If the limiting factor is relatively rapid clearance, then a drug with a slow off-rate might be preferred therapeutically. Dissociation rate is concentration independent (s^{-1}) and can only be influenced by structural changes to the lead compound. Structure-activity-relationship (SAR) studies^{4,5} can be improved by plotting the structures of the lead series in an $k_{on}/k_{off}/K_D$ -map.

In this lecture, I will discuss the effects of different on/off-rate combinations in relation to the bioavailable concentration of leads and how this influences binding to the target protein and the residence time of the drug in the binding site. Examples will be given to highlight the importance of on-rate – Without "on" there is no "off"!

REFERENCES

1. Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov.* 2006;5(9):730-9
2. Gabrielsson J, Hugues D, Gillberg, P-G, Bredberg U, Benthem B, Duker G. Early integration of pharmacokinetic and dynamic reasoning is essential for optimal development of lead compounds: strategic considerations. *Drug Discovery Today*, 2009;14(7/8):58-372.
3. Andersson K, Löfås S, Karlsson R, Hämmäläinen MD. Label-free kinetics binding data as a decisive element in drug discovery. *Expert opinion on drug discovery* 2006;1(5):439-446.
4. Markgren P-O, Schaal W, Hämmäläinen MD, Karlén A, Hallberg A, Samuelsson B, Danielson UH. Relationships between structure and interaction kinetics for HIV-1 protease inhibitors, *J Med Chem.* 2002;45:5430-9.
5. Huber W. A new strategy for improved secondary screening and lead optimization using high-resolution SPR characterization of compound-target interactions. *J Mol Recognit.* 2005;8(4):273-81.

POSTER COMMUNICATIONS – PC.013

SELECTIVE INHIBITORS OF MMP-12, A TARGET IN INFLAMMATION : FROM CHEMISTRY TO THERAPEUTIC APPLICATIONS

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Following the disappointment of the clinical trials of early broad-spectrum synthetic inhibitors of matrix metalloproteinase (MMPs), the field is now resurgent focusing on the development of inhibitors able to fully discriminate between the different members of the MMP family. MMP-12 has been involved in the development of several human pathologies like atherosclerosis, aneurysm and COPD, observations justifying the development of selective MMP-12 inhibitors.

Extremely potent inhibitors of MMP-12 have been developed by the screening of phosphinic peptide libraries. In order to understand the molecular determinants responsible for the observed inhibitor selectivity, X-ray, isothermal titration microcalorimetry, NMR and MMP-12 mutant studies have been performed. Altogether, these new data open new vistas for developing selective inhibitors towards other MMPs. The *in vivo* activity of one MMP-12 selective inhibitor has been evaluated in mouse model of atherosclerosis. Inhibitor treatment was observed to affect both plaque initiation and progression at multiple sites, thus MMP-12 specific inhibition may prove useful for the treatment of progressive atherosclerosis in human.

POSTER COMMUNICATIONS – PC.014

POTENT, SELECTIVE AND PLASMA-STABLE INHIBITORS OF THE PSD-95/NMDA RECEPTOR INTERACTION

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The protein-protein interaction between the *N*-methyl-D-aspartate (NMDA) receptor and the intracellular scaffolding protein postsynaptic density protein-95 (PSD-95) is a potential target for treatment of ischemic brain diseases. Extensive activation of the NMDA receptor by glutamate, as seen in excitotoxicity, plays a key role in several brain diseases. However, development of drugs that directly modulate the NMDA receptor has been difficult.¹ Instead, inhibition of the ternary complex of the NMDA receptor, neuronal nitric oxide synthase (nNOS), and PSD-95 attenuates glutamate induced cell death by impairing the functional link between Ca²⁺ entry and NO production without affecting vital synaptic transmission. Thus, PSD-95 constitutes a promising target for neuroprotective drugs. Uncoupling of PSD-95 and the NMDA receptor subunit, GluN2B, has previously been achieved by a 20-mer peptide (Tat-N2B), which mimics the C-terminal of GluN2B and thereby inhibits the nNOS-NMDA receptor linkage by binding to two protein domains of PSD-95 – the PDZ1 and PDZ2 domains. This peptide is currently in clinical trials as a potential treatment of stroke.²

We have identified two novel types of compounds as potent inhibitors of the PSD-95/NMDA receptor interaction: *N*-alkylated tetrapeptides³ and dimeric ligands⁴, respectively. In the first approach a compound was identified that is 20-fold more potent towards PSD-95 than the clinical candidate Tat-N2B.¹ In the other approach, two peptide ligands have been dimerized whereby extremely potent compounds were generated with affinities as low as 10 nM – approximately 1000-fold more potent than the peptide in clinical development. The two series were optimized for human blood plasma-stability and is currently undergoing investigations focusing on membrane permeability and biological activity.

REFERENCES

1. F. Gardoni, M. Di Luca, *Eur. J. Pharmacol.* 2006, 545, 2.
2. M. Aarts, Y. Liu, L. Liu, S. Besshoh, M. Arundine, J.W. Gurd, Y. T. Wang, M.W. Salter, M. Tymianski, *Science* 2002, 298, 846.
3. A. Bach, C. N. Chi, T. B. Olsen, S.W. Pedersen, M. U. Røder, G. F. Pang, R. P. Clausen, P. Jemth, K. Strømgaard, *J. Med. Chem.* 2008, 51, 6450.

4. A. Bach, C. N. Chi, G. F. Pang, L. Olsen, A. S. Kristensen, P. Jemth, and K. Strømgaard, *Angew. Chem. Int. Ed.* 2009, 48, 9685.

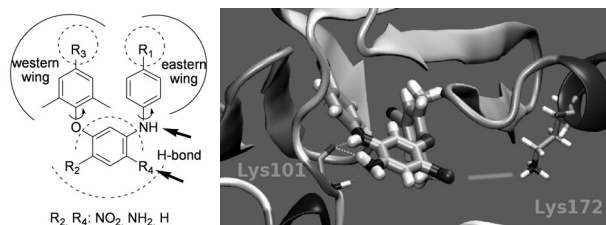
POSTER COMMUNICATIONS – PC.015

DIARYLANILINE DERIVATIVES AS A NOVEL CLASS OF HIV-1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

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Structure-based drug design and isosteric replacement were applied to the synthesis of diarylaniline (*N*,5-diarylbenzene-1,2-diamine) derivatives, which were evaluated against wild-type HIV-1 and drug-resistant viral strains. The most promising compound **37** showed significant EC₅₀ values of 0.003–0.032 μM against wild-type HIV-1, which were better than that of TMC125 in the same assay, and EC₅₀ values of 0.005–0.604 μM against several drug-resistant strains. Current results also revealed important structure-activity relationship (SAR) conclusions for diarylanilines and strongly support our hypothesis that an NH₂ group on the central benzene ring *ortho* to the aniline moiety is crucial for interaction with K101 of the NNRTI binding site in HIV-1 RT, likely by forming H-bonds with K101. Furthermore, molecular modeling studies with MM/GBSA technology demonstrated the rationality of our hypothesis. Overall, the results in this study were promising for the discovery of diarylaniline derivatives as a novel class of next-generation HIV-1 NNRTI agents.



37, R₁ = R₃ = CN, R₂ = NO₂, R₄ = NH₂

EC₅₀ 0.003 μM (WT), 0.005–0.604 μM (MDR strains)

ACKNOWLEDGMENTS

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POSTER COMMUNICATIONS – PC.016

A PHOTOACTIVABLE PROBE TO IDENTIFY PROTEINS IMPLIED IN IODIDE TRANSPORT

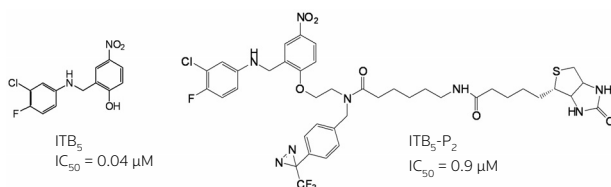
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The Na^+/I^- symporter (NIS) is a membrane glycoprotein essentially found in thyroid cells which is responsible for plasmatic iodide uptake into the cytoplasm¹. This transport is at the basis of an emerging approach using radioiodide for cancer diagnosis and treatment. However, cancer cells naturally or artificially overexpressing the NIS present in many cases a greatly reduced activity of this protein. A better understanding of the still poorly defined post-traductionnal regulation mechanisms of the NIS could consequently allow us to reinduce the activity of the NIS in these cells and to open new prospects for the treatment of thyroid and non-thyroid cancers.

To study these mechanisms, our laboratory set up a direct chemical genetics strategy whose first step was to look for small organic molecules being able to inhibit iodide transport. After a high-throughput screening, 10 inhibitors were identified with IC_{50} values in the submicromolar range². The second step consists in isolating the target proteins of these molecules. The considered method, namely affinity photolabelling, is based on the use of photoactivable analogues which can covalently bind their target proteins under light irradiation. These analogues are then coupled to a molecule allowing for the isolation of the complex formed by affinity chromatography.

We started with our most active inhibitor (ITB_5). The study of the structure-activity relationship of this compound allowed to design and to synthesize a probe ($\text{ITB}_5\text{-P}_2$) which contains a photoactivable group and a biotin molecule and which displays a satisfactory inhibitory activity ($\text{IC}_{50} = 0.9 \mu\text{M}$). Preliminary experiments gave promising results. The photolysis of $\text{ITB}_5\text{-P}_2$ in the presence of living cells has led to the specific labelling of a single protein (molecular weight $\sim 25 \text{ kDa}$). These results as well as the different methods currently tested to isolate and identify this protein will be presented.



REFERENCES

1. Dai G, Levy O, Amzel L.M, Carrasco N : **The mediator of thyroidal iodide accumulation : the sodium/iodide symporter**. *Handbook of biological physics*. Elsevier Press : Amsterdam, 1996, 15-22.
2. Lecat-Guillet N, Goulven M, Lopez R, Pourcher T, Rousseau B, Ambroise Y : **Small-Molecule Inhibitors of Sodium Iodide Symporter Function**. *ChemBiochem* 2008, **9**:889-895

POSTER COMMUNICATIONS – PC.017

NUCLEOPHILIC SUBSTITUTION AT THE C3a TERTIARY CARBON OF THE BROMOPYRROLOINDOLE SCAFFOLD

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The hexahydropyrrolo[2,3-b]indole scaffold is a common structural element of a variety of natural products that possesses a wide range of biological activities (**Fig. 1**).¹ This molecular framework is, therefore, particularly attractive for the preparation of compound libraries for medicinal chemistry programmes. Furthermore, alkaloids containing the hexahydropyrrolo[2,3-b]indole molecular framework have been identified as potent and selective protein-protein interaction (PPI) inhibitors.² In the modern drug discovery era, targeting PPIs offers the opportunity to identify more selective drugs.

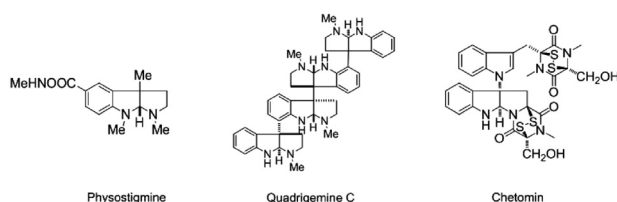
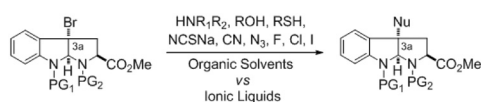


Figure 1. Examples of natural products containing the pyrroloindole molecular framework.

To date, the striking architecture of these pyrroloindole-based alkaloids has generated considerable interest within the synthetic community.³ For the preparation of compound libraries, functionalisation at the C3a position is critical and, although explored for total syntheses, the scope at present remains limited.⁴ Progress on synthetic studies to nucleophilic substitution at C3a to introduce a broader range of groups will be presented (**Scheme 1**).



Scheme 1. Nucleophilic substitution at C3a.

REFERENCES

1. (a) Hino, T.; Nakagawa, M. *Alkaloids* **1988**, 34, 1-75. (b) Anthoni, U.; Christophersen, C.; Nielsen, P. H. *Alkaloids* **1999**, 13, 163-236.
2. Zinzalla, G.; Thurston, D.E. *Future Med. Chem.* **2009**, 1, 65.
3. Crich, D.; Banerjee, A. *Acc. Chem. Res.* **2007**, 40, 151 – 161.
4. Espejo, V. R.; Rainier, J.D. *J. Am. Chem. Soc.* **2008**, 130, 12894-12895.

POSTER COMMUNICATIONS – PC.018

STRUCTURE–MEMBRANE INTERACTIVITY
RELATIONSHIP OF LOCAL ANAESTHETICS
ASSOCIATED WITH THEIR STEREOSELECTIVE
CARDIOTOXICITY

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Local anaesthetics display cardiotoxic capacities by an accidental intravenous injection or an absolute overdose. *S*(–)-Bupivacaine and *S*(–)-ropivacaine have been reported to be less toxic than their *R*(+)-enantiomeric counterparts and racemic mixture [1]. In mechanistic pharmacotoxicology, local anaesthetics interact not only with cardiomyocyte channels and receptors but also with membrane lipids to modify the physicochemical properties of lipid bilayers [2,3]. In order to find a clue to understand the cardiotoxic stereoselectivity, we studied the structure–membrane interactivity relationship of local anaesthetic stereoisomers with attention to chiral membrane components.

Liposomal membranes were prepared with cardiolipin, phospholipids and cholesterol of varying compositions, and cardiomyocyte-mimetic membranes with a mixture of 40 mol% cholesterol, 10 mol% cardiolipin, 47 mol% different phospholipids and 3 mol% sphingomyelin [3]. Bupivacaine and ropivacaine (*S*(–), *R*(+)-enantiomers and racemate) were reacted with these membrane preparations at the cardiotoxically relevant concentration of 200 μ M [4], and then their membrane interactivities were comparatively determined by measuring fluorescence polarisation [5].

All the tested drugs interacted with lipid bilayers to increase the membrane fluidity. Despite having two chiral centres, cardiolipin was not effective in discriminating the induced membrane fluidisation between *S*(–)- and *R*(+)-enantiomers. In contrast, increasing cholesterol (5–45 mol%) in membranes produced the different membrane interactions of *S*(–)-, racemic and *R*(+)-anaesthetics, but not in the absence of cholesterol. When lipid bilayers contained 40 mol% and more cholesterol, local anaesthetics fluidised such membranes with the potency being *S*(–) < racemic < *R*(+)-bupivacaine and being *S*(–) < *R*(+)-ropivacaine.

Bupivacaine (racemate), levobupivacaine (*S*(–)-enantiomer) and ropivacaine (*S*(–)-enantiomer) interacted with cardiomyocyte-mimetic membranes in the decreasing order of intensity. The rank order of membrane interactivity agreed with that of cardiotoxicity [1]. Their opposite absolute configurations allow enantiomers to be discriminated by the interaction with another chiral molecule. The enantioselectivity was increased with elevating the molar ratio of cholesterol in membranes. Membrane cholesterol is responsible for the enantiomeric discrimination because it has higher chirality compared with the glycerol backbone of phospholipids including cardiolipin. The stereoselective membrane interactivity determined by cholesterol may be associated with the stereoselective cardiotoxicity of local anaesthetics.

The stereostructure–membrane interactivity relationship characterised in the present study supports the development and clinical use of *S*(–)-enantiomers to reduce the toxic effects of piperidine local anaesthetics on cardiovascular systems.

REFERENCES

1. Heavner JE. *Reg Anesth Pain Med*, **27**: 545–555, 2002.
2. Önyüksel H, Sethi V, Weinberg GL, Dudeja PK, Rubinstein I. *Chem Biol Interact*, **169**: 154–159, 2007.
3. Tsuchiya H, Ueno T, Mizogami M, Takakura K. *Chem Biol Interact*, **183**: 19–24, 2010.
4. Groban L, Deal DD, Vernon JC, James RL, Butterworth J. *Anesth Analg*, **92**: 37–43, 2001.
5. Tsuchiya H, Mizogami M, Takakura K. *J Chromatogr A*, **1073**: 303–308, 2005.

POSTER COMMUNICATIONS – PC.019

DESIGN, SYNTHESIS AND BIOLOGICAL
EVALUATION OF SPIRO [CHROMANE-2,4'-
PIPERIDINE] HYDROXAMIC ACID
DERIVATIVES AS STRUCTURALLY NOVEL
HISTONE DEACETYLASE (HDAC) INHIBITORS

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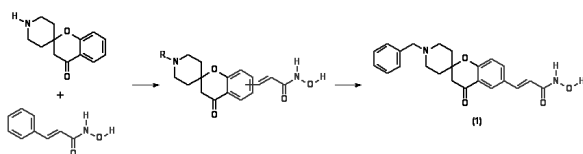
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The post-translational acetylation status of chromatin is regulated by the competing activities of two enzymatic classes: histone

acetyl transferases (HATs) and histone deacetylases (HDACs). Acetylation of histones is generally associated with gene activation; conversely, deacetylation is associated with gene silencing, including a decrease in the expression of tumor suppressor genes¹. Inhibition of HDAC activity can reverse this epigenetic silencing making histone deacetylases (HDACs) an attractive anticancer target. SAHA (suberoylanilide hydroxamic acid), the first HDAC inhibitor approved by the FDA in 2006 for the treatment of cutaneous T-cell lymphoma (CTCL), validates this approach for the treatment of cancer and several other HDAC inhibitors are currently at different stages of clinical development².

In the present study, we report the design, synthesis and biological evaluation of a new series of spiro[chromane-2,4'-piperidine] hydroxamic acid derivatives. We sought to combine a 'privileged scaffold', spiro[chromane-2,4'-piperidine], with minimal structures able to inhibit histone deacetylase enzymes³. The compounds were evaluated for their ability to inhibit nuclear extract HDACs as well as for their in vitro antiproliferative activity on different tumor cell lines. This work resulted in the discovery of spirocycle derivative (**1**) that showed good oral bioavailability and tumor growth inhibition in a HCT116 murine xenograft model.



REFERENCES

1. J. E. Bolden, M. J. Peart, R. W. Johnstone *Nat. Rev. Drug Discov.* **2006**, *5*, 769-784.
2. A. A. Lane, B. A. Chabner. *Journal of Clinical Oncology* **2009**, *27*, 5459-5468.
3. F. Thaler, A. Colombo, A. Mai, R. Amici, C. Bigogno R. Boggio, A. Cappa, S. Carrara, T. Cataudella, F. Fusar, E. Gianti, S.J. di Ventimiglia, M. Moroni, D. Munari, G. Pain, N. Regalia, L. Sartori, S. Vultaggio, G. Dondio, S. Gagliardi, S. Minucci, C. Mercurio, M Varasi, M., *Journal of Medicinal Chemistry* **2010**, *53*, 822-839.

POSTER COMMUNICATIONS – PC.020

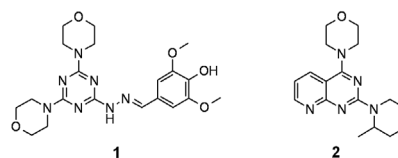
THE IDENTIFICATION OF CLINICAL CANDIDATE, AZD8055: A POTENT, SELECTIVE SMALL MOLECULE INHIBITOR OF mTOR KINASE

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The mammalian target of rapamycin (mTOR) is a key target in the development of antitumour therapies¹. Activated by growth factor/mitogenic stimulation activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway mTOR is a central

regulator of cell growth and proliferation. This PI3K-Akt-mTOR pathway is one of the most frequently dysregulated pathways in cancer². The known mTOR inhibitor Rapamycin and its analogues (such as Everolimus and Temsirolimus) bind to the FKBP12/rapamycin complex binding domain (FRB), resulting in suppression of signalling to the downstream targets p70S6K and 4E-BP1³. However, it has recently been shown that mTOR can exist in an alternative, rapamycin insensitive, complex that signals to Akt⁴. Therefore, it is proposed that a compound directly targeting the kinase domain of mTOR would inhibit signalling through both the rapamycin sensitive complex (mTORC1) and the rapamycin insensitive complex (mTORC2) and as such the compound would exhibit a different spectrum of activity compared with rapamycin.



Two alternative approaches to identify selective inhibitors of the mTOR kinase domain resulted in the identification of two distinct lead series, (1) and (2)⁵. The identification and optimisation of these series will be described culminating in the discovery of AZD8055, which has been shown to be a potent and selective inhibitor of both complexes of mTOR (mTORC1 and mTORC2) and demonstrates dose dependant tumour growth inhibition in xenograft studies. AZD8055 is currently undergoing clinical evaluation as a potential cancer therapy.

REFERENCES

1. Huang, S.; Houghton, P. J. *Curr Opin Pharmacol.* **2003**, *3*, 371.
2. Yap, T.A.; Garrett, M.D.; Walton, M.I.; Raynaud, F.; de Bono, J.S.; Workman, P. *Curr. Opin. Pharmacol.* **2008**, *8*, 393.
3. Fingar, D.C.; Salama, S.; Tsou, C.; Harlow, E.; Blenis, J. *Genes Dev.* **2002**, *16*, 1472.
4. Sarbassov, D.D.; Ali, S.M.; Sabatini, D.M. *Curr. Opin. Cell Biol.* **2005**, *17*, 596.
5. Menear, K. A; *et al*, *Bioorg. Med. chem. Lett.* **2009**, *19*, 5898; Malagu, K.; *et al*, *Bioorg. Med. chem. Lett.* **2009**, *19*, 5950.

POSTER COMMUNICATIONS – PC.021

ARYL PIPERAZINE AND PYRROLIDINE AS ANTIMALARIAL AGENTS SYNTHESIS AND INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

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The 2009 Word Malaria Report indicated that 108 countries showed endemic malaria¹. The search for novel drugs with inexpensive and easy synthetic methods against specific parasites is an important goal for antimalarial drug discovery.

Taking into account the antimalarial activity shown by aminealcohol derivatives², piperazine and pyrrolidine derivatives have been developed and evaluated as growth inhibitors of Chloroquine-resistant *Plasmodium falciparum* strains (FCR-3).

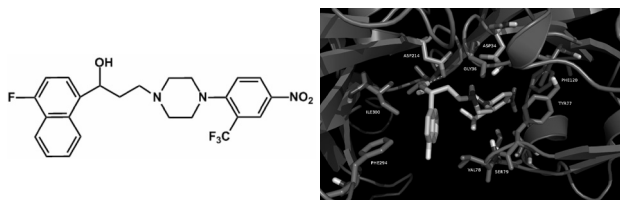
Five of the aryl alcohol assayed inhibited the parasite growth in 50% at a concentration <10 µM.

These studies have demonstrated a direct relationship between antiplasmodial activity and the presence of aryl alcohol, a four carbon aliphatic chain and a fluorine atom in the aryl alcohol.

Complementary toxicity studies have shown low toxicity for the most active compound (Fig. 1a). This compound is 20 to 40 times more active in FCR-3 strain against tumoural cells.

In spite of a favourable physical chemical profile of intestinal absorption, the low solubility by oral administration reduces *in vivo* assay activity.

Finally, computational and docking studies (Fig. 1b) have developed the compound affinity for *Plasmodium* enzyme, Plasmeprin II. This fact suggests an alternative action mechanism, different from Chloroquine.



Figures 1a, 1b: 1-(4-fluoronaphthyl)-3-[4-(4-nitro-2-trifluoromethylphenyl) piperazin-1-yl]propan-1-ol.

REFERENCES

1. 2009 Word Malaria Report. www.who.int.
2. Pérez-Silanes, L., Berrade, R.N., García-Sánchez, A., Mendoza, S., Galiano, B., Martín-P.Solórzano, J.J., Nogal-Ruiz, A.R., Martínez-Fernández, I., Aldana, Monge. A., 2009. New 1-aryl-3-substituted propanol derivatives as antimalarial agents. *Molecules* 14, 4120-4135.

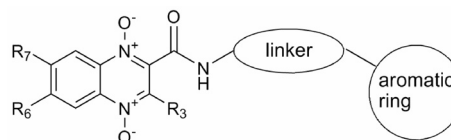
POSTER COMMUNICATIONS – PC.022

QUINOXALINE-2-CARBOXAMIDE 1,4-DI-N-OXIDE DERIVATIVES AS ANTI-TUBERCULOSIS AGENTS: SYNTHESIS, BIOLOGICAL EVALUATION AND ELECTROCHEMICAL STUDIES

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The global epidemiology of TB, particularly MDR-TB and XDR-TB, is unknown and the true magnitude of the problem is most likely underestimated, thereby encouraging the search for new anti-tuberculosis agents¹. As a continuation of our research in quinoxaline 1,4-di-N-oxide and with the aim of establishing structural requirements for the design of new anti-tuberculosis drugs, seventy-nine quinoxaline-2-carboxamide 1,4-di-N-oxide derivatives were prepared according to the following structure^{2,3}:



All of the synthesized compounds were evaluated for *in vitro* anti-tuberculosis activity following the screening program for the discovery of new drugs for the treatment of tuberculosis carried out by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF).

To gain insight into the mechanism of action and based on the results, twenty-six derivatives were selected in order to study the redox properties of these substances, using cyclic voltammetry and first derivative cyclic voltammetry in DMF with TBAP as supporting electrolyte. For all of the compounds studied, two cyclic voltammetric waves were observed during reduction.

The influence of molecular structure on reduction potential is addressed and it can be said that some relationships exist between reduction potential and reported antimicrobial activity. For the compounds in the present study, electrochemical reduction in DMF is consistent with reduction of the N-oxide functionality for forming a radical anion⁴. The second reduction wave observed could be due to the formation of the dianion, or to reduction of the product formed from a chemical step involving the radical anion⁵. The latter is supported by the irreversibility of the first reduction wave. The exact mechanism, however, was not further investigated.

This work has been carried out with the financial support of the PIUNA project from University of Navarra. We wish to express our gratitude to the TAACF for the evaluation of the anti-tuberculosis activity through. E. M. is indebted to the La Rioja Government for a grant.

REFERENCES

1. Global TB Control WHO REPORT 2009. www.who.int/tb/publications/global_report/2009/pdf/full_report.pdf
2. S. Ancizu, E. Moreno, et al., Bioorg. Med. Chem. (2010) doi:10.1016/j.bmc.2010.02.024
3. E. Moreno, S. Ancizu, et al., Eur. J. Med. Chem. *sent*
4. P. W. Crawford, R. G. Scamehorn, et al., Chem.-Biol. Interactions 60, (1986), 67.
5. K. R. Barqawi and M. A. Atfah, Electrochim. Acta 32, (1987), 597.

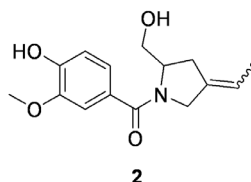
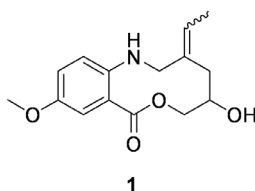
POSTER COMMUNICATIONS – PC.023

SYNTHESIS AND STRUCTURE DETERMINATION OF CH0035

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CH0035, was isolated from the extract of the marine Actinomycete BL-54, and presented cytotoxicity against three human tumor cell lines: A459, HT-29 and MDA-231. Its structure was determined by NMR spectroscopy and it is based in a 10 membered lactone with an exocyclic double bond fused with an aromatic ring (**1**). Synthesis was used at this stage to confirm the structure of CH0035.



Our synthetic results using two alternative routes will be discussed. Both routes converged in one intermediate that was converted by Wittig olefination and macrocyclisation to the proposed structure **1**. The main goal of our synthesis is the alkylation of a weakly nucleophilic aniline by this two different methods, which are based on a reductive amination and on a nucleophilic substitution.

The synthetic product was compared with the isolated natural product and the chemical shifts were slightly different. As a con-

sequence a new proposal for the structure were mandatory. The new proposed structure should have similar predicted chemical shifts, and the main difference between the former and the latter should be the connectivity between the aromatic system and the alkyl chain. A new structure was proposed, consisting on a pyrrolidine ring with an exocyclic double bond linked to an aromatic ring by an amide bond (**2**). The new synthesis will be also discussed as well as the comparison of both structures.

POSTER COMMUNICATIONS – PC.024

POST-COLUMN AFFINITY TECHNOLOGIES FOR LIGAND SCREENING IN MIXTURES FOR THE ACETYLCHOLINE BINDING PROTEIN

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The Acetylcholine Binding Protein (AChBP) is considered an excellent analog for the ligand-binding domain of neuronal nicotinic Acetylcholine Receptors. Its stability and solubility in water allowed the development of an on-line bioaffinity analysis technology. For this, a tracer ligand which displays enhanced fluorescence in the active site of AChBP was designed and synthesized. Evaluation and optimization was performed in convenient microplate reader format and subsequently transferred to the on-line format. The high reproducibility of the system led to the possibility of estimating the affinity of ligands injected in one known concentration. Furthermore, the strength of the on-line bioaffinity analysis system was highlighted with the analysis of biologically active mixtures in gradient HPLC mode with parallel high resolution mass spectrometry (MS) for compound identification.

Since the on-line technology relies on fast post-column incubation times, it cannot efficiently analyze ligands with low a k_{on} . Therefore, a bioaffinity analysis system allowing long post-column incubation times was developed. It is centralized around a contact-less post-column spotter technology capable of rapidly and accurately depositing HPLC eluent onto another platform (e.g. a 1536 microtiter well plate). The technology overcomes the hurdle of traditional low resolution fractionation strategies by tailoring the fractionation frequency to chromatographic peaks.

During the spotting process, biochemical assay constituents were simultaneously mixed in and spotted with HPLC eluent in 1536 microtiter well plates. The strength and possibilities of the spotter were demonstrated by running a post-column at-line spotted AChBP bioaffinity assay with 2 second fractions, each comprising only ~300 nl of eluent from the HPLC separation. Bioaffinities of ligands (also with low a k_{on}) in mixtures could be matched with their identities by correlating reconstructed bioaffinity chromatograms after plate reader analysis with parallel MS chromatograms.

POSTER COMMUNICATIONS – PC.026

SYNTHESIS AND SUBSEQUENT NMR STUDIES OF TRKA BINDERS TOWARDS A NOVEL THERAPEUTIC TO TREAT ALZHEIMER'S DISEASE

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The loss of cholinergic neurons in the basal forebrain has been observed in Alzheimer's disease, resulting in cognitive decline. Such cholinergic neurons are dependent on nerve growth factor (NGF) for survival. NGF binds to the TrkA receptor, dimerises two of these receptors and activates intracellular signalling¹. The fact that NGF is involved in the neuronal survival makes it extremely attractive therapeutically. Administration of NGF has been shown to have therapeutic potential, however in practical terms it would require invasive and expensive surgery². It would be more desirable to design a small orally administered drug able to mimic the way in which NGF binds to its receptor and its effects *in vivo*³.



The crystal structure of NGF dimerising two TrkA receptors⁴.

We have previously prepared a series of small molecule binders which can bind the TrkA receptor and have more recently identified a small molecule capable of binding the TrkA receptor and stimulating downstream signalling *in vitro*. This compound will act as our lead compound for developing an orally available TrkA agonist in treating Alzheimer's disease.

The synthesis of our small molecule binders is currently being explored. HSQC NMR is also acting as a powerful tool in eluci-

dating structure-activity relationships and in the design of our binders. The results of this ongoing work will be presented.

REFERENCES

1. Jing, S., Tapley, P. And Barbacid, M., *Neuron*, **1992**, 9, 1067-79.
2. Tuszyński, M. H., Thal. L., Pay. M., Salmon, D. P. *et al*, *Nat. Med.*, **2005**, 11, 551-5.
3. Dawbarn, D., Fahey, M., Watson, J., Tyler, S., Shoemark, D., Sessions, R., Zhang, R., Brady, L., Willis, C., Allen, S. J., *Biochem. Soc. Trans.*, **2006**, 34, 587-90.
4. Wiesmann, C., Ultsch, M. H., Bass, S. H., de Vos, A. M., *Nature*, **1999**, 401, 184-8.

POSTER COMMUNICATIONS – PC.027

MOLECULAR FRAGMENTS AS PROBES FOR STRUCTURE SELECTIVITY RELATIONSHIP ANALYSIS

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Target selectivity or specificity has long been considered a hallmark of biological recognition processes. However, the use of computational methodologies to study and predict the selectivity of target-ligand interactions is still in its infancy. We have introduced molecular fragment-based approaches to analyze target selectivity. Hierarchical fragmentation and knowledge-based design strategies were applied to systematically extract well-defined molecular fragments from different compound sets that could serve as molecular selectivity markers. Furthermore, a new type of atom environment descriptors were designed to distinguish compounds having different target selectivity profiles. These descriptors, termed Bonded Atom Pairs (BAPs), represent conceptually new atom pair descriptors with high chemical information content. BAPs combine different types of structural information including element type, hybridization states, aliphatic/aromatic character, and cyclic/acyclic structural motifs. When applied to molecular selectivity analysis, BAPs and conventional atom pair descriptors were found to capture complementary structural information. Moreover, BAPs accurately distinguished between compounds having different selectivity and hence extend the current repertoire of topological molecular descriptors.

REFERENCES

1. Ahmed H. E. A, Vogt M & Bajorath J. Design and evaluation of bonded atom pair descriptors. *J. Chem. Inf. Model.*, in press.
2. Ahmed H. E. A. and Bajorath J. Methods for computer-aided chemical biology. Part 5: Rationalizing the selectivity of cathepsin inhibitors on the basis of molecular fragments and topological feature distributions. *Chem. Biol. Drug Des.*, 74:129-141, 2009.

POSTER COMMUNICATIONS – PC.028

BENZOAZEPINONES AS MODULATORS OF γ -SECRETASE

Christian Fischer, Susan Zultanski, Hua Zhou, Joey Methot, Colby Brown, Elizabeth Kelley, Sanjiv Shah, Bethany Hughes, Christopher Moxham, Nathan Bays, Bo Pan, Zhenhua Wu, Lily Moy, Jonathan Cruz, Deborah Walker, George Nikov, Sujal Deshmukh, Thomas Miller, Benito Munoz, Mark Goulet, Mark Shearman

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which in 2009 affected over 5 million patients in the US and more than 25 million worldwide¹. Current standard of care relies on symptomatic treatment, which only provides short-term stabilization and to date no disease modifying therapy has been clinically validated. The widely accepted amyloid hypothesis describes oligomers of $A\beta$, specifically $A\beta_{42}$, as the neurotoxic species leading to neurodegeneration and ultimately AD². γ -Secretase modulators (GSMs) are a novel therapeutic approach for the treatment of AD. GSMs modulate APP cleavage by γ -secretase (Figure 1) to decrease $A\beta_{42}$, increase $A\beta_{38}$ whilst not affecting the cleavage of other substrates, such as Notch (Figure 2)³.

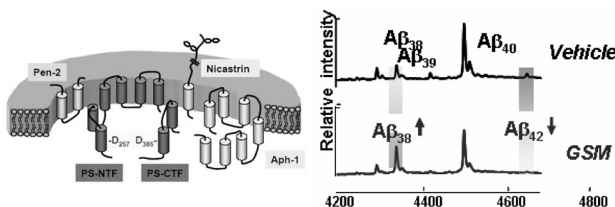
Figure1: γ -Secretase complex

Figure 2: GSM mode of action

This poster will discuss the medicinal chemistry strategy on triazole GSMs, specifically our recent advances in the benzoazepinone series (Figure 3)⁴. Detailed SAR studies and the medicinal chemistry lead optimization strategy will be presented. This effort has led to the identification of several potent and selective GSMs which we present herein detailing their *in vitro* and *in vivo* properties.

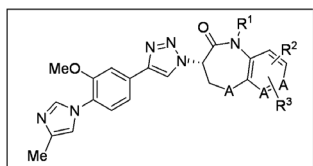


Figure 3: Benzoazepinone GSM

REFERENCES

1. Alzheimer's Association 2009 Alzheimer's disease facts and figures http://www.alz.org/national/documents/report_alzfactsfigures2009.pdf
2. Imbimbo, B. P. *Curr. Top. Med. Chem.* **2008**, 8, 54.
3. Peretto, I.; La Porta, E. *Curr. Top. Med. Chem.* **2008**, 8, 38.
4. Fischer, C.; Munoz, B.; Zultanski, S.; Methot, J.; Zhou, H.; Brown, W. C. *PCT Int. Appl.* (2008), WO 2008156580.

POSTER COMMUNICATIONS – PC.030

ANTIMALARIAL 4(1H)PYRIDONES

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4(1H)Pyridones (Fig 1) are novel potent and selective inhibitors of Plasmodium electron transport chain at the cytochrome bc1 level (Complex III). Although Plasmodium makes ATP predominantly by anaerobic fermentation in the cytoplasm, its mitochondria plays additional roles as the cellular compartment where essential reactions take place, such as, for example, reactions involved in heme and pyrimidine biosynthesis, the latter being the only source of pyrimidines in Plasmodium species. In summary, the presence of an intact mitochondria, energised by a functioning electron transport chain is likely to be essential for the parasite.

Atovaquone (GSK, Malarone®) and antimalarial 4(1H)Pyridones display the same pattern of inhibition of cytochrome bc1, providing strong circumstantial evidence that the site of action of 4(1H)Pyridones is complex III. However, as 4(1H)Pyridones are active against Atovaquone-resistant strains, their binding site in complex III is not identical to that of Atovaquone, but it could still be in cytochrome bc1.

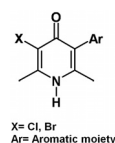


Fig 1

POSTER COMMUNICATIONS – **PC.031****RATIONAL DESIGN, SYNTHESIS AND MECHANISM OF ACTION OF NOVEL ANTIDIABETIC ETHOXYBENZO-THIAZOL DERIVATIVES***Ella Meltzer- Mats¹, Shlomo Sasson² and Arie Gruzman¹*

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Type 2 diabetes has been defined as a worldwide epidemic by the World Health Organization. The WHO estimated that more than 220 million people worldwide have diabetes. Over time this disease can damage the heart, blood vessels, eyes, kidneys, nerves and cause death. Type 2 diabetes is a chronic disease with a major pathophysiological symptom: decreasing glucose utilization in peripheral tissues such as muscle and fat due to insulin resistance. Modern antidiabetic drug therapy aims at a strict regulation of glucose homeostasis. However, monotherapy and combined therapy with oral agents often fail to achieve near-normoglycemia; hence the frequent need for insulin treatment. Therefore, the search for novel antidiabetic drugs is intense. Recently, we design and synthesized new line of compounds (ethoxybenzo-thiazol derivatives) with potential antidiabetic activity. According to our preliminary results these compounds increased the rate of glucose transport and utilization in peripheral tissues, most notably in skeletal muscle, which is the main consumer of glucose. Stimulation of intracellular glucose transport was induced via activation (phosphorylation) of key enzyme in energy metabolism: 5'-AMP-activated protein kinase (AMPK), since in its active form, it induces glucose transporter-4 (GLUT4) translocation to the plasma membrane of insulin sensitive cells and thus increases glucose influx in a non-insulin-dependent manner. This mechanism is distinct from insulin action, therefore it can be targeted by ethoxybenzo-thiazol derivatives that exert their effect even in the face of severe insulin resistance, such as found in obese type 2 diabetic patients.

POSTER COMMUNICATIONS – **PC.032****DEVELOPMENT OF NEW DRUGS FOR TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)***Omer Green and Arie Gruzman*

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive muscle weakness and

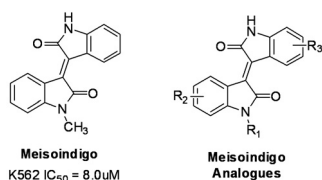
reflective loss of upper and lower motor neurons, predominantly in the spinal cord. Tremendous advances have been made in understanding the pathogenesis of ALS. However, these advances have not led to significant results in the development of an effective drug therapy. Unfortunately, beside riluzole which can prolong the life of ALS patients for an estimated three months, no other compounds have yet to have been developed. Thus, the development of anti-ALS drugs remains urgent. Mutations in the SOD1 are most common mutations that lead to familial ALS. The formation of SOD1-rich inclusions in the spinal cord is an early and prominent feature of SOD1-linked familial ALS in human patients and animal models of this disease. These inclusions have been shown to consist of SOD1-rich fibrils, suggesting that the conversion of soluble SOD1 into amyloid fibrils may play an important role in the etiology of familial ALS. SOD1 is also present in the inclusions found in spinal cords of sporadic ALS patients. SOD1 is degraded by at least two pathways in lysosomes and in the proteasome. Quick and sufficient clearance of mutant SOD1 reduces its motor neuron toxicity. Therefore, it has become clear, that drugs that prevent aggregation of proteins (especially SOD-1) in motor neurons or induce their appropriate clearance may be beneficial for treating ALS patients in general, and in particular patients with SOD-1 linked familial ALS. In search for compounds that will be able to prevent aggregation and accelerate degradation of misfolded SOD1 ALS affected motor neurons, we have found that the chemical chaperone trimethylamine N-oxide (TMAO) stimulates degradation of the disease-linked species of SOD1 by activation of its Cathepsin D cleavage. TMAO per se has unfavorable pharmacokinetic parameters to become a useful drug: the concentrations required to achieve its biological effect are rather high, TMAO reaches all organelles in the cell non-selectively, when we are interested in selective targeting in lysosomes, where Cathepsin D is localized. We, therefore, aimed at synthesizing potent lysosome targeted TMAO derivatives with favorable pharmacokinetic parameters.

POSTER COMMUNICATIONS – **PC.033****LEAD OPTIMIZATION STUDY OF MEISOINDIGO AGAINST CHRONIC MYELOGENOUS LEUKEMIA***Xi-Kai Wee, Clement Jun-Wen Ong, Mei-Lin Go*

Department of Pharmacy, Faculty of Science, National University of Singapore. 18 Science Drive 4, Singapore 117543.

Meisoindigo (1-methyl-isoidindigo) has been used as a Chinese medication for chronic myelogenous leukemia (CML) for many years¹. Meisoindigo was developed from a lead optimization effort based on a structurally similar compound named indirubin.² Indirubin is the active entity of the traditional Chinese herbal prescription "Danggui-Longhui Wan" for the treatment of chronic myelogenous leukemia². We investigated lead optimization efforts on the meisoindigo scaffold to probe structure activity relationships. A series of functionalized isoidindigos was synthesized by a modified acid-catalyzed aldol condensation

between substituted isatins and oxindoles assisted by microwave irradiation³. The compounds were then evaluated for their anti-proliferative activity on chronic myelogenous leukemic lymphoblast K562 cell line³. While meisindigo (IC_{50} = 8.0 μ M) contains a methyl group at position 1, a range of substituents was tolerated at this position, giving rise to compounds with close to 5 fold improvement in anti-proliferative activities. For instance, 1-phenpropyl-isoidindigo gave an antiproliferative IC_{50} value of 1.8 μ M. Meisindigo analogs with substitutions on the aromatic rings of the scaffold were also evaluated. Substitution at position 6' was found to be the most promising with the 6'-methoxy analogue of meisindigo being the most active (IC_{50} = 3.2 μ M). Substitution at the 7'-position generally led to poorer solubility while substitution on other positions did not yield any analogue that is better than meisindigo. Of the 65 compounds investigated, the most promising compound identified to date is more than 15 fold more potent than meisindigo as an anti-proliferative agent on K562 cells. A screen for kinase inhibitory activity of selected compounds showed that they did not target cyclin dependent kinases (CDKs) as previously thought³, although several receptor tyrosine kinases were possible targets.



REFERENCES

- Xiao, Z., Hao, Y., Liu, B. and Qian, L., *Leuk. Lymphoma*. **2002**, 43, 1763-1768.
- Xiao, Z., Hao, Y. *Indirubin, the red shade of indigo*. **2006**, 20, 203-208.
- Wee, X.K., Yeo, W.K., Zhang, B., Tan, V.B.C., Lim, K.M., Tay, T.E., Go, M.L. *Bioorg. Med. Chem.* **2009**, 17, 7562-7571.

POSTER COMMUNICATIONS – PC.034

AMINOPHOSPHOLIPID GLYCATION. STUDY OF THE REACTIVITY OF 2-AMINOETHYLPHENETHYLPHOSPHATE WITH GLYCATING COMPOUNDS

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Non-enzymatic glycation of biomolecules has been implicated in the pathophysiology of aging and diabetes¹. In most studies of glycation, the amino components have been proteins, amino acids or peptides². Several studies have demonstrated that carbohydrates can also modify aminophospholipids by reacting

with their amino groups both *in vivo*³ and *in vitro*⁴. Such reactions have been suggested to promote lipid oxidation as a result of glycation. As aminophospholipids are important components of cell membranes, their glycation could have a significant effect on membrane permeability and fluidity⁵. The complex structure of natural aminophospholipids makes it difficult to study the chemical changes that accompany their glycation. For that reason, in the present work, we have synthesised 2-aminoethylphenethylphosphate, a low molecular weight analogue of natural phosphatidylethanolamines⁶ and compared its glycation with that of a peptide (Ac-Phe-Lys) and pyridoxamine, an inhibitor of formation of advanced glycation and lipoxidation end-products⁷. We have conducted the kinetic study, under physiological pH and temperature conditions, of the reactions of 2-aminoethylphenethylphosphate with various glycation carbonyl compounds (acetol, arabinose, and glucose) by using high performance liquid chromatography-UV/Vis. The formation rate constants for Schiff bases of the aminophospholipid analogue and the carbonyl compounds are of the same order of magnitude as those determined for the Schiff bases of the peptide Ac-Phe-Lys⁸. However, the values obtained for Schiff bases of pyridoxamine are five times greater than that for the aminophospholipid analogue that could explain pyridoxamine ability to compete with amino residues in aminophospholipids glycation.

REFERENCES

- Thorpe S R, and Baynes J W *Drugs Aging*, 1996, 2, 69-77.
- Horvat S, and Jakas A J. *Peptide Sci.* 2004, 10, 119-137.
- Nakagawa K, Oak J H, Higuchi O, Tsuzuki T, Oikawa S, Otani H, Mune M, Cai H and Miyazawa T J. *Lipid Res.* 2005, 46, 2514-2524.
- Oak J; Nakagawa K; and Miyazawa T. *FEBS Lett.* 2000, 481, 26-30.
- Levi V, Villamil A M, Castello P R, Rossi J P, and Gonzalez-Flecha F L *Biochem. J.* 2008, 416, 145-152.
- Breitling-Utzmann C M, Unger A, Friedl D A, and Lederer M O *Arch. Biochem. Biophys.* 2001, 391, 245-254.
- Onorato J M, Jenkins A J, Thorpe S R, and Baynes J W *J Biol Chem* 2000, 275, 21177-21184.
- Adrover M, Vilanova B, Frau J, Muñoz F, and Donoso J *Amino Acids* 2009, 36, 437-448.

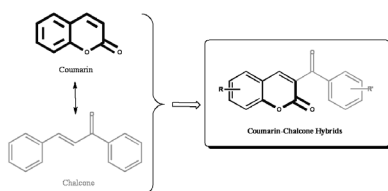
POSTER COMMUNICATIONS – PC.035

RATIONAL DESIGN AND SYNTHESIS OF COUMARIN-CHALCONE DERIVATIVES AS CHEMOTHERAPEUTIC AGENTS

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Chemotherapy, in its most general sense, is the treatment of diseases by chemicals especially by killing micro-organisms or cancerous cells. Nowadays are known a wide range of different chemotherapeutic agents. One of our aims in the last years has been the development of new tools and methodologies for drug discovery. Following this aim we designed hybrid molecules coming from two natural occurring compounds: coumarin and chalcones. Both separated compounds have well known pharmacological activities such as antibacterial antitumor, anti-inflammatory, antithrombotic, cardio protectors or enzymatic inhibitors^{1,2}.



Including the double bond of the chalcone moiety in a coumarinic structure we will be able to obtain compounds whose scaffold will include both pharmacophores, with a semi-rigid chalcone. In the 3-benzoylcoumarin scaffold can be introduced atoms, groups or fragments of very different nature in variable number and position. With the aim of selecting the most appropriated series of compounds in order to make the synthesis and the pharmacological evaluation, we pretend to help us by using rational design based on QSAR methodologies.

Having got good QSAR models for different chemotherapeutic activities, even also multi-target models, we use them for doing a virtual screening and also for improving the pharmacological profile of a lead compound. According to the QSAR models, we selected a series of compounds and we have developed simple, efficient and generalized procedures to synthesize them using a Knoevenagel type reaction as key step³. All results will be further discussed and showed in our communication.

REFERENCES

1. Ni, L.; Meng, C. Q. and Sikorski, J. A., *Expert Opin. Ther. Patents*, **2004**, 14(12), 1669.
2. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L. and Uriarte, E., *Current Med. Chem.*, **2005**, 12, 887
3. Frédéric, R.; Rovert, S.; Charlier, C.; Ruyck, J. de; Wouters, J.; Pirote, B.; Marsereel, B. and Pochet, L., *J. Med. Chem.*, **2005**, 48, 7592

POSTER COMMUNICATIONS – PC.036

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY FROM DIFFERENT SAMPLES OF MEDICINAL PLANT *GALPHIMIA GLAUCA* CAV. AND STRUCTURAL ELUCIDATION USING SPECTROPHOTOMETRY AND HPLC-ESI-MS

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According to epidemiological studies, availability and consumption of antioxidants in plants is closely related to the prevention of various degenerative diseases. Phenolic compounds are attracting considerable interest in the field of chemistry, food and medicine due to their promising antioxidant potential and other useful functions. Thryallis [*Galphimia glauca*, (Cav.) Malpighiaceae] [Syn. *Thryallis glauca* (Poir.) O. Kuntze], commonly known as "calderona amarilla", has been widely used in Mexican traditional medicine and homoeopathic preparations. Pharmacological investigations have reported sedative, (CNS) depressant, anticonvulsant, antiasthmatic, antiallergic properties, hypothermic activity, barbiturate potentiation, protection against strychnine- and leptazol-induced convulsions properties. As different antioxidants can act in vivo through different mechanisms, single method for determination of total antioxidant capacity is insufficient [1].

The present investigations deal with the determination of total antioxidant capacity (TAC), total polyphenolic content (TPC) and further structural identification of the major constituents from samples of *Galphimia glauca*, after fractionating extracts (microwave assisted extraction in 50 % ethanol) using polyamide column, to yield six different fractions following a modified protocol [2]. Three sensitive, speedy, and specific methodologies (DPPH [3], FRAP [1], TEAC [4]) have been adopted for the assessment of TAC and Folin Ciocalteu's method [5] was used for the determination of TPC.

Percent inhibition and IC 50, FRAP [Fe⁺² g/g sample], TEAC [trolox g/g sample], AEAC [Ascorbic acid g/g sample] values, calculated using ascorbic acid, trolox and FeSO₄ as standards, revealed that fraction 3 and 4 in all samples proved to be most potent antioxidants in comparison to others. A directly proportional relationship between TPC and TAC was found to exist. All three methods used to determine TAC values exhibited same tendency, showing that the mechanism of action of these antioxidants was nearly same. Mass spectrometric investigations showed that fractions rich in TPC and consequently most potent antioxidants, consisted of multiple galloyl derivatives of quinic acid and oligomers/polymers of flavanols.

REFERENCES

1. Nicoletta Pellegrinim, Mauro Serafini (2003) J. Nutr. 133: 2812-2819.
2. Ulla Svedström, (2006) J. of Chromatogr. A. 1112: 103-111.
3. Rajbir Singh et al. (2007) Food Chemistry, 103: 505-511.
4. Susan Cheung and Joseph Tai (2005) Oncology Reports, 14: 1331-1336.
5. Singleton and Rossi (1965) Am. J. Enol. Vitic. 16: 144

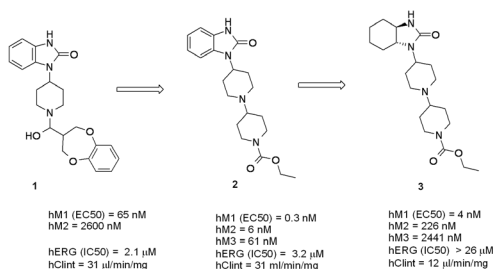
POSTER COMMUNICATIONS – PC.037

DISCOVERY AND PRECLINICAL EVALUATION OF M1 SELECTIVE AGONISTS

Vijayaratnam Santhakumar, Yun-Xing Cheng, Jennifer Laird, Shujuan Jin, Xuehong Luo, Nektaria Markoglou, Mehrnaz Pousaraf, and Mirek Tomaszewski

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M1 agonists have therapeutic potential as cognitive enhancing agents and disease modifying agents for Alzheimer's disease as demonstrated by preclinical and clinical studies. 3-(4-piperidyl)-1H-benzimidazol-2-ones (example: compound 1) were identified as M1 selective agonists by high throughput screening. Replacement of the aromatic benzimidazolone core (compound 2) structure with the aliphatic octahydro benzimidazolone core (compound 3) improved selectivity against M2 and reduced hERG activity. SAR analysis showed that aryl alkyl groups and amide groups at the piperidine ring improve selectivity against M2 but aryl alkyl groups increase hERG activity and amide groups increase efflux. Removal of the hydrogen bond donor at the benzimidazolone ring reduced efflux however resulted in reduced metabolic stability or reduced selectivity against M2. Introduction of quaternary methyl group at the piperidine ring and replacement of ethyl carbamate group with isopropyl carbamate improve M2 selectivity and hERG profiles. Four candidates suitable for pre-clinical evaluation of in vivo efficacy and safety profiles were identified.



POSTER COMMUNICATIONS – PC.038

DE NOVO DESIGN AND SYNTHESIS OF POTENTIAL CXCR4 INHIBITORS

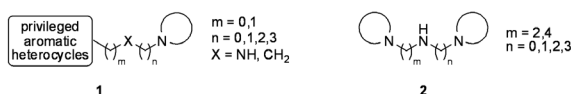
Laia Ros-Blanco¹, Violeta I. Pérez-Nueno¹, Llorenç Nieto¹, Maria Pau Mena², José A. Esté², Bonaventura Clotet², Jordi Teixidó¹, José I. Borrell¹

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HIV cell fusion and entry coreceptors CXCR4 and CCR5 have been validated as targets for therapeutic intervention¹. Nowadays, there is one entry inhibitor (Maraviroc, Pfizer) and one fusion inhibitor (Enfuvirtide, Roche Pharmaceuticals) available for the treatment of HIV infection. However, none of them inhibits CXCR4 coreceptor. Our group previously described a combinatorial library of analogues of the currently most active *lead* AMD3100, whose polinitrogenated building block consisted of a nitrogen-containing heterocycle (piperidine, piperazine, morpholine and pyrrolidine among others), a (CH₂)_n (n = 0, 1, 2, 3) spacer and a terminal amine group. Library selection led to the identification of a *lead* with nanomolar activity against HIV².

Looking for new potential CXCR4 inhibitors, a *de novo* structure based drug design was carried out with LUDI package from Discovery Studio³, using a 3D homology model of the coreceptor⁴ (built from the available template structure of bovine rhodopsin, since no CXCR4 crystal structure exists), to obtain a new virtual library of compounds. LUDI interaction sites were generated on CXCR4 binding pocket according to the key binding residues (Asp171, Asp262, Glu288), pointed by directed mutagenesis⁵. This approach yielded a total of 200 new ligands, based on the 20 highest-ranked scaffolds from standard calculations. The most relevant fragments of the resulting library were "privileged" nitrogen-containing aromatic cycles (like indole and iminodibenzyl), connected by spacers with difficult synthetic feasibility. For this reason, a more synthetically accessible library of compounds was designed, focusing on both polinitrogenated building blocks previously described by our group and *de novo* selected fragments (and their commercial availability). Complex spacers were substituted by hydrocarbonated chains. Virtual screening approaches (docking, pharmacophore modelling, shape matching and similarity searches) were performed to select the best potential active ligands to be synthesized and tested. Final structures **1** and **2** can be obtained by reductive amination or by secondary amine alkylation, amide synthesis and reduction.

Synthetic strategies and anti-HIV activity of selected new potential inhibitors will be presented.



REFERENCES

1. Bleul *et al.*, *Nature* **1996**, 382, 829-833; Deng *et al.*, *Nature* **1996**, 381, 661-666
2. Pettersson *et al.*, *ChemMedChem* **2008**, 3, 1549-57
3. LUDI, Discovery Studio, Version 2.5, Accelrys Inc., **2009**
4. Pérez-Nueno *et al.*, *J.Chem.Inf.Model.* **2008**, 48, 509-533
5. Zhou *et al.*, *J.Biol.Chem.* **2001**, 276, 42826-42833

POSTER COMMUNICATIONS – PC.039

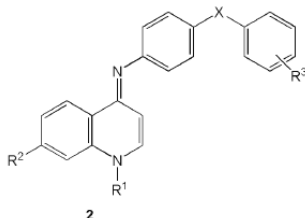
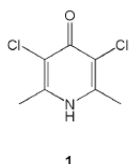
SYNTHESIS AND SAR OF QUINOLONIMINES AS POTENTIAL ANTIMALARIALS

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Malaria is a serious global health problem, with devastating social and economic consequences in countries where the disease is endemic, due to the rapid emergence and spread of parasites that are resistant to well-established antimalarial drugs [1]. Although continued attempts to develop a vaccine for malaria are ongoing, drugs continue to be the only treatment option [2]. As a result, there is an urgent need for novel drugs, preferably acting on underexploited parasite targets in order to delay or overcome the selection of clinical resistance [3,4].

Cytochrome *bc_L*, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [5]. Clopidol, **1**, is a known *bc_L* complex inhibitor and has been taken as a lead for structural optimization [6]. Based in the 4(1*H*)-pyridone scaffold we recently designed and synthesized potential isomers containing the pyridin-4 amines core [7,8]. Now we report the synthesis of related quinolin-4 amines, **2**. These were obtained in good or excellent yields and their activity found to be in the low or sub-micromolar range against *Plasmodium falciparum* W2 strain. A docking study was also carried out to predict the major interactions of the ligand with the binding pocket. SAR will be discussed.



REFERENCES

1. Martinelli, A. *et al.*, *Mini-Rev Med Chem* **2008**, 8, 201-212
2. Nwaka S, *et al.*, *Nat Rev Drug Discov* **2006**, 5, 941-955
3. Arav-Boger, R. *et al.*, *Annu Rev Pharmacol Toxicol* **2005**, 45, 565-585

4. Fidock, D.A. *et al.*, *Trends Parasitol* **2008**, 24, 537-544
5. Rodrigues, T. *et al.*, *Curr Med Chem* **2010**, 17, 929-956
6. Yeates, C. L. *et al.*, *J Med Chem* **2008**, 51, 2845-2852
7. Rodrigues, T. *et al.*, *Int J Quantum Chem* **2010**, accepted
8. Rodrigues, T. *et al.*, *Bioorg Med Chem Lett* **2009**, 19, 3476-3480

POSTER COMMUNICATIONS – PC.040

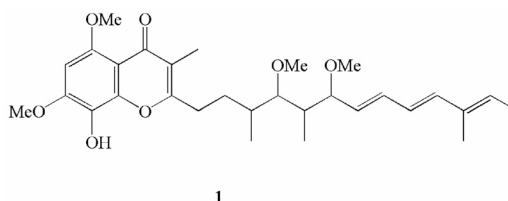
SYNTHESIS AND SAR OF FLAVONES AS POTENTIAL ANTIMALARIAL SCAFFOLD

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Malaria is an infectious disease with serious social and economic repercussions mainly in endemic areas. The rapid emergence and spread of parasites that are resistant to well-established antimalarial drugs poses a great challenge in finding efficacious chemotherapeutic agents [1]. Drugs continue to be the only treatment option, despite ongoing attempts to develop a vaccine for malaria [2]. As a result, there is an urgent need for novel drugs, preferably acting on underexploited parasite targets in order to delay or overcome the selection of clinical resistance [3,4].

Cytochrome *bc_L*, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [5]. Stigmatellin, **1**, is an antibiotic presenting the flavone core and significant antimalarial activity through inhibition of the mitochondrial electron transport chain [5]. Here we report the synthesis of a series of flavones through a one-pot Baker-Venkataraman procedure. These displayed antimalarial activity against the *Plasmodium falciparum* W2 strain in the micromolar range. The SAR and future directions will be discussed.



REFERENCES

1. Martinelli, A. *et al.*, *Mini-Rev Med Chem* **2008**, 8, 201-212
2. Nwaka S, *et al.*, *Nat Rev Drug Discov* **2006**, 5, 941-955
3. Arav-Boger, R. *et al.*, *Annu Rev Pharmacol Toxicol* **2005**, 45, 565-585
4. Fidock, D.A. *et al.*, *Trends Parasitol* **2008**, 24, 537-544
5. Rodrigues, T. *et al.*, *Curr Med Chem* **2010**, 17, 929-956

POSTER COMMUNICATIONS – PC.042

SULFONATES OF N-TRIAZINYLAMMONIUM SALTS AS A NEW COUPLING REAGENTS FOR AMYLIN SYNTHESIS

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Activation of carboxylic acids, for the formation of amide or ester bonds, is a key step in the synthesis of a large number of bioorganic molecules, particularly in the case of peptide synthesis. Recently, systematic exploration of structural diversity offered by incorporation of non-coded amino acids into the peptide chain has been developed into one of the most efficient strategies used in designing new analogues of pharmaceutically active peptides, inspired the search for the new, more versatile and general activation reagents and procedures. This demand is of the prime importance in the case of so-called "difficult couplings" involving strongly hindered amino acids or peptide sequences coiled into non-reactive conformations.

In the search for improved coupling reagents we found that triazine based "superactive esters" superior to the most popular coupling reagents which are based on the chemistry of the benzotriazole or azabenzotriazole ring system [1]. Taking advantage of the modular structure of triazine reagents allowing the fine tuning of their properties, we attempted to develop the new family of them by treatment of 2-chloro-4,6-dimethoxy-1,3,5-triazine with salts of N-methylmorpholine, N-methylpiperidine and N-methylpyrrolidine with toluene-4-sulfonic acid, methanesulfonic acid, trifluoromethanesulfonic acid and camphorosulfonic acid respectively. We found all of them useful for activation of carboxylic components and peptide synthesis in solution and SPPS using all basic N-protecting groups: Z-, Boc-, and Fmoc-. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium toluene-4-sulfonate selected after screening experiments as most versatile coupling reagent was used in SPPS synthesis of Islet Amyloid Polypeptide – amylin and their analogues with α , α -amino acids replacing L-amino acids residues in the native structure.

REFERENCES

1. a) König, W.; and Geiger, R.; Chem. Ber. 1970, 103, 788-798; b) Carpino, L. A. J. Am. Chem. Soc. 1993, 115, 4397-4398; c) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Tetrahedron Lett. 1975, 16(14), 1219-1222; d) Coste, J.; Le-Nguyen, B.; Castro, B. Tetrahedron Lett., 1990, 31, 205-208; e) Carpino, L.A.; Imazumi, H.; El-Faham, A.; Ferrer, F.J.; Zhang, C.; Lee, Y.; Foxman, B.M.; Henklein, P.; Hanay, C.; Mugge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. Angew. Chem. Int. Ed. 2002, 41, 441-445; f) Li, P.; Xu, J. C. J. Chem. Soc., Perkin 2, 2001, 113-120. g) Carpino, L. A.; Henklein, P.; Foxman, B. M.; Abdelmoty, I.; Costisella, B.; Wray, V.; Domke, T.; El-Faham, A.; Muegge, C. J. Org. Chem. 2001, 66, 5245-5247.

POSTER COMMUNICATIONS – PC.043

SYNTHESIS OF N,N'-DISUBSTITUTED-2-(4-(2-AMINOETHYL)-PHENYL)ETHYLAMINES AS A POTENTIAL CXCR4 CORECEPTOR INHIBITORS

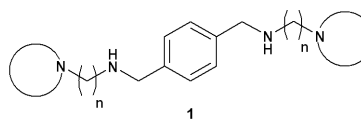
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HIV cell fusion and entry have been validated as targets for therapeutic intervention against infection^[1]. Nowadays, FDA have approved Enfuvirtide (Hoffmann-La Roche & Trimeris) as a fusion inhibitor and Maraviroc (Pfizer) as a CCR5 entry coreceptor antagonist.

Bicyclams were the first low-molecular-weight compounds to show specific interaction with CXCR4, another entry coreceptor. The most potent bicyclam was AMD3100, in which the two cyclam moieties are tethered by a 1,4-phenylenebis(methylene) bridge. It was withdrawn from clinical trials owing to its lack of oral bioavailability and cardiotoxicity.

As a part of our research in this field, we have developed a series of new non-cyclam polynitrogenated CXCR4 coreceptor inhibitors. The structure of these compounds (**1**) is constituted by two commercially available nitrogenated building blocks (consisting of a nitrogen-containing heterocyclic system, a $(CH_2)_n$ ($n = 2, 3$) spacer and a terminal amine) tethered by a 1,4-phenylenebis(methylene) bridge^[2].



These molecules were tested for anti-VIH activity and some of them present high anti-HIV activity. These good results, prompted us to explore variations around this kind of structures. Among these variations, we have designed and synthesized the corresponding next higher homologous systems. Thus, instead of using 1,4-phenylenebis(methylene) as a bridge, we have selected 1,4-phenylenebis(ethylene) to link the two amine moieties.

The subsequent anti-HIV *in vitro* testing led to the identification of some highly active compounds. In some cases the results obtained were similar to those achieved for compounds **1**.

REFERENCES

1. Bleul *et al.* Nature. 1996, 382, 829-833; b) Deng *et al.* Nature. 1996, 381, 661-666.
2. Pettersson *et al.* ChemMedChem. 2008, 3 (10), 1549-1557.

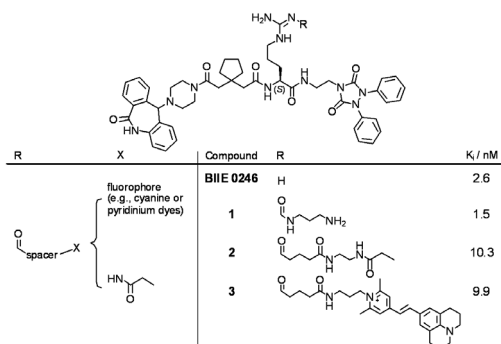
POSTER COMMUNICATIONS – PC.044

NONPEPTIDIC FLUORESCENCE- AND RADIOLIGANDS AS PHARMACOLOGICAL TOOLS FOR THE NEUROPEPTIDE Y₂ RECEPTOR

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As shown previously for argininamide-type neuropeptide Y (NPY) Y₁ (Y₁R) and Y₂ receptor (Y₂R) antagonists, the introduction of various electron-withdrawing groups into the N^G-position is well tolerated and may be used to attach radiolabels or fluorescent dyes^[1-3]. Based on the (S)-argininamide BIIIE 0246^[4], the first highly potent and selective nonpeptidic Y₂R antagonist, we synthesized N^G-substituted acylguanidine bioisosters as fluorescence- and radioligands. The functionalization of BIIIE 0246 with different ω-amino acyl substituents yielded precursors for the preparation of variously labeled Y₂R antagonists. Binding affinities and functional activities were investigated in a flow cytometric binding assay and in a spectrofluorimetric Ca²⁺ assay^[5], respectively. The new substances are highly potent Y₂R antagonists comparable or even superior to the parent compound BIIIE 0246. The high affinity fluorescence ligands proved to be applicable in confocal laser scanning microscopy. Additionally, due to Y₂R selectivity, these fluorescent nonpeptides are suitable to replace the universal NPY receptor ligand Cy5-NPY in flow cytometric binding studies. [³H]-labeled argininamide-type Y₂R selective antagonists can be used instead of radio-labeled NPY derivatives in radioligand binding studies or for the quantification of Y₂R's, and should be applicable to autoradiography.



REFERENCES

1. Keller, M., et al., *J. Med. Chem.* **2008**, 51, 8168-8172.
2. Schneider, E., et al., *ChemBioChem* **2007**, 8, 1981-1988.
3. Brennauer, A., PhD thesis, University of Regensburg, **2006**.
4. Doods, H., et al., *Eur. J. Pharmacol.* **1999**, 384, R 3-5.
5. Ziemek, R., et al., *Eur. J. Pharmacol.* **2006**, 551, 10-18.

POSTER COMMUNICATIONS – PC.046

SYNTHESIS OF NEW MORPHOLINE DERIVATIVES OF PHENCYCLIDINE WITH MORE ANALGESIC EFFECTS

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Phencyclidine (1-(1-phenylcyclohexyl) piperidine, PCP, **I**) has shown analgesic effects. Some of its derivatives have been synthesized and their biological properties have been studied. In this research, (1-[1-(4-methylphenyl) (cyclohexyl)]-4-morpholine, **II**), (1-[1-(2-methoxyphenyl) (cyclohexyl)]-4-morpholine, **III**), (1-[1-(2-methyl-4-hydroxyphenyl) (cyclohexyl)]-4-morpholine, **IV**) as three analogues of PCP with a methyl, methoxy, methyl-hydroxyl groups on the aromatic ring and morpholine instead of piperidine ring, were synthesized and their analgesic effects were examined on rats using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute chemical and chronic pain) tests and compared to PCP and control groups. As indicated in our previous works on this family [1, 2], incorporation of methyl and methoxy groups on the aromatic ring increased electron distribution and dipole moments properties because of the high electron donating character of these groups. Also incorporation of hydroxyl group on the phenyl ring and morpholine instead of piperidine ring increased the hydrophilic, polarity and solubility properties of these analogues.

The results showed that all of the new synthesized compounds were effective in acute thermal and chronic pain but not effective in acute chemical pain compared to the PCP and control groups.

Key words: Phencyclidine- Tail immersion test- Formalin test- Methyl, Methoxy, Methyl-hydroxyl morpholine derivatives of PCP.

REFERENCES

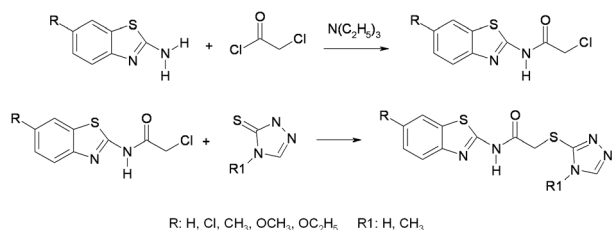
1. A. Ahmadi et al., *Arzneimittelforschung*.2009;59(4):202-206.
2. A. Ahmadi et al., *Arzneimittelforschung*.2009;59(12).

POSTER COMMUNICATIONS – PC.047

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEW BENZOTHIAZOLYL-AMIDES, DERIVATIVES OF 1,2,4-TRIAZOL-3-THIONE

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The enhancement of bacterial resistance of pathogens to currently available antibiotics constitutes a serious public health threat. So, intensive efforts are underway worldwide to develop new antimicrobial agents [1]. For this purpose, ten new N-(benzothiazol-2-yl)-2-(1,2,4-triazol-3-yl-sulfanyl)acetamide derivatives were synthesized by reacting 2-chloro-N-(benzothiazol-2-yl)acetamides with 1,2,4-triazol-3-thione derivatives in acetone [2,3]. The chemical structures of the synthesized compounds were elucidated by IR, ¹H-NMR, FAB⁺-MS spectral data. The compounds were evaluated for antimicrobial activity. Promising results were observed.



REFERENCES

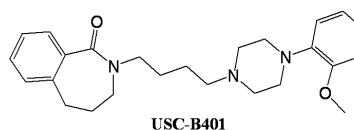
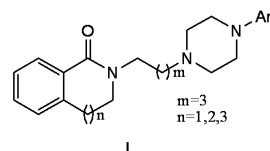
1. C. Franchini, M. Muraglia, F. Corbo, M.A. Florio, A.D Mola, A. Rosato, R. Matucci, M. Nesi, F.V. Bambeke, C. Vitali, Arch. Pharm. Chem. Life Sci. 342, 605-613, 2009.
2. G. Turan-Zitouni, Ş. Demirayak, A. Özdemir, Z.A. Kaplancıklı, M.T. Yıldız, Eur. J. Med. Chem., 39(3), 267-272, 2004.
3. G. Turan-Zitouni, Z.A. Kaplancıklı, Ü. Uçucu, A. Özdemir, P. Chevallet, Y. Tunalı, Phosphorus, Sulfur, and Silicon and the Related Elements, 179(11), 2183-21

POSTER COMMUNICATIONS – PC.049

AROMATIC RING FUNCTIONALIZATION OF BENZOLACTAM DERIVATIVES: NEW POTENT DOPAMINE D₃ RECEPTOR LIGANDSRaquel Ortega¹, Christian F. Masaguer¹, Harald Huebner², Peter Gmeiner².¹ Department of Organic Chemistry. Faculty of Pharmacy. University of Santiago de Compostela. 15782-Santiago de Compostela, Spain.² Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, D-91052 Erlangen, Germany.

Since the discovery of the dopamine D₃ receptor by Sokoloff et al in 1990¹, intensive effort has been directed toward the development of selective ligands for this receptor to help definitively separate D₃ actions from those mediated by the D₂ receptors, in order to elucidate the function and potential therapeutic advantages of targeting D₃ receptors. Subsequent studies evidence that dopamine D₃ receptor is an interesting target in different CNS diseases such as schizophrenia^{2,3}, Parkinson⁴, drug-induced dyskinesia⁵, and drug abuse⁶.

Joining to these efforts, we have synthesized a new series (I) based on a benzolactam scaffold, which maintains three characteristic elements of many dopamine D₃ receptor antagonist: 1) an amine moiety, 2) a spacer, usually a linear alkyl chain, and 3) a hydrophobic residue, often connected through an amide bond.



Owing to the excellent affinity results of some of this compounds such as **USC-B401**⁷ together with the docking simulation studies and SAR analysis, some modifications have been made on the benzolactam moiety with the addition of different substituents. The new synthesized compounds will allow us to evaluate some of the structural requirements in this series for high affinity and selectivity binding at the D₃ receptor.

ACKNOWLEDGMENT

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REFERENCES

1. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. *Nature* **1990**, 347, 146

2. Joyce, J. N.; Millan, M. J. *Drug Discov. Today* **2005**, 10, 917.
3. Richtand, N. M.; Woods, S. C.; Berger, S. P.; Strakowski, S. M. *Neurosci. Biobehav. Rev.* **2001**, 25, 427.
4. Joyce, J. N.; Ryoo, H. L.; Beach, T. B.; Caviness, J. N.; Stacy, M.; Gurevich, E. V.; Reiser, M.; Adler, C. H. *Brain Res.* **2002**, 955, 138.
5. Bezard, E.; Ferry, S.; Mach, U.; Stark, H.; Leriche, L.; Boraud, T.; Gross, C.; Sokoloff, P. *Nat. Med.* **2003**, 9, 762.
6. Heidebreder, C. A.; Gardner, E. L.; Xi, Z.-X.; Thanos, P. K.; Mugnaini, M.; Hagan, J. J.; Ashby, C. R. *Brain Res. Rev.* **2005**, 49, 77.
7. Ortega, R.; Raviña, E.; Masaguer, C.F.; Areias, F.; Brea, J.; Loza, M.I.; López, L.; Selent, J.; Pastor, M.; Sanz, F. *Bioorg. Med. Chem. Lett.* **2009**, 19, 1773.

POSTER COMMUNICATIONS – PC.050

SMALL QUATERNARY AChE INHIBITORS FOR MG TREATMENT – SYNTHESIS, IN VITRO SCREENING AND MOLECULAR DOCKING

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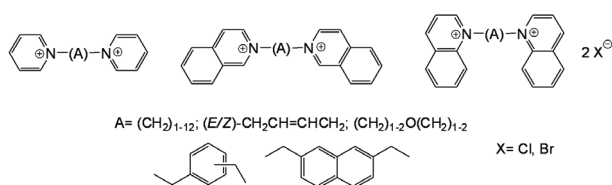
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Keywords: AChE, small inhibitor, Myasthenia gravis, synthesis, in vitro, docking

Small quaternary acetylcholinesterase (AChE) inhibitors are used (e.g. pyridostigmine bromide, neostigmine bromide, ambenonium dichloride) are used for early Myasthenia gravis treatment [1]. Their peripheral effect allows sufficient quantity of acetylcholine available for decreasing amount of AChE receptors. Their side effects including gastrointestinal effects (nausea, intestinal obstruction), increased bronchial secretion, cardiac arrhythmia or cholinergic crisis are well described and limit their dosage and long-term treatment.

Novel small quaternary inhibitors were designed as peripherally acting compounds (pyridinium, quinolinium and isoquinolinium analogues) [2,3]. The *in vitro* ability was determined using IC₅₀ values and kinetic constants. Inhibitory ability towards AChE was found superior to used drugs and non-competitive mechanism of inhibition was confirmed by kinetic parameters and molecular docking.



REFERENCES

1. Garcia-Carrasco, M. et al. *Autoimmun. Rev.* 2007, 6, 373.
2. Musilek, K. et al. *Bioorg. Med. Chem. Lett.* 2010, 20, 1763.
3. Komloova, M. et al. *Curr. Med. Chem.* 2010, in press.

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POSTER COMMUNICATIONS – PC.051

THE MULTIPLE EFFECTS OF CYP17 INHIBITORS ON PROSTATE CANCER CELLS

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Cytochrome P450 17 α -hydroxylase, C_{17,20}-lyase (CYP17) catalyses androgen biosynthesis in the human body^{1, 2} and its inhibition is an important strategy for PC treatment as a way to lower androgen levels and thus stop disease progression. The non steroidal antifungal compound ketoconazole was reported to cause gynecomastia in male patients and became the first drug to be used clinically for PC treatment. In line with ketoconazole, several steroidal and non steroidal compounds have been synthesized and evaluated as CYP17 inhibitors in the hope of finding more potent molecules with a better side effects' profile³.

Pre-clinical evaluation of several compounds designed as CYP17 inhibitors has shown that some of these compounds interact with other molecular targets such as the androgen receptor (AR) which is involved in disease progression, and might therefore bear additional interest as promiscuous agents for PC treatment³⁻⁵. We present our efforts and results in the synthesis of two series of novel androstane derivatives, generally designated as indazole derivatives and C17 2'-methylimidazole derived carbamates. Some of these compounds were found to inhibit CYP17 and also bind to the AR, blocking AR-mediated transcription^{6, 7}. Moreover, they inhibited the proliferation of the most common PC cell line models, namely LNCaP, PC-3 and LAPC4 cells.

ACKNOWLEDGMENTS

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REFERENCES

1. S. Nakajin; J. E. Shively; P. M. Yuan; P. F. Hall, Microsomal Cytochrome P-450 from Neonatal Pig Testis: Two Enzymatic Activities (17 α -Hydroxylase and C_{17,20}-Lyase) Associated with One Protein. *Biochemistry* **1981**, 20, (14), 4037-42.
2. P. F. Hall, Cytochrome P-450 C_{21sc}: One Enzyme with Two Actions: Hydroxylase and Lyase. *J. Steroid Biochem. Mol. Biol.* **1991**, 40, (4-6), 527-32.
3. V. M. Moreira; J. A. R. Salvador; T. S. Vasaitis; V. C. Njar, CYP17 Inhibitors for Prostate Cancer Treatment - An Update. *Curr. Med. Chem.* **2008**, 15, 868-899.
4. V. C. Njar; A. M. Brodie, Inhibitors of 17 α -Hydroxylase/17,20-Lyase (CYP17): Potential Agents for the Treatment of Prostate Cancer. *Curr. Pharm. Des.* **1999**, 5, (3), 163-80.
5. R. W. Hartmann; P. B. Ehmer; S. Haidar; M. Hector; J. Jose; C. D. Klein; S. B. Seidel; T. F. Sergejew; B. G. Wachall; G. A. Wachter; Y. Zhuang, Inhibition of CYP 17, a New Strategy for the Treatment of Prostate Cancer. *Arch. Pharm. Pharm. Med. Chem.* **2002**, 335, (4), 119-28.
6. V. M. Moreira; T. S. Vasaitis; V. C. Njar; J. A. R. Salvador, Synthesis and evaluation of novel 17-indazole androstene derivatives designed as CYP17 inhibitors. *Steroids* **2007**, 72, 939-948.
7. V. M. Moreira; T. S. Vasaitis; Z. Guo; V. C. Njar; J. A. R. Salvador, Synthesis of Novel C17 Steroidal Carbamates. Studies on CYP17 Action, Androgen Receptor Binding and Function, and Prostate Cancer Cell Growth. *Steroids* **2008**, 73, 1217-1227.

POSTER COMMUNICATIONS – PC.052

LACTATE DEHYDROGENASE A (LDH-A) INHIBITORS AS STARVATION AGENTS AGAINST INVASIVE HYPOXIC TUMOURS

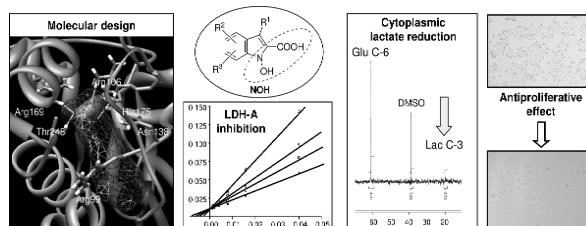
C. Granchi¹, S. Roy¹, T. Tuccinardi¹, A. Martinelli¹, M. Macchia¹, M. Lanza², L. Betti², G. Giannaccini², A. Lucacchini², L. G. León³, E. Giovannetti³, G. J. Peters³, R. Palchoudhuri⁴, P. J. Hergenrother⁴, F. Minutolo¹

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Hypoxic tumours, normally resistant to chemotherapy and radiotherapy, are often characterized by a metabolic switch ("Warburg effect") from normal oxidative phosphorylation to anaerobic glycolysis. This ensures a sufficient energy supply from glucose, even in hypoxic environments. One of the key enzymes of anaerobic glycolysis, the muscle isoform of lactate dehydrogenase (LDH-A), is over-expressed by metastatic cancer cells and is linked to the vitality of tumours in hypoxia. This enzyme constitutes a new target for anticancer agents, since its inhibition leads to a cut in energy supply to cancer, thus reducing its metastatic and invasive potential¹. LDH-A is regarded as a safe target since hereditary LDH-A deficiency causes a certain

level of myopathy only after intense anaerobic exercise, whereas it does not provoke any symptoms under ordinary circumstances². Nowadays, only a few LDH inhibitors have been developed, mainly because there was no evidence so far for health benefits deriving from this action³. Some catechol-type derivatives, originally designed as antimalarial agents, have very recently proved that LDH-A inhibition by small molecules represses tumour progression⁴.

We have discovered new carboxy-substituted N-hydroxyheterocycles (**NOH**), which are efficient and selective LDH-A inhibitors, with minimal inhibition of the heart isoform (LDH-B). Their activity in cells was confirmed by ¹³C NMR experiments measuring the reduction of the cytoplasmic lactate production in tumour cells, and they also proved to be efficient antiproliferative agents *in vitro*.



REFERENCES

1. V. R. Fantin, J. St-Pierre, P. Leder. *Cancer Cell.* **2006**, 9, 425.
2. T. Kanno, K. Sudo, M. Maekawa, Y. Nishimura, M. Ukita, K. Fukutake. *Clin. Chim. Acta* **1988**, 173, 89.
3. C. Granchi, S. Bertini, M. Macchia, F. Minutolo. *Curr. Med. Chem.* **2010**, 17, 672.
4. A. Le, C.R. Cooper, A.M. Gouw, et al. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, 107, 2037.

POSTER COMMUNICATIONS – PC.053

IMIDAZOLIUM-BASED FRAMEWORKS. II. A GENERAL COUNTERANION EXCHANGE PROCEDURE FOR ILS

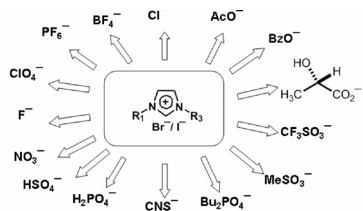
Immaculada Dinarès, Anna Ibáñez, Neus Mesquida and Ermitas Alcalde

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The incorporation of imidazolium quaternary salts in a wide array of cationic and oligocationic systems situate them at the cross-roads of multidisciplinary fields in chemistry leading toward ionic liquids (ILs), anion recognition chemistry and N-heterocyclic carbenes¹. Simple imidazolium quaternary salts with a low melting point have become a green alternative to conventional organic solvents¹ with applications in the chemical industry³. Additionally, some ILs are also emerging as active pharmaceuti-

cal ingredients (APIs)⁴. Systematic anion variation could be a useful tool in the sustainable design of 'task specific ionic liquids'.

As part of our ongoing research into imidazolium-based frameworks, we recently reported an efficient and practical procedure using anion-exchange resins to obtain ionic liquids based on imidazolium salts with the selected anion counterpart quantitatively⁵.



We herein describe our current efforts directed to broadening this protocol to obtain an increased number of anions and cations, using non-aqueous media for the loading and exchange procedures.

ACKNOWLEDGMENTS

This research was supported by the D.G.I. (M.E.C.) Project CTQ2006-1182/BQU. Thanks are also due to the AGAUR, Grup de Recerca Consolidat 2009SGR562 (G.C.)

REFERENCES

1. E. Alcalde, I. Dinarès, N. Mesquida. Imidazolium based receptors. In Topics in Heterocyclic Chemistry: Anion Complexation by Heterocycle Based Receptors. Springer-Verlag, Heidelberg, 2010. Due: November 2010.
2. T. Welton. Ionic liquids in synthesis. P. Wasserscheid and T. Welton, Eds. Wiley-VCH, Weinheim, 2008.
3. N. V. Plechkova, K. R. Seddon. Chem. Soc. Rev. 2008, 37, 123–150.
4. J. Stoimenovski, D. R. MacFarlane, K. Bica, R. D. Rogers. Pharmaceutical Research. 2010, 27, 521–526.
5. I. Dinarès, C. García de Miguel, A. Ibañez, N. Mesquida, E. Alcalde. Green Chem. 2009, 11, 1507–1510.

POSTER COMMUNICATIONS – PC.054

PDE7 INHIBITORS AS NEW POTENTIAL DRUGS FOR THE THERAPY OF SPINAL CORD INJURY

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Primary traumatic mechanical injury to the spinal cord (SCI) causes the death of a number of neurons that to date can neither be recovered nor regenerated. However, neurons continue to die for hours after SCI, and this represents a potentially avoidable event. Local inflammatory response in the injured spinal cord contributes significantly to the evolution of this secondary damage.

During the last years our group has been involved in the design, synthesis and evaluation of PDE7 inhibitors as new innovative drugs for several neurological disorders [1, 2].

Our working hypothesis is based on two different facts. Firstly, neuroinflammation is modulated by cAMP levels, thus the key role for phosphodiesterases (PDEs), which hydrolyze cAMP, is undoubtedly demonstrated. On the other hand, PDE7 is expressed simultaneously on leukocytes and on brain, highlighting the potential crucial role of PDE7 as drug target for neuroinflammation.

Here we present two chemically diverse families of PDE7 inhibitors, designed using computational techniques such as virtual screening and neuronal networks. We report their biological profile, and their efficacy in an experimental SCI model induced by the application of vascular clips (force of 24 g) to the dura via a four-level T5–T8 laminectomy.

We have selected two candidates, namely S14 and VP1.15, as PDE7 inhibitors with IC₅₀ values in the micromolar range. These compounds increase cAMP production both in macrophage and neuronal cell lines. Moreover, the inflammatory response induced by LPS treatment in both types of cells was reduced after being treated with these PDE7 inhibitors. The inflammatory response was completely reversed by treatment of the cells with the membrane-permeable inhibitor of cAMP Rp-cAMP, suggesting that the cAMP signaling pathway mediates the effects of both S14 and VP1.15 compounds.

Regarding drug like properties, compounds were able to cross the blood brain barrier using parallel artificial membranes (PAMPA) methodology. SCI in mice resulted in severe trauma characterized by edema, neutrophil infiltration, and production of a range of inflammatory mediators, tissue damage, and apoptosis. Treatment of the mice with S14 and VP1.15, two PDE7 inhibitors, significantly reduced the degree of spinal cord inflammation, tissue injury (histological score), and TNF- α , IL-6, COX-2 and iNOS expression. All these data together, led us to propose PDE7 inhibitors, and specifically S14 and VP1.15, as potential drug candidates to be further studied for the treatment of SCI.

REFERENCES

1. Castro A, Jerez MJ, Gil C, Martínez A. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: advances in the development of specific phosphodiesterase inhibitors. Med Res Rev. 2005 25:229-44.
2. Gil C, Campillo NE, Perez DI, Martínez A. PDE7 inhibitors as new drugs for neurological and inflammatory disorders. Exp. Opin. Ther. Patents 2008, 18:1127-1139

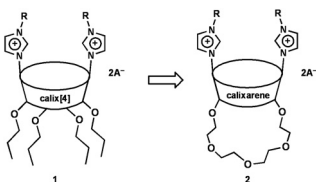
POSTER COMMUNICATIONS – PC.055

IMIDAZOLIUM-BASED FRAMEWORKS. I.
DICATIONIC SYSTEMS FOR ANION
RECOGNITION*Neus Mesquida, Anna Ibáñez and Immaculada Dinarès*

Laboratori de Química Orgànica, Departament de Farmacologia i Química Terapèutica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, 08028-Barcelona, Spain.

The development of new receptors, sensors and transporters for anionic species continues to be a rich area of research due to the number of fundamental roles played by anions in biological and chemical processes. In the past few years, imidazolium functionality has gained a place among the classical anion binding functional groups and has emerged as an attractive starting point for the design of abiotic anion receptors and selective molecular sensors^{1,2}. In parallel, calixarenes are particularly attractive scaffolds for receptor development, the macrocyclic core being available in a variety of sizes, easily pre-organized into a number of topographies and readily selectively functionalized for the apposite introduction of ligands³. These qualities have given a biological perspective to the rapidly growing field of Bionanotechnology, whose aim is to develop new tools for biology, new biomaterials, selective sensors and supramolecular devices for clinical analysis, new therapeutics and smart drug delivery systems.

We recently published the anion recognition studies of **1**-type bis(imidazolium)-calixarenes⁴. Continuing our research into imidazolium-based frameworks, we herein report the binding properties of **2**-type calixarene crowns with bis(imidazolium) salts directly bonded to the upper rim and a polyether chain tethered across the lower rim.



ACKNOWLEDGMENTS

This research was supported by the D.G.I. (M.E.C.) Project CTQ2006-1182/BQU. Thanks are also due to the AGAUR, Grup de Recerca Consolidat 2009SGR562 (G.C.)

REFERENCES

1. E. Alcalde, I. Dinarès, N. Mesquida. Imidazolium based receptors. In Topics in Heterocyclic Chemistry: Anion Complexation by Heterocycle Based Receptors. Springer-Verlag, Heidelberg, 2010. Due: November 2010.
2. C. Caltagirone, P. A. Gale. Chem. Soc. Rev. 2009, 520–563.
3. L. Baldini, A. Casnati, F. Sansone, R. Ungaro. Chem. Soc. Rev. 2007, 36, 254–266.

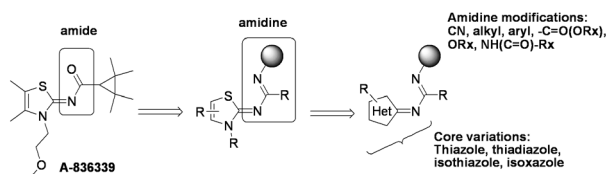
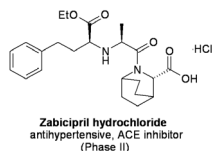
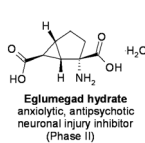
4. I. Dinarès, C. Garcia de Miguel C, N. Mesquida, E. Alcalde. J. Org. Chem. 2009, 482–485.

POSTER COMMUNICATIONS – PC.056

CONFORMATIONAL RESTRICTION IN DRUG
DESIGN: THE AMINO ACID CASE*Igor V. Komarov¹, Oleksandr O. Grygorenko^{1,2}, Dmytro S. Radchenko², Oleksiy S. Artamonov², Nataliya A. Kopylova¹, Pavel K. Mykhailiuk^{1,2}, Andrey A. Tolmachev²*

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Contemporary medicinal chemistry tends to narrow the search area of the chemical space dealing preferably with compounds which have high probability of displaying desirable pharmacokinetic and pharmacodynamic properties. The Lipinski's "Rules of Five" impose such limitations on the chemical space, defining the "drug-like" compounds. Still, the drug-like chemical space, as defined today, is very large. A recent paper on enumeration of small drug-like organic molecules containing up to 13 atoms of C, N, O, S, and Cl reported more than 970 million compounds [1]. There is an obvious need to use further limitations and concepts in drug design, narrowing the search area for new drugs. One of such concepts is decrease of conformational mobility or rigidification of drug candidate and building block molecules [2]. In principle, conformationally restricted molecules, due to their pre-organization, can be better ligands for biological targets, thus displaying more selective and pronounced biological activity, comparing to flexible analogues. We performed analysis of the literature and MDL Drug Data Report Database in order to investigate the use of a particular class of the building blocks – conformationally restricted amino acids (CRAAs) in drug design. The analysis showed that cyclic and quaternary α -amino acids are intensively used for construction of new drug candidates; however, bi- and polycyclic amino acids and their derivatives are under-represented among the actively studied compounds. Only a few compounds of this type reached clinical stage of development, for example, eglumegad and zabicipril. At the same time, bi- and polycyclic CRAAs have very large structural variability which allows fine-tuning the structure and properties of the drug candidate to the need of the design. We suggest that one of the reasons which hinder the use the bicyclic CRAAs in drug design is the lack of efficient and practical syntheses of them, and concentrated our efforts on improving the known and developing new approaches to this valuable class of building blocks. A number of methods were developed; they are based on intramolecular cyclization of different types, tandem Strecker reaction – intramolecular nucleophilic substitution, ylide addition to C=C double bonds, photochemical [2+2] cycloaddition, [3+2] dipolar cycloaddition for the construction of conformationally restricted scaffolds.



REFERENCES

1. Blum, L.C.; Reymond, J.-L. *J. Am. Chem. Soc.* **2009**, 131, 8732.
2. Mann A. Conformational restriction and/or steric hindrance in medicinal chemistry. In: *Practice of Medicinal Chemistry (2nd Edition)* Editor: Wermuth, C. G. Elsevier, London, **2003**, 233-250.

POSTER COMMUNICATIONS – PC.057

DESIGN, SYNTHESIS, AND INITIAL PHARMACOLOGY OF SEVERAL NEW AMIDINE CLASSES OF SELECTIVE CB₂ AGONISTS

Teodozyj Kolasa, Meena Patel, Jennifer Frost, Steven Latshaw, Xueqing Wang, Arturo Perez-Medrano, Sridhar Peddi, Tongmei Li, Bo Liu, William Carroll, Michael Dart, Anthony Daza, George Grayson, Yihong Fan, Loan Miller, Lanlan Li, Bradley Hooker, Tiffany Garrison, Odile El-Kouhen, Jill Wetter, Gin Hsieh, Betty Yao and Michael Meyer

Neuroscience Research, GPRD, Abbott Laboratories, Abbott Park, IL 60064

Both CB₁ and CB₂ cannabinoid receptor agonists have demonstrated antinociceptive properties. The clinical utility of mixed cannabinoid agonists for the treatment of pain is limited by central effects (psychoactivity, hypoactivity and hypothermia) and tolerance due to activation of CB₁ receptors. A distinct benefit of highly selective CB₂ agonists is their ability to display robust effects across a range of preclinical models of inflammatory, post-operative and neuropathic pain, without the undesirable CNS-mediated side effects associated with central CB₁ receptor activation. Clinical validation for the CB₂ target has not been achieved, and research continues to be directed toward the identification of CB₂ agonists for effective management of pathological pain in humans.

Previously we reported the pharmacological activity of the thiazolylidene amide A-836339, a selective CB₂ agonist. Replacement of the amide group in the thiazole series with a cyanoamidine led to compounds that retained high levels of CB₂ affinity and improved levels of selectivity vs. the CB₁ receptor. To expand SAR investigations around this new structural class of CB₂ agonists, additional modifications of the amidine moiety and variations of the core were desired.

Here we describe the two synthetic approaches to these various amidine compound classes. The first, which allowed access to the thiazole- and thiadiazole-based amidines, involved transformation of the amide to the corresponding thioamide followed by amination in the presence of a mercury salt. An alternate method was required for preparation of isoxazolidene- and isothiazolylidene-cyanoamidines. The second approach was designed to prepare cyanoamidines of all core variants, and involved the reaction of the heterocycle-imine with dimethyl cyanocarbonimidodithiate. The resulting carbamimidodithiate intermediate was coupled with arylboronic acids in the presence of palladium (0) and copper (I) to afford the desired cyanoamidine analogs. A variety of amidine derivatives were synthesized and characterized in assays to evaluate cannabinoid receptor binding affinity, selectivity, and agonist activity. Several new, highly selective amidine CB₂ ligands have been identified that possess favorable PK properties and robust efficacy in animal pain models.

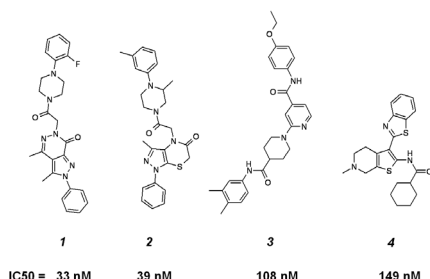
POSTER COMMUNICATIONS – PC.058

DISCOVERY AND OPTIMIZATION OF POTENT AND SELECTIVE SMO/HEDGEHOG PATHWAY INHIBITOR

Alexander Khvat, Sergey Tkachenko, Frederick Lakner, Elena Dubrovskaya, Ilya Okun, Alexander Ivachtchenko

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Hedgehog signalling is activated in a diverse spectrum of cancers, and its pharmacological blockade has emerged as an anti-tumour strategy. Several Hedgehog inhibitors were developed and advanced into clinical trials. So far the main mechanism for all compounds in clinical trials is an inhibition of the pathway activity on the level of activating receptor, Smoothened (Smo). We report here various new Smo/Hh pathway antagonists (e.g., hits 1-5) that were discovered by implementation of Targeted Diversity™ strategy for discovery of small molecules, inhibitors of signalling pathways. Targeted Diversity™ has been created as a universal tool for the design of high quality libraries of drug-like compounds that have been focused against multiple biological targets. These targets may be congener or 'orthogonal' (non-overlapping) and include (a) different classes of targets, (b) distinct, structurally unrelated branches of the same target class and (c) independent targets.



Development of SAR for different classes of discovered hits 1-5 led to the discovery of *CD-005-0002*, a novel potent inhibitor of the hedgehog pathway. The compound was optimized for potency, selectivity, PK and drug-like properties by modifications of the core and amide portion of the molecule. *CD-05-0002* is a potent hedgehog pathway inhibitor (IC₅₀ = 28 nM) that not only competes in Smo binding with bodipy-cyclopamine but also has activity on yet unknown targets below SMO level. Compound does not show any activity against a panel of 96 kinases as well as WNT and NFκB pathways. *CD-05-0002* reduces mGluR expression in different cell-based models and produces tumor regression in MiaPaca-2 and Panc-1 mouse models.

The mechanism of action and ADME characteristics of *CD-05-0002* will be discussed.

POSTER COMMUNICATIONS – PC.059

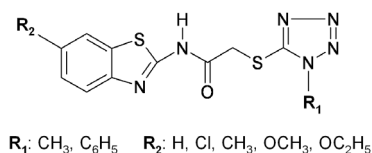
SYNTHESIS AND ANTINOCICEPTIVE ACTIVITY OF SOME ACETAMIDE DERIVATIVES

Zafer Asim Kaplancikli¹, Gulhan Turan-Zitouni¹, Ahmet Ozdemir¹, Mehlika Dilek Altintop¹, Ozgur Devrim Can²

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The experimental researches for the development of safer and more effective analgesic agents are a great deal of interest for many researchers. In the chemical literature, derivatives of acetamide have been described with the antinociceptive activity [1,2].

In the present study, some acetamide derivatives were synthesized to investigate their potential antinociceptive activities. The N-(benzothiazol-2-yl)-2-(1-substituted-¹H-tetrazol-5-ylthio)acetamide derivatives were synthesized by reacting 2-(chloroacetyl)benzothiazole derivatives with tetrazol-5-thioles. The chemical structures of the compounds were elucidated by ¹H NMR and FAB+-MS spectral data and Elemental analyses. The prepared compounds were investigated for their antinociceptive activity using by hot plate and acetic acid-induced writhing tests.



REFERENCES

1. T. Önkol, D. S. Dogruer, S. Ito, M. F. Sahin, "Synthesis and Antinociceptive Activity of (5-Chloro-2-benzothiazolinon-3-yl)acetamide Derivatives", *Archiv der Pharmazie*, 333(10), 337 – 340, (2000).
2. An-Chih Chang, Alan Cowan, Akira E. Takemori, Philip S. Portoghesi, Aspartic Acid Conjugates of 2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(3-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide: Opioid Receptor Agonists with Limited Access to the Central Nervous System, *J. Med. Chem.*, 39, 4478-4482, (1996).

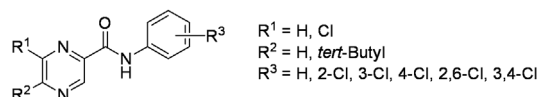
POSTER COMMUNICATIONS – PC.060

STRUCTURE-PROPERTY RELATIONSHIPS IN SERIES OF CHLORINE-SUBSTITUTED PYRAZINECARBOXANILIDES AS BIOLOGICALLY ACTIVE COMPOUNDS

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Compounds possessing -NHCO- moiety simulating a peptide bond in their molecule show a broad range of biological effects. Various ring-substituted anilides of pyrazine-2-carboxylic acid were prepared and evaluated as potential antimicrobial and herbicidal agents or abiotic elicitors [1-7]. Twenty substituted N-phenylpyrazine-2-carboxamides were analysed using the RP-HPLC method for the lipophilicity measurement. Lipophilicity is one of the most important physical properties of biologically active compounds. It influences the transport of a molecule through cellular membranes, because drugs cross biological barriers most frequently through passive transport, which strongly depends on their lipophilicity. Lipophilicity also characterizes drug solubility.



In the present study the correlation between the RP-HPLC retention parameter log *k* and log *P* data calculated in various ways is shown, and the relationships between the lipophilicity and the

chemical structure of the studied compounds are discussed. The distributive parameters π of various substituents are listed for the mentioned studied compounds. The determined π parameters of substituents can be used for describing relationships between physico-chemical properties and biological activity of prepared ring-substituted pyrazine-based compounds. Although all the discussed compounds are relatively simple structures substituted within the series only by chlorine, interesting intramolecular interactions influencing lipophilicity were observed probably due to the presence of a pyrazine ring and a carboxamide moiety.

The study was supported by MSM0021620822.

REFERENCES

1. Dolezal, M.; Vicik, R.; Miletin, M.; Kralova, K. *Chem. Pap.* **2000**, *54*, 245.
2. Dolezal, M.; Miletin, M.; Kunes, J.; Kralova, K. *Molecules* **2002**, *7*, 363.
3. Dolezal, M.; Palek, L.; Vinsova, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Molecules* **2006**, *11*, 242.
4. Dolezal, M.; Tumova, L.; Kesetovicova, D.; Tuma, J.; Kralova, K. *Molecules* **2007**, *12*, 2589.
5. Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Eur. J. Med. Chem.* **2008**, *43*, 1105.
6. Dolezal, M.; Zitko, J.; Kesetovicova, D.; Kunes, J.; Svobodova, M. *Molecules* **2009**, *14*, 4180.
7. Tumova, L.; Tuma, J.; Megusar, K.; Dolezal, M. *Molecules* **2010**, *15*, 331.

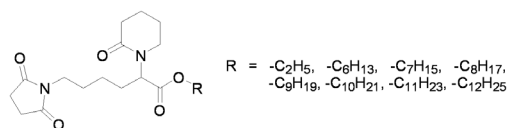
POSTER COMMUNICATIONS – PC.061

PREPARATION AND PHYSICO-CHEMICAL PROPERTIES OF ALKYL-6-(2,5-DIOXOPYRROLIDIN-1-YL)-2-(2-OXOPIPERIDIN-1-YL) HEXANOATES

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Transdermal penetration enhancers are special pharmaceutical excipients that interact with skin components to increase penetration of drugs from the topical dosage forms to blood circulation. Numerous compounds have been evaluated as penetration enhancers and many potential sites and modes of action have been identified for them [1]. This is a follow-up paper to our previous articles [2-5] and deals with synthesis, physico-chemical properties and 3D structures of below mentioned compounds derived from 6-aminohexanoic acid prepared as potential transdermal penetration enhancers.



The aim of modification of the molecule ester part with linear alkyl chains was an increase of lipophilicity and a consequent increase of penetration/carrier activities. Lipophilicity is one of the most important physical properties both for biologically active compounds and for excipients; it influences the transport of a molecule through cellular membranes. The lipophilicity (nonpolarity) of enhancers was determined and the corresponding relationship between enhancer nonpolarity and penetration enhancement potency is under further investigation.

This study was supported by the Ministry of Education of the Czech Republic MSM 6215712403.

REFERENCES

1. Williams, A.C.; Barry, B.W. *Adv. Drug Deliv. Rev.* **2004**, *56*, 603.
2. Brychtova, K.; Farsa, O.; Csollei, J. *ECSOC-12* **2008**, c005.
3. Brychtova, K.; Farsa, O.; Csollei, J. *Lett. Org. Chem.* **2009**, *6*, 25.
4. Brychtova, K.; Dittrichova, S.; Slaba, B.; Placek, L.; Opatrilova, R.; Jampilek, J.; Csollei, J. *ECSOC-13* **2009**, c010.
5. Brychtova, K.; Jampilek, J.; Opatrilova, R.; Raich, I.; Farsa, O.; Csollei, J. *Bioorg. Med. Chem.* **2010**, *18*, 73.

POSTER COMMUNICATIONS – PC.062

PLASMODIUM FALCIPARUM AMINOACYL-tRNA SYNTHETASES AS TARGETS IN THE SEARCH FOR NEW ANTIMALARIAL DRUGS

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Malaria is a wide spread infectious disease caused by the parasite Plasmodium. Every year 350 to 500 million of people are infected of which 1 to 3 million, mainly young children, die¹. Although there are a number of antimalarial drugs on the market^{2,3}, the rapid developing/developed resistance of the parasite to these drugs makes that the search for new medicines against Malaria an urgent necessity.

Aminoacyl-tRNA synthetases (aaRS) are important enzymes in the protein synthesis machinery of living organisms⁴. They catalyze the esterification of a specific amino acid or its precursor to one of all its compatible cognate tRNAs to form an aminoacyl-

tRNA in a two step process as shown in figure 1. The first step is the binding of ATP to the corresponding amino acid (AA) to form an aminoacyl-adenylate and the release of inorganic pyrophosphate (PP_i). In the second step the amino acid is transferred from the AA-AMP complex to the tRNA.



Figure 1: Two step mechanism of aaRS mediated esterification of AA with tRNA

Mephitis is a consortium of 8 research groups from 5 different countries⁵. The main goal of the consortium is to significantly advance the understanding of protein synthesis in *Plasmodium* and to contribute to the effort on drug discovery against malaria, taking the aminoacyl-tRNA synthetases of *Plasmodium falciparum* as potential drug targets. To do so, three different areas of research are covered, e.g. evolution and structural biology, molecular biology and biochemistry and drug design and discovery.

In this paper we will discuss the latest developments on the design and synthesis of new inhibitors of *Plasmodium falciparum* tRNA synthetases and related proteins.

REFERENCES

1. <http://en.wikipedia.org/wiki/Malaria>
2. M. Schlitzer, *Arch. Pharm. Chem. Life Sci.* **2008**, 341, 149-163.
3. "The Elusive Malaria Vaccine: Miracle or Mirage?" by Irwin W. Sherman, ASM Press, Washington DC, **2009**
4. M. Ibba; D. Söll *Annu. Rev. Biochem.* **2000**, 69, 617-650
5. <http://www.mephitis.eu>

POSTER COMMUNICATIONS – PC.063

DESIGNED MULTIPLE LIGANDS AS PHARMALOGICAL TOOLS TO DETECT A_{2A}-D₂ RECEPTOR HETEROMERS

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Due to a better understanding of the pathogenesis of diseases in the recent decades, drug discovery has gradually shifted from a

phenotype-based endeavor to today's single-molecular-target centered approach¹. As a result, it has become and still is aimed mainly at the discovery of small molecules able to aim a single protein target, which is thought to be responsible for a certain disease². This one-molecule, one-target approach has resulted in the discovery of a large number of successful drugs. Nevertheless, a highly selective ligand for a specific target does not always result in a highly efficient drug. This might be due to, for example, a complex mechanism of the disease in which multiple pharmacological targets play a role. One approach to overcome this problem is the use of Designed Multiple Ligands (DMLs)³⁻⁵. DMLs drugs are single molecules in which two drugs have been combined. Consequently, this molecule can interact specifically on multiple targets of relevance to a disease (targeted polypharmacology).

Recently we have published a series of DMLs which could be used as pharmacological tools in the detection of heteromized Adenosine A_{2a} (A_{2A}R) and Dopamine D₂ (D₂R) receptors⁶. Additionally, these DMLs can be potential new drug candidates against Parkinson's Disease (PD). It has been demonstrated that in the stratum the interactions between D₂ Dopamine Receptor (D₂R) and A_{2a} Adenosine Receptor (A_{2A}R) are antagonistic and have shown implications in neurological illnesses such as Parkinson's Disease (PD) and its potential treatment⁷⁻⁹.

In this poster we present the recent results obtained in the design and synthesis of new DMLs and their subsequent pharmacological characterization.

REFERENCES

1. P. T. Lansbury; *Nat. Rev. Neurosci.* **2004**, 5 (Suppl.), S51-S53.
2. S. Frantz; *Nature* **2005**, 437, 942-943.
3. R. Morphy et al.; *J. Med. Chem.* **2005**, 48(21), 6523-6543.
4. R. Morphy et al.; *Drug Disc. Today* **2004**, 9(15), 641-651.
5. A. Cavalli et al.; *J. Med. Chem.* **2008**, 51(3), 347-372.
6. A. Soriano et al.; *J. Med. Chem.* **2009**, 52(18), 5590-5602.
7. K. Fuxe et al.; *Neurology* **2003**, 61, S19-S23.
8. P. Svenningsson et al.; *Neurology* **2003**, 61, S5-S9.
9. M. Deogaonkar et al.; *Brain Res. Rev.* **2005**, 50, 156-168.

POSTER COMMUNICATIONS – PC.064

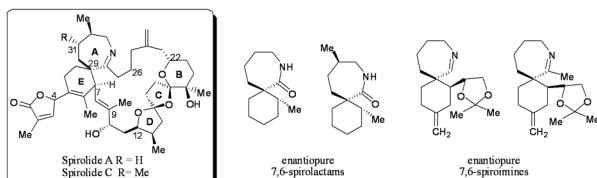
SYNTHETIC STUDIES TOWARDS THE SPIROIMINE UNIT OF THE SPIROLIDES

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The spirolide family is a class of marine toxins isolated from the digestive glands of contaminated mussels (*Mytilus edulis*), scallops (*Placopecten magellanicus*) and phytoplankton from the south-eastern coast of Nova Scotia in Canada¹. The spirolides

are metabolites of the dinoflagellate *Alexandrium ostenfeldii* and *A. peruvianum*. To date fourteen members of the spirolide family have been identified from contaminated shellfish extracts and cultures of toxic strains of *A. ostenfeldii* around the world. The spirolides are considered to be fast-acting neurotoxins due to their pronounced effects in the mouse bioassay. They are also antagonists of the nicotinic and muscarinic acetylcholine receptors as well as weak activators of L-type calcium channels, rendering these compounds possible candidates for the treatment of cardiovascular disorders². The spirolides contain a bis-spiroacetal unit, previously synthesised by the Ishihara³ and Brimble groups⁴, and an unusual 7,6-spiroimine moiety that has not yet been synthesised. The spiroimine moiety of the spirolides is thought to be the pharmacophore⁵. The first part of our studies aims to establish the quaternary centre C29 in the spirolides using an enantioselective Birch reductive alkylation. This strategy provided access to a wide range of enantiopure bicyclic spiro-lactams⁶. Unfortunately, the lactam functionality could not be converted to an imine. Subsequent, an improved retrosynthetic pathway, making use of a Diels-Alder reaction⁷, allows the construction at the same time of both C29 and C7 stereocentre in the spirolides. Further elaboration of the Diels-Alder adduct affords access to the key ketoamine or aldamine intermediate, in good overall yield. Final cyclisation of the advanced intermediate provides the first synthesis of an enantiopure 7,6-bicyclic spiroimine containing both C7 and C29 stereocentres of the spirolides. Further stability studies of the aldimine and ketimine are in progress.



REFERENCES

- Hu, T.; Curtis, J. M.; Oshima, Y.; Quilliam, M. A.; Walter, J. A.; Watson-Wright, W. M.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1995** 2159-2161.
- Guéret, S. M.; Brimble, M. A. *Mar. Drugs* **2010**, ASAP.
- Ishihara, J.; Ishizaka, T.; Suzuki, T.; Hatakeyama, S. *Tetrahedron Lett.* **2004**, 45, 7855-7858.
- Meilert, K.; Brimble, M. A. *Org. Biomol. Chem.* **2006**, 4, 2184-2192.
- Hu, T. M.; Curtis, J. M.; Walter, J. A.; Wright, J. L. C. *Tetrahedron Lett.* **1996**, 37, 7671-7674.
- Guéret, S. M.; O'Connor, P. D.; Brimble, M. A. *Org. Lett.* **2009**, 11, 963-966.
- Asano, M.; Inoue, M.; Watanabe, K.; Abe, H.; Katoh, T. *J. Org. Chem.* **2006**, 71, 6942-6951.

POSTER COMMUNICATIONS – PC.065

CANNABINOIDS AS MULTITARGET DRUGS FOR ALZHEIMER DISEASE

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Neurodegenerative diseases are an increasing public concern, being currently the eighth cause of disease burden for developed regions. According to the World Health Organization (WHO), neurodegenerative diseases will become the world's second leading cause of death by the middle of the century, overtaking cancer. This fact will have a dramatic impact in medical care, as well as important social and economic implications, unless more effective preventive procedures or treatments are available. Alzheimer's disease (AD), characterized by cortical amyloid plaques and neurofibrillary tangles is the most common cause of dementia, accounting for probably 50-75% of all dementias worldwide, followed by vascular dementia, mixed dementia, and Lewy body dementia¹.

The targeting of multiple receptors through a single drug molecule is gaining increasing acceptance. This multifunctional drug paradigm strongly challenges the widely held assumption that single-target agents are better to "dirty drugs" in therapy. Although the single-target approach currently remains the major drug discovery strategy in pharmaceutical companies, there is increasing recognition of the limitations of such an approach for complex diseases².

During the last years there are more evidences about the implications of the cannabinoid system on neurodegenerative processes such as excessive glutamate production, oxidative stress and neuroinflammation. Moreover, several evidences indicate the relation that exists between the cannabinoid and the cholinergic systems.

Regarding to this, the aim of our study has been to development a new family of multitarget drugs with cannabinoid and cholinergic properties for the treatment of neurodegenerative diseases as Alzheimer disease or Lewy body dementia. The key steps for this task have been the design of the new family of drugs using docking tools, the synthesis and the biological study of cannabinoid and cholinergic properties. Thus, we have obtained a new family of 3-indazolyl ether derivatives as innovative strategy for treatment of Alzheimer disease³.

REFERENCES

- Campillo N.E.; Páez J.A. Cannabinoid System in Neurodegeneration. New Perspectives in Alzheimer's disease. *Mini Rev. Med. Chem.* **2009**, 9 (5), 539-59

2. Van der Schyf, C. J.; Youdim, M. B. Multifunctional drugs as neurotherapeutics. *Neurotherapeutics* **2009**, 6, 1-3.
3. Paéz J.A, Campillo, N.E, González P., Pérez C., Arán V.J., Martín M. I., Girón R., Sánchez E. M. Una nueva familia de éteres de 3-indazolilo con propiedades cannabinoides y/o colinérgicas. ESP. P200930775. **2009**. CSIC- Universidad Rey Juan Carlos.

POSTER COMMUNICATIONS – PC.066

A DIBROMOPYRIDO[2,3-D]PYRIMIDINE AS A GENERAL PLATFORM FOR THE DEVELOPMENT OF PROTEIN KINASE INHIBITORS

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Since the beginning of nineties, the research in the field of human signalling pathways has continuously provided hundreds of new therapeutic targets. Among those, protein kinases have proved to be highly relevant in cancer, immunological, nervous, endocrine and cardiac diseases, diabetes, Alzheimer¹, asthma², arteriosclerosis, leukemia³, arthritis⁴.

As a result of this growing knowledge about human transduction machinery, different inhibitory strategies have been proposed and studied: only few of them have succeeded. Some small molecules have been described as protein kinase inhibitors and potential therapeutic agents, some of the more promising candidates belonging to pyrido[2,3-*d*]pyrimidine family.

Traditionally, the heterocyclic moiety of this kind of compounds is synthesized in several steps, the required substituents being progressively introduced^{5,6}. Thus, starting from a pyrimidine ring bearing some of the final substitution pattern, the formation of the pyridine ring is achieved by condensation with reagents carrying another part of final substitution pattern. That is to say, diversity should be progressively introduced thus limiting the number of accessible products. In addition, some chemical incompatibilities could also limit the accessible chemical diversity.

As part of our research in the field of potential tyrosine kinase inhibitors, we have disclosed a series of straightforward methodologies for the synthesis of pyrido[2,3-*d*]pyrimidines which are based on a Michael addition between α,β -unsaturated esters and active methylene compounds, followed by cyclization with guanidines and amidines^{7,8}.

Herein we describe a new three-step protocol for the synthesis of a dibromo substituted 2-arylaminopyrido[2,3-*d*] pyrimidine scaffold in which the formation of pyridopyrimidine skeleton is achieved in one step from an α,β -unsaturated ester, malononitrile and an arylsubstituted guanidine. The subsequent regioselective bromination afforded, in two steps and in high yield the

aforementioned dibromosubstituted pyrido[2,3-*d*]pyrimidine. The two bromine atoms are easily and selectively replaced by carbon, nitrogen and oxygen substituents by standard Suzuki and Ullmann protocols, thus achieving new and already described biologically active pyrido[2,3-*d*]pyrimidines substituted in positions C6, C4 and in the phenylamino ring present in C2.

REFERENCES

1. Ho, G. J. *et al.*, *Current Drug Targets: Inflammation & Allergy*, **2005**, 4(2), 247
2. Wong, W. F., *Current Opinion in Pharmacology*, **2005**, 5(3), 264
3. Noriko, U., *Cancer & chemotherapy*, **2005**, 32(3), 297
4. Collett, M. S. *et al.*, *Proc. Natl Acad. Sci. USA*, **1978**, 75, 2021
5. Klutchko, S. R. *et al.*, *Journal of Medicinal Chemistry*, **1998**, 41(17), 3276
6. Schroeder, M. C. *et al.*, *Journal of Medicinal Chemistry*, **2001**, 44(12), 1915
7. Mont, N. *et al.*, *Molecular Diversity*, **2003**, 7, 153
8. Berzosa, X. *et al.*, *Journal of Organic Chemistry*, **2010**, 75, 487

POSTER COMMUNICATIONS – PC.067

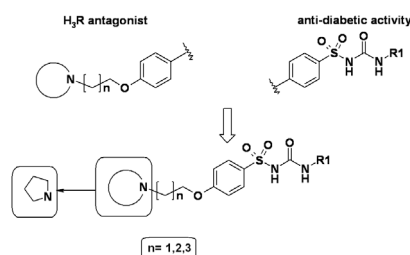
DUAL HISTAMINE H₃ ANTAGONISTS/ANTI-DIABETIC AGENTS: DESIGN, SYNTHESIS, BIOLOGICAL EVALUATION AND SAR

Silvia Galiano, Saioa Ancizu, Nerea Castrillo, Javier Ceras, Silvia Pérez-Silanes, Ignacio Aldana, Antonio Monge

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More than 400 million people suffer from obesity worldwide, making it one of today's global health issue. Hand in hand with the obesity rates is the rapid widespread of type 2 diabetes emerging in a new epidemic: the diabetes¹.

The combination of antagonism at histamine H₃ receptors² and the stimulation of insulin secretion through the sulfonylurea system³ has been proposed as an approach of new dual therapeutic agents for the treatment of obesity and type 2 diabetes mellitus.



Based on this strategy and as part of our ongoing programme to develop new anti-obesity drugs⁴, we have designed and synthesized a new series of non-imidazole derivatives, based on a basic pyrrolidine ring connected through an alkyl spacer of variable length to a phenoxysulfonylurea moiety. These compounds were initially evaluated for histamine H₃ receptor binding affinities suggesting that a propoxy chain linker between the amine and the core ring could be essential to the optimal binding affinity. The results confirmed these derivatives as a promising starting point for the design of new H₃-antagonists with anti-diabetic activity.

REFERENCES

1. Diabetes Prevention Program Research Group. *N. Engl. J. Med.* **2002**, 346, 393–403.
2. Malmlof, K.; Hastrup, S.; Wulff, B. S.; Hansen, B. C.; Peschke, B.; Jeppesen, C. B.; Hohlweg, R.; Rimvall, K. *Biochem. Pharmacol.* **2007**, 73, 1237.
3. Nissen, S.E.; Nicholls, S.J.; Wolsky, K.; Nesto, R.; Kupfer, S.; Pérez, A.; Jure, H.; Delarochellière, R.; Staniloae, C.S.; Mabromatis, K.; Saw, J.; Hu, B.; Lincoff, A.M.; Tuzcu, E.M. *JAMA* **2008**, 299(13), 1561-1573
4. Galiano, S.; Ceras, J.; Cirauqui, N.; Pérez, S.; Juanenea, L.; Rivera, G.; Aldana, I.; Monge, A. *Bioorg. Med.Chem.* **2007**, 15, 3896-3911

POSTER COMMUNICATIONS – **PC.068**

P-PIPERIDINYLLALKYL-PHENOXYSULFONYLUREAS: NEW DERIVATIVES AS DUAL H₃ RECEPTOR ANTAGONISTS AND ANTI-DIABETIC ACTIVITY

Nerea Castrillo, Saioa Ancizu, Silvia Galiano, Javier Ceras, Silvia Pérez-Silanes, Ignacio Aldana and Antonio Monge

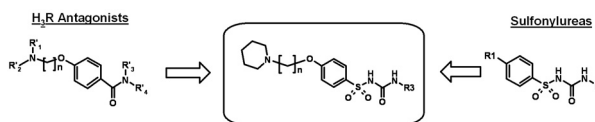
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A new threat has appeared in worldwide health: the diabetes epidemic. The World Health Organization estimated in 2005 that 1.1 million people died as a result of diabetes¹. Due to obesity is the major risk factor in type 2 diabetes, it's necessary to develop new treatments to the prevention in this epidemic.

It is known that histamine H₃ receptor antagonists are able to promote weight loss or prevent weight gain. Although most H₃ receptor antagonists had an imidazol moiety in their structure, recent studies have shown that N-containing non-aromatic heterocycles act as a potent anti-obesity agents². A-331440 is a novel non-imidazol-based antagonist of the histamine H₃ receptor which apparently reduces weight³.

The American Diabetes Association has suggested that sulfonylurea system is clinically significant due to its anti-diabetic properties. Glimepiride is the first third-generation sulfonylurea approved as oral anti-diabetic drug⁴.

Based on current findings, our research group proposed new compounds with dual activity as anti-obesity and anti-diabetic agents. Our aim was the design and synthesis of 22 new p-piperidinyllalkylphenoxysulfonylurea derivatives in order to evaluate the biological activity against H₃R and their affinity with hERG receptor.



ACKNOWLEDGMENTS

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REFERENCES

1. Nath, D., Heemels, M.T., Anson, L. *Nature* **(2006)**, 444 (7121), 839.
2. Meier, G.et al. *Eur. J. Pharm. Sci.* **(2001)**, 13, 249-259.
3. Hancock, A.A. *Curr. Opin. Investig.* **(2003)**, 4, 1190-1197.
4. Nissen, S.E.; Nicholls, S.J.; Wolsky, K.; Nesto, R.; Kupfer, S.; Pérez, A.; Jure, H.; Delarochellière, R.; Staniloae, C.S.; Mabromatis, K.; Saw, J.; Hu, B.; Lincoff, A.M.; Tuzcu, E.M. *JAMA* **(2008)**, 299(13), 1561-1573.

POSTER COMMUNICATIONS – **PC.069**

DOCKING OF SUBSTRATES AND INHIBITORS INTO THE SUBSTRATE BINDING SITE OF TWO SEROTONIN TRANSPORTER MODELS

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The serotonin transporter (SERT) belongs to the neurotransmitter:sodium symporter (NSS) family. Located in the membranes of presynaptic neurons, the transporter removes serotonin (5-HT) from the synaptic cleft, thus reducing the number of neurotransmitters available for interaction with postsynaptic receptors. The transporter is targeted by two of the main classes of antidepressant drugs - the selective serotonin reuptake inhibitors (SSRIs) and the tricyclic antidepressants (TCAs) - and is also the target for psychostimulants such as cocaine, MDMA (ecstasy), and d-amphetamine. The three-dimensional structure of SERT has not yet been determined. However, the first three-

dimensional structure of a NSS family member, the *Aquifex* aeolicus leucine transporter, LeuT, is available both in an occluded conformation (1) as well as in an outward-facing conformation (2). Based on a comprehensive alignment of NSS family members (3), two different homology models of SERT representing two different conformational states were generated using the ICM program package and the two LeuT crystal structures as templates and the models were used for docking of a set of known substrates and several inhibitors into the putative substrate binding site. Our results indicated that the bacterial leucine transporter crystal structures are reliable templates for generating SERT homology models for subsequent docking of substrates and inhibitors. The docking indicated that D98 is a key amino acid for binding both substrate and inhibitors. Transmembrane helices 1, 3, 6 and 8 were involved in interactions with substrates and inhibitors in both SERT models.

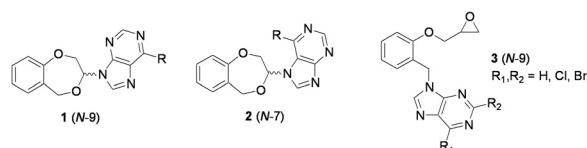
ACKNOWLEDGMENTS

This study was partly supported by a grant PNRF-103-AI-1/07 through the Norwegian Financial Mechanism and the Norwegian Research Foundation.

REFERENCES

1. Yamashita A., Singh S.K., Kawate T. et al. *Nature* 2005; 437:215-223.
2. Singh SK, Piscitelli CL, Yamashita A, Guaux E. *Science* 2008; 322(5908):1655-61.
3. Beuming T, Shi L, Javitch JA, Weinstein H. *Mol Pharmacol* 2006; 70:1630-42.

compounds with the advantage of selective obtaining the *N*-9' (1) or *N*-7' (2) regioisomers simplifying their isolation².



Figure

Trying to increase the anticancer activity of compounds **1** their structure was modified as followed: a) The salicyl alcohol fragment (present in the 2,3-dihydro-5H-1,4-benzodioxepin fragment) and the purine of **1** were maintained; b) The interaction of xenobiotics with DNA and the resulting adducts have been studied extensively, in order to understand how they exhibit their cytotoxic and carcinogenic properties³. It is within this philosophy that our group has focused on simple epoxides on which until now only little research has been performed. As a lead moiety the phenyl glycidyl ether was chosen (Figure).

We describe herein the synthesis and biological evaluation of a series of substituted 9-(2-oxiranylmethoxybenzyl)-9H-purines **3**.

REFERENCES

1. M.C. Núñez, M.G. Pavani, M. Díaz-Gavilán, et al. *Tetrahedron*, **2006**, 62, 11724-11733.
2. A. Conejo-García, M.C. Núñez, J.A. Marchal, et al. *Eur. J. Med. Chem.*, **2008**, 43, 1742-1748.
3. H. Bartsch. *Mutat. Res.*, 1996, 340, 67-79.

POSTER COMMUNICATIONS – PC.070

DI- AND TRI-SUBSTITUTED PURINES WITH THE PHENYL GLYCIDYL ETHER MOIETY: SYNTHESIS AND ANTICANCER ACTIVITIES

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Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge in the 21st century. Therefore, the development of new drugs against cancer belongs among the priorities of the development of science and fundamental research. Alkylating agents have been used for the treatment of cancer for over six decades and yet their repertoire continues to grow.

Studies on the synthesis and pharmacological evaluation of (RS)-6-substituted-7 or 9-(2,3-dihydro-5H-1,4-benzodioxepin-3-yl)-7H- or -9H-purines were published.¹ The microwave-assisted organic synthesis has provided faster access to the target

POSTER COMMUNICATIONS – PC.071

ENANTIOMERS OF 9-[1-(*p*-NITROBENZENESULFONYL)-1,2,3,5-TETRAHYDRO-4,1-BENZOXAZEPIN-3-YL]-2,6-DICHLORO-9H-PURINE, AS ANTICANCER AGENTS

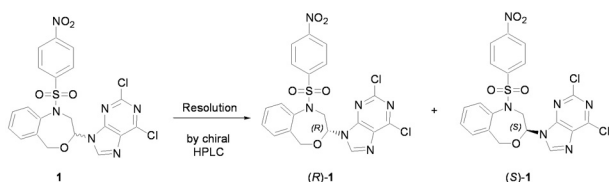
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Knowledge of the molecular signalling pathways that stimulate tumours has led to the identification of both Epidermal Growth Factor Receptor (EGFR) and Vascular Endothelial Growth Factor (VEGF) as key components implicated in the regulation of the tumour proliferation and angiogenesis, respectively. The dual inhibition of both molecular signalling pathways such as EGFR and VEGF may solve the multidrug resistance (MDR) problem and promote synergism¹. There are about ten scientific publications that deal with the dual EGFR and VEGF inhibition, and all of them concentrate in the period 2004-2010, which supports

the innovative character of the therapeutic approach. The drugs used are structures derived from 4-anilinoquinazolines, such as gefitinib and erlotinib, and monoclonal antibodies such as trastuzumab, used alone or in combination with other drugs. Nevertheless, only two homochiral drugs have been employed: a) (-)-gossypol, which is a polyphenol derived from the cotton plant (gender *Gossypium*, family *Malvaceae*)²; and b) AEE788, a synthetic product prepared in Novartis³. AEE788 presents the basic structure of 7H-pyrrolo[2,3-d]pyrimidine, that can be considered a bioisostere of the quinazoline moiety.

In this communication both enantiomers of a new tetracyclic prototype, such as 9-[1-(p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepin-3-yl]-2,6-dichloro-9H-purine, (R)-**1** and (S)-**1** are shown. Such homochiral drugs display a dual EGFR and VEGF inhibition, with production of an elevated level of apoptosis in tumour cells and revealing neither acute nor chronic toxicity.



Figure

REFERENCES

1. G.N. Naumov, M.B. Nilsson, T. Cascone, *et al.*, *Clin. Cancer Res.*, **2009**, *15*, 3484-3494.
2. R.M. Mohammad, S. Wang, S. Banerjee, *et al.*, *Pancreas*, **2005**, *31*, 317-324.
3. T. Sasaki, Y. Kitadai, T. Nakamura, *et al.*, *Neoplasia*, **2007**, *9*, 1066-1077.

POSTER COMMUNICATIONS – PC.072

INFLUENCE OF THE LINKER IN THE BIOLOGICAL ACTIVITY OF MONOCATIONIC INHIBITORS OF CHOK

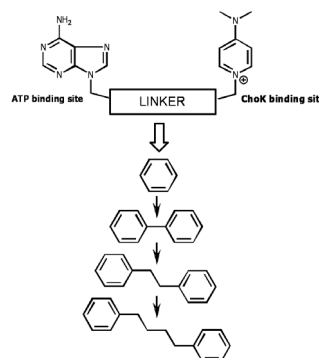
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Choline kinase (ChoK), the enzyme responsible for the generation of phosphorylcholine (PCho), is involved in cell proliferation. Studies using nuclear magnetic resonance (NMR) techniques have demonstrated elevated levels of PCho in human tumoral tissues with respect to normal ones, including breast, colon, lung, and prostate tumors [1]. Inhibition of ChoK has been

demonstrated to be a novel efficient antitumor strategy in onco-gene transformed cells [2].

Docking studies on the homology model of human ChoK based on the crystal structure of ChoK from *C. elegans* indicate that bis-cationic inhibitors can bind to the binding sites of ATP and choline [3]. Therefore we have design monocationic structures with a positive charge that could be stabilized into the choline binding site and a noncharged aromatic group that mimics the ATP adenine moiety connected by an appropriated linker. Here we report that, according to their inhibitory activities against human ChoK, the enzymatic inhibitory potency is closely related to the size of the linker being the 1,4-diphenylbutane the most suitable.



REFERENCES

1. Smith, T. A. D.; Bush, C.; Jameson, C. *et al.* *NMR Biomed.* **1993**, *6*, 318-323.
2. Hernández-Alcoceba, R.; Saniger, L.; Campos, J. *et al.* *Oncogene*, **1997**, *15*, 2289-2301.
3. Milanese, L.; Espinosa, A.; Campos, J. *et al.* *ChemMedChem*. **2006**, *1*, 1216-1228.

POSTER COMMUNICATIONS – PC.073

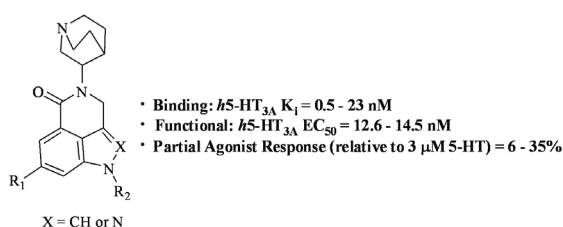
NOVEL 5-HT₃ RECEPTOR PARTIAL AGONISTS FOR THE POTENTIAL TREATMENT OF IRRITABLE BOWEL SYNDROME

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Partial activation of the 5-HT₃ receptor is an emerging strategy for the potential treatment of irritable bowel syndrome (IBS), a debilitating functional disorder of the bowel, which diminishes

quality of life for an estimated 7-12% of adults. The 5-HT₃ receptor is a pentameric, ligand-gated cation channel found in both the enteric and central nervous systems and has been a successful drug target for the treatment of chemotherapy-induced nausea and vomiting (CINV) and more recently for IBS. Initial drug development campaigns focused on the development of 5-HT₃ receptor antagonists as potential treatments for diarrhea predominant IBS (IBS-D). Although clinically successful, the first agent in this class to reach market (alosetron) has limited use due to severe constipation and rare instances of ischemic colitis (IC). Nevertheless, the 5-HT₃ receptor remains an attractive IBS drug target as shown by the subsequent approval in Japan of the 5-HT₃ receptor antagonist ramosetron (a repurposed CINV agent). 5-HT₃ receptor antagonists like ramosetron are believed effective in patients with IBS-D due to effects on visceral sensitivity, colonic transit and secretion, which lead to improvements in abdominal discomfort and stool consistency in patients. Targeting compounds that partially activate the 5-HT₃ receptor would minimize the potential for severe constipation or IC and may result in therapeutics that are applicable to a broader array of IBS patient populations (e.g. IBS-D, IBS-C (Constipation predominant) and IBS-M (Mixed; fluctuating between bouts of diarrhea and constipation)). Attenuation of the excessive serotonergic activity of the 5-HT₃ receptor without completely abolishing channel function is hypothesized to control or normalize gastroenteric function and to provide a novel, more clinically acceptable treatment of IBS. Two new chemical series bearing indazole and indole cores have been identified with nanomolar binding affinity for the h5-HT_{3A} receptor. A range of partial agonist activities in HEK cells expressing the h5-HT_{3A} receptor are reported. Excellent 5-HT₃ receptor selectivity, favorable eADME and good oral *in vivo* potency in the murine von Bezold-Jarisch reflex model is exemplified thereby indicating the series to have potential utility as improved IBS agents.



POSTER COMMUNICATIONS – PC.074

STUDY OF A NEW APPROACH FOR POTENT AND SELECTIVE MAO INHIBITORS

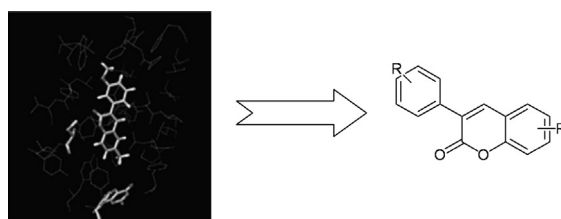
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Coumarins are a large family of compounds widely used in numerous applications¹. This is an important reason to study and develop different modifications on the coumarin skeleton. Phenylcoumarins are synthetic compounds in which an additional phenyl ring is present in any position of the pyrone or the benzenic ring of the coumarin's nucleus². For the last years, we have been studying deeply the 3-phenylcoumarin's scaffold. The inclusion of the resveratrol skeleton makes these molecules an interesting group to study. Recent studies pay special attention to their antioxidant, anticarcinogenic and enzymatic inhibition properties. In regard to the monoamine oxidase (MAO) inhibition, the recent findings revealed that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in the coumarin and 3-phenyl rings^{2,3}.

To a better understanding of the structure/activity relationships of MAO inhibitory activity and selectivity, and to improve them, in the present communication we report the design, synthesis and biological study of a new series of 3-phenylcoumarin derivatives.



The preliminary results of pharmacological study using recombinant human MAO-A and MAO-B isoenzymes indicated that some of the synthesized compounds are powerful and selective MAO-B inhibitors with IC₅₀ values in the low nanomolar-picomolar range. The present modifications, which we are studying more deeply, lead to improve coumarin's pharmacologic profile in the Parkinson's disease.

REFERENCES

- Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.*, **2005**, *12*, 887.
- Matos, M.J., Viña, D., Quezada, E., Picciau, C., Delogu, G., Orallo, F., Santana, L., Uriarte, E. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 3268.
- Matos, M.J., Viña, D., Picciau, C., Orallo, F., Santana, L., Uriarte, E. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 5053.

POSTER COMMUNICATIONS – PC.075

HOMOCHIRAL PURINE DERIVATIVES:
ANTICANCER ACTIVITIES

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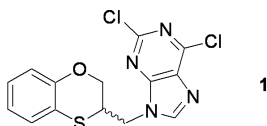
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The intrinsically chiral and non-racemic nature of the living world often results in its different interactions with the enantiomers of a given substance. If this substance is a drug, it might well be that only one of the two isomers is capable of exerting the desired therapeutic effect. The other may be inert (but must still be metabolized by the organism), harmful (causing even dramatic biological damages), or responsible for side effects that are difficult to predict.

Well aware of this fact and stimulated by the new policy statements issued by the regulatory agencies, the pharmaceutical industry has, over the past decade or so, systematically begun to develop chiral drugs in enantiometrically pure forms^{1,2}.

A series of eleven (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purines was obtained by applying the Mitsunobu protocol from (*RS*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol (**1**).

The racemic mixture (**1**) was resolved in its enantiomers: (*S*)-**1** (Retention time = 14.7 min) and (*R*)-**1** (Retention time = 17.5 min), using a CHIRALPAK® IA semipreparative column and a hexane/*i*PrOH mixture as eluent. The absolute configurations of the stereocentres were determined by X-ray diffraction. (*R*)-**1** and (*S*)-**1** show different antiproliferative activities against the human breast cancer cell line MDA-MB-231.



Several drugs are now marketed or being developed as single enantiomers in place of a previous racemic mixture, a process known as "chiral switching". It is easier to understand "pure" as opposed to "mixture" pharmacology but whether the promise of chiral (and metabolite) switches will translate into real clinical advances remains to be seen.

REFERENCES

1. M.C. Núñez; M.E. García-Rubiño; A. Conejo-García; O. Cruz-López; M. Kimatrai; M.A. Gallo; A. Espinosa; J.M. Campos. *Curr. Med. Chem.*, **2009**, 16, 2064-2074.

2. M.C. Núñez; M. Díaz-Gavilán; A. Conejo-García; O. Cruz-López; M.A. Gallo; A. Espinosa; J.M. Campos. *Curr. Med. Chem.*, **2008**, 15, 2614-2631.

POSTER COMMUNICATIONS – PC.076

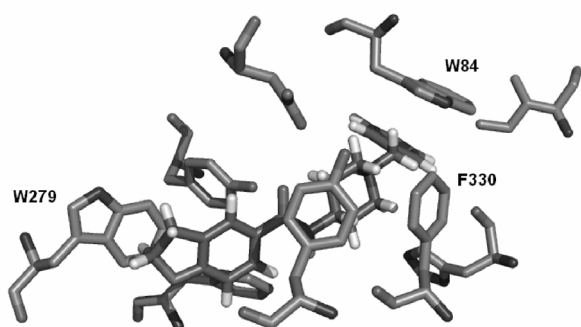
STRUCTURE-BASED SEARCH FOR NEW
INHIBITORS OF CHOLINESTERASES

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Alzheimer's disease (AD) is an irreversible, progressive, neurodegenerative disorder with memory and thinking disturbances. Due to the cholinergic hypothesis explaining the cognitive deficits, inhibitors of acetylcholinesterase (AChE) and also butyrylcholinesterase (BuChE) were introduced for the symptomatic treatment of mild and moderate AD [1]. Donepezil, rivastigmine and galantamine can increase cholinergic neurotransmission and lead to learning and memory improvement. They can also affect different mechanisms such as β -amyloid aggregation, regional cerebral blood flow, tau phosphorylation and oxidative stress which lead to the development of the disease [2,3]. Therefore, the search for new cholinesterase inhibitors is undertaken by many laboratories [4].

The aim of our study was to search for potential new inhibitors of cholinesterases with structure-based computational techniques. Based on properties of known inhibitors and requirements for blood-brain barrier penetration, the ZINC database was filtered for compounds fulfilling the following conditions: $1 \leq \log P \leq 3$, $1 \leq \text{charge} \leq 3$, rotatable bonds ≤ 8 , $1 \leq \text{H-bond donors} \leq 5$, $1 \leq \text{H-bond acceptors} \leq 10$, $100 \leq \text{MW} \leq 500$, $\text{PSA} \leq 90$. Almost 65 000 compounds passed these filters. Pharmacophore models were created for reference ligands such as donepezil, decamethonium and tacrine and used for virtual screening of the selected compounds. This resulted in a limited amount of well-fitting molecules. These compounds and some derivatives of compounds synthesized previously in our lab were used in a second screening which was based on a docking and scoring procedure optimized on the basis of known cholinesterase complexes. The top-ranked results were visually inspected to check for possible interactions, to assess the quality of the fitting and to suggest further chemical modifications. This enabled us to select potential hits which will be synthesized and tested for activity against AChE and BuChE *in vitro* with the spectrometric method of Ellman [5] and with electrophoretically mediated microanalysis [6].



One of the selected potential hits docked into AChE: Illustration of the suggested binding mode at the active site.

REFERENCES

1. Giacobini, E. *Neurochem. Res.* 2003, 28, 515.
2. Ballard, C. G. et al. *Curr. Alzheimer Res.* 2005, 2, 307.
3. Munoz, F. J.; Inestrosa, N. C. *FEBS Lett.* 1999, 450, 205.
4. Musiat, A.; Bajda, M.; Malawska, B. *Curr. Med. Chem.* 2007, 14, 2654.
5. Ellman, G. L.; Courtney, K.D.; Andres, V.; Feather-Stone, R. M. *Biochem. Pharmacol.* 1961, 7, 88.
6. Bajda, M.; Więckowska A.; Malawska B. *Acta Pol. Pharm.* 2009, 66, 357.

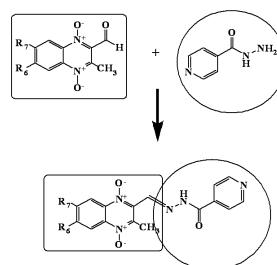
POSTER COMMUNICATIONS – PC.077

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW ISONICOTINIC HYDRAZIDE QUINOXALINE 1,4-DIOXIDE DERIVATIVES AS ANTITUBERCULAR AGENTS

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Tuberculosis (TB) is one of the most common infectious diseases known by the mankind. About 32% of the world's population is infected by *Mycobacterium tuberculosis*, with about 8 million new cases per year. The re-emergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB-cases. In this sense, the discovery of new and promising compounds with antimycobacterial activity remains essential for the control and prevention of TB (1).



As a continuation of our research in quinoxalines 1,4-di-N-oxide and with the aim of identifying new antitubercular drug candidates a new series of compounds have been prepared. Structure-based design has been used to obtain new lead molecules through the incorporation of isoniazid, one of the most effective first line anti-TB drugs, in a quinoxaline-1,4-di-N-oxide derivative (2).

Nine new quinoxalines 1,4-di-N-oxide derivatives containing isoniazid pharmacophore were prepared by a microwave assisted method optimized by our group in order to reduce the reaction times and increase the reaction yield. It has been necessary to optimize a new flash chromatography method due to the unexpected results obtained by the traditional chromatography purification methods.

In order to study the structure-activity relationship (SAR) of these compounds we use the Osiris program for calculating the fragment-based druglikeness of the compounds also using isoniazide and pirazinamide as reference compounds due to its structural closeness with our derivatives. With the aim of determining the antitubercular activity of the quinoxaline derivatives, the synthesized compounds have been sent to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF).

Taking into account the promising profile and the biological values of these compounds, it can be said that they should be considered for further experimental research.

ACKNOWLEDGMENTS

We wish to express our gratitude to the PIUNA project from the University of Navarra and the Antimicrobial Acquisition and Coordinating Facility for carrying out the biological assays through research and development contracts. Enrique Torres is indebted to the Navarra Government for a grant.

REFERENCES

1. WHO/HTM/TB/2008.393. WHO Report 2008
2. R. Villar, E. Vicente et al. *In vitro and in vivo* antimycobacterial activities of ketone and amide derivatives of quinoxaline 1,4-di-N-oxide. *J. Antimicrob. Chemother.* **2008**, 62, 547-554.

POSTER COMMUNICATIONS – PC.078

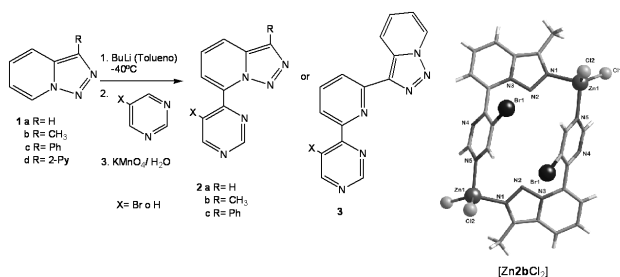
NEW PYRIMIDINYLTRIAZOLOPYRIDINES.
INTERACTION WITH NUCLEIC ACIDS

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Over the last four decades, intense research has focused on the effects of small organic compounds that noncovalently bind to nucleic acids, either by intercalation or groove binding. Classical intercalators typically have fused polyaromatic¹, while unfused polyaromatic compounds have been considered in literature as nonclassical intercalators².

In this work we have synthesized a series of compounds with the structure 7-(pyrimidin-4-yl)-[1,2,3]triazolo[1,5-a]pyridine **2** and 3-[6-(pyrimidin-4-yl)-pyridin-2-yl]-[1,2,3]triazolo[1,5-a]pyridine **3** both with fused and unfused aromatic rings. The synthesis of these compounds has been achieved by regioselective lithiation of the 7 position of [1,2,3]triazolo[1,5-a]pyridines **1**, and further reaction with the corresponding pyrimidine (Scheme 1). The reaction of 7-lithium-[1,2,3]triazolo[1,5-a]pyridine with electrophiles has been deeply studied,³ however it is the first time in which this reaction gives arylation products. All the obtained products have fluorescent properties, with λ emission between 474 and 500 nm.



Scheme 1

Figure 1

It has been reported that several metal complexes having polyaromatic ligands, such as metalloporphyrins⁴, bind to DNA. In this work we have prepared Zn^{II} complexes with ligands **2** and **3**, with the aim of studying their interaction with DNA (Figure 1).

Preliminary assays of DNA binding, by ethidium bromide quenching assay, show that compounds **2a** (X= Br) and **2b** (X= Br) can interact with DNA, while thermal denaturation assays show that complex **4** can destabilize the double helix of DNA.

REFERENCES

1. W.D. Wilson, Reversible interactions of small molecules with nucleic acids, in: M. Gait, M. Blackburn (Eds.), *The Chemistry and Biology of Nucleic Acids*, Oxford University Press, Oxford, England, **1990** (chapter 8).
2. L. Strekowski, B. Wilson, *Mutation Research*, **623**, **2007**, 3.
3. For example: a) G. Jones, D. R. Sliskovic, *Tetrahedron Lett* **1980**, 21, 4529. b) B. Abarca, R. Ballesteros, R. Ballesteros-Garrido, F. Colobert, F. R. Leroux, *Tetrahedron* **2007**, **63**, 10479.
4. M. Asadi, E. Safaei, B. Ranjbar, L. Hasani, *New J. Chem.*, **2004**, 1227.

POSTER COMMUNICATIONS – PC.079

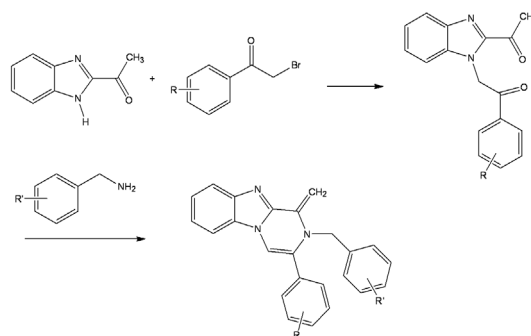
THE SYNTHESIS AND ANTICANCER ACTIVITY
OF SOME 1,2,3-TRISUBSTITUTED PYRAZINO-
BENZIMIDAZOLE DERIVATIVES

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The synthesis of some new pyrazino[1,2-a]benzimidazole derivatives and investigation of their anticancer activities were aimed in this work. Thus, 2-acetylbenzimidazole was reacted with 2-bromoacetophenone derivatives to give 1-(2-phenyl-2-oxoethyl)-2-aryloyl-4,5-diarylbenzimidazoles. These diketone compounds were reacted with appropriate benzylamine in acetic acid to obtain 1-methylene-2-benzyl-3-aryl-1,2-dihydropyrazino[1,2-a]benzimidazole derivatives. The structure of the compounds obtained were elucidated by using IR and ¹H-NMR spectral data and elemental analysis results.

All of the compounds were offered to American National Cancer Institute (NCI). Anticancer activities of the selected compounds were evaluated *in vitro* against approximately sixty human cell lines derived from nine neoplastic diseases and the results were given as growth percentage values. It was observed that the diketones were remarkable active compounds with low growth percentage. The most active compound was 1-[2-(3-chlorophenyl-2-oxoethyl)]-2-acetylbenzimidazole with the growth percentage of 24.53.



REFERENCES

1. Demirayak, Ş., Abu Mohsen, U., Karaburun, A.Ç., Synthesis and anti-cancer and anti-HIV testing of some pyrazino[1,2-a]benzimidazole derivatives, *Eur. J. Med. Chem.*, 37, 255-260 (2002).
2. Demirayak, Ş., Kayagil, I., Yurttas, L., Synthesis of Some 1,3-diarylpyrazino[1,2-a]benzimidazole derivatives by using either Rrflux or microwave irradiation method and investigation their Anticancer activities, *Eur. J. Med. Chem.*, Unpublished work.

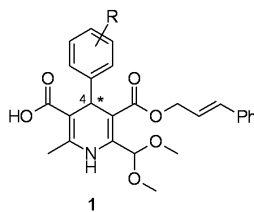
POSTER COMMUNICATIONS – PC.080

SYNTHESIS AND BIOLOGICAL EVALUATIONS OF OPTICALLY PURE 1,4-DIHYDROPYRIDINE-3-CARBOXYLIC ACID DERIVATIVES AS INHIBITORS FOR N-TYPE CALCIUM CHANNELS

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The N-type calcium channel (Ca_v2.2) is a well-established and characterized subtype of the voltage-dependent calcium channels (VDCCs), and distributes on the endings of central and peripheral nerves to control calcium influx into cells in response to changes in membrane potential. The activation of this channel triggers various physiological events such as neuronal excitability and secretion of neurotransmitters (ex. glutamate, substance P or CGRP) in strong association with the pathological process of neuropathic pain. Thus, blockade of the N-type VDCC has been considered as a promising therapeutic target for the pathological states, and many efforts have been made to discover potent small-molecule N-type VDCC blockers as candidates of next generation analgesics.¹ To serve this purpose, we have performed structure–activity relationship (SAR) studies for a unique 1,4-dihydropyridine-3-carboxylate compounds to find an effective and selective N-type calcium channel inhibitor with the least influence on cardiovascular system.² The latest SAR study on the 4-position of 1,4-dihydropyridine structure (**1**) will be disclosed with synthetic procedure and biological evaluations of two enantiomers.



REFERENCES

1. (a) Yamamoto, T.; Takahara, A. *Curr. Top. Med. Chem.* **2009**, 9, 377.; (b) Belardetti, F.; Zamponi, G. W. *Curr. Opin. Investig. Drugs* **2008**, 9, 707.
2. (a) Yamamoto, T.; Niwa, S.; Ohno, S.; Onishi, T.; Matsueda, H.; Koganei, H.; Uneyama, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.*, **2006**, 16, 798. (b) Yamamoto, T.; Niwa, S.; Iwayama, S.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Yamamoto, H.; Shoji, M. *Bioorg. Med. Chem.* **2006**, 14, 5333; (c) Yamamoto, T.; Niwa, S.; Ohno, S.; Tokumasu, M.; Masuzawa, Y.; Nakanishi, C.; Nakajo, A.; Onishi, T.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.* **2008**, 18, 4813.

POSTER COMMUNICATIONS – PC.081

A NOVEL CLASS OF DISUBSTITUTED ADAMANTYL DERIVATIVES AS POTENT MULTIDRUG RESISTANCE REVERSAL AGENTS

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A series of novel disubstituted adamantyl derivatives was conceived and synthesized as potent Multidrug resistance (MDR) reversal agents. MDR, which is a potentially surmountable obstacle to effective chemotherapy of cancer, is associated with excessively high expression of P-glycoprotein (P-gp) in cancer cells. P-gp, a plasma membrane transporter, which extrudes chemotherapeutic agents out of cells, can be considered as a therapeutic target. Here the design of target molecules was based on rational small molecules screening from in-house chemical library using an image-based high-throughput screening assay in a P-gp overexpressing MDR sarcoma cell line. The SAR studies on P-gp reversal activity indicated some adamantyl derivatives were more than 10-fold more potent than verapamil without considerable intrinsic cytotoxicity.

REFERENCES

1. Michael M. Gottesman, Tito Fojo and Susan E. Bates, *Nature Reviews Cancer*, **2002**, 2, 48-58.
2. Tripta Bansal, Naseem Akhtar, Manu Jaggi, Roop K. Khar and Sushama Talegaonkar, *Drug Discovery Today*, **2009**, 14 (21-22), 1067-1074.
3. Kyung Hoon Min, Yan Xia, Eun Kyung Kim, Yinglan Jin, Navneet Kaur, Eun Seon Kim, Dae Kyong Kim, Hwa Young Jung, Yongseok Choi, Mi-Kyung Park, Yong Ki Min, Kiho Lee, Kyeong Lee, *Bioorganic & Medicinal Chemistry Letters*, **2009**, 19, 5376–5379.

POSTER COMMUNICATIONS – PC.083

NEW HISTAPRODIFEN-DERIVED H₁-RECEPTOR AGONISTS

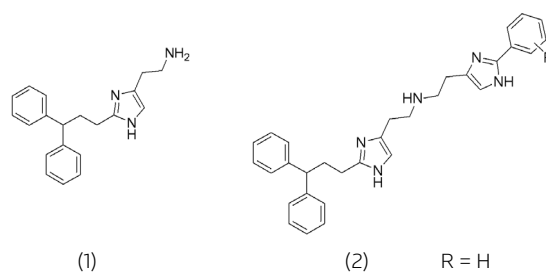
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In the histamine-receptor family (H₁, H₂, H₃, and H₄, respectively), the H₁-receptor subtype plays an essential role in the physiology of allergic reactions (e. g., rhinitis, flush) and in the control of sleep and waking state. Although this receptor is known for a long time and specific H₁-receptor antagonists have been introduced in the therapeutic market thirty years ago, the precise molecular binding mode of ligands and the binding pocket still remain to be elucidated. In our approach, we develop specific, highly potent histamine H₁-receptor agonists as pharmacological tools in order to get a deeper insight into the binding mode and to deduce further structure-activity relationships.

Histaprodiven (**1**)-type H₁-receptor agonists¹ offer an interesting starting point for these molecular investigations. Especially *suprahistaprodiven*, a “dimer” of histaprodiven and histamine which displays 36-fold higher activity than histamine, is an important milestone within this new class of H₁-receptor agonists². The idea to synthesize a hybrid-molecule of histaprodiven and the H₁-receptor agonist 2-phenylhistamine led to a new potent and selective H₁-agonistic compound showing 42-fold higher potency than histamine³. This agonist is also referred to as *phenoprodiven* (**2**). Searching for further structure-activity relationships, 2-phenylhistamines with different monosubstitution patterns (e. g., R = -CH₃, -OCH₃, -Hal) in the phenyl moiety – mostly H₁-receptor agonists by themselves – have been coupled with the conventional histaprodiven moiety in order to create a small library of *phenoprodiven*-derivatives. In the presented project the influence of different regioisomeric substituents in the 2-phenylimidazole moiety on H₁-receptor agonist potency and intrinsic activity is examined, and the results are compared to structure-activity relationships in the 2-phenylhistamine series. The *meta*-fluoro derivative of **2** was 47-fold more potent than histamine.

The syntheses included a reductive amination of an aldehyde containing the histaprodiven moiety with the appropriate 2-phenylhistamine as well as a microwave assisted *Suzuki* coupling, delivering the required 2-phenylhistamines⁴. Functional pharmacological characterisation was performed by means of the classical guinea-pig ileum H₁-receptor assay¹.



REFERENCES

1. Elz, S. et al., *J. Med.Chem.* **2000**, 43, 1071.
2. Menghin, S. et al., *J. Med. Chem.* **2003**, 46, 5458.
3. Striegl, B., *PhD thesis, University of Regensburg*, **2006**.
4. Skoumbourdis, A. et al., *Tetrahedron Lett.* **2007**, 48, 9140.

POSTER COMMUNICATIONS – PC.084

EXPLORING THE RIGID DIAMINE CHEMICAL SPACE: THEORY AND PRACTICE

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Dmytro S. Radchenko,^{1,2} Igor V. Komarov,^{1,2} and Andrey A. Tolmachev,^{1,2}

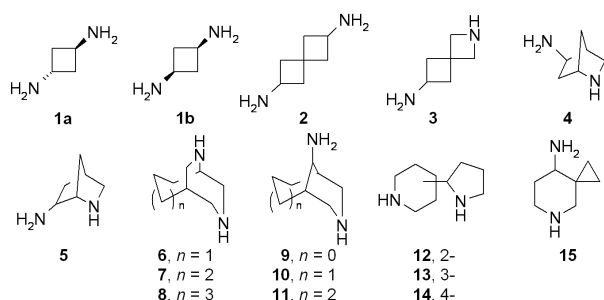
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Molecular rigidity has been repeatedly outlined as a distinguishing feature of approved drugs and natural products. Conformationally rigid aliphatic diamines exemplify privileged structures among other small molecules of importance to drug discovery. These building blocks have been widely used in drug discovery; in particular, more than 15 marketed drugs comprise residues of bicyclic diamines.

In this work, the theoretically available part of chemical space covered by rigid diamines was explored. It has been found that the known compounds comprise only a small part of all theoretically possible diamines, although even in case of known compounds, the diversity is quite large. MDL DDR data were analyzed for rigid diamines described in open or patent literature according to several parameters characterizing topology and spatial structure of the scaffolds.

Conformationally rigid diamines **1 – 15** either not described in the literature or recognized as hardly accessible by chemical synthesis using previously reported procedures were selected by us from the theoretically available chemical space. The practical approaches to the synthesis of these diamines as selectively monoprotected derivatives were developed. In the case of diamines **1 – 3**, spatial structure was also evaluated by X-Ray diffraction studies.



REFERENCES

- Shivanyuk, A. N.; Volochnyuk, D. M.; Komarov, I. V.; Nazarenko, K. G.; Radchenko, D. S.; Kostyuk, A.; Tolmachev, A. A. *Chem. Today* **2007**, 25, 12–13.
- Mityuk, A. P.; Denisenko, A. V.; Dacenko, O. P.; Grygorenko, O. O.; Mykhailiuk, P. K.; Volochnyuk, D. M.; Shishkin, O. V.; Tolmachev, A. A. *Synthesis* **2010**, 493–497.
- Mityuk, A. P.; Denisenko, A. V.; Dacenko, O. P.; Grygorenko, O. O.; Mykhailiuk, P. K.; Volochnyuk, D. M.; Tolmachev, A. A. *Tetrahedron Lett.* **2010**, 51, 1790–1792.

POSTER COMMUNICATIONS – PC.085

INHIBITION OF PEROXIDASE GENE EXPRESSION AND ENZYMATIC ACTIVITY BY RESVERATROL-COUMARIN HYBRID DERIVATIVES

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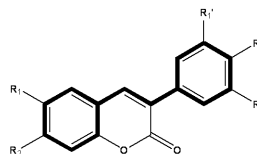
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Heme peroxidases are widespread in eukaryotes and prokaryotes and have a pivotal role in biology. [1] Both mammalian and plant peroxidases have been reported to produce aromatic oxyl radicals from several aromatic substrates and reactive oxygen species as part of a defense mechanism against pathogens. [2]

However, such reactive species generated by peroxidase-catalyzed reactions can also contribute to progressive human tissue damage in chronic inflammatory diseases as atherosclerosis, asthma and rheumatoid arthritis, which makes peroxidases interesting pharmacological targets. [3]

Considering the antioxidant and anti-inflammatory activities reported for *t*-resveratrol [4] and some coumarins [5], a series of resveratrol-coumarin hybrid derivatives have been evaluated as

inhibitors of enzymatic activity of peroxidases (horseradish peroxidase and peroxidases of HL-60 cells) and myeloperoxidase (MPO) gene expression.



T-resveratrol and some of the studied compounds caused significant inhibition of both peroxidase activity (HRP and peroxidase of HL-60 lysates). Not big differences could be found between inhibition of HRP or peroxidases of HL-60 lysates. Additionally, these compounds downregulated HL-60 MPO mRNA levels and at 100 μ M, some of them completely suppressed MPO gene expression.

Results obtained in this study may be helpful to understand better the interactions between resveratrol-coumarin hybrids and peroxidase catalytic site.

REFERENCES

- Everse, J.; Everse, K. E.; Grisham, M. B. In *Peroxidases in Chemistry and Biology*; CRC Press: Boca Raton, FL, 1991.
- Takahama, U.; Yoshitama, K. *J. Plant Res.* **1998**, 111, 97.
- Obinger, C. *Arch. Biochem. Biophys.* **2006**, 445, 197.
- Castro, R.; Lamas, J.; Morais, P.; Sanmartín, M.L.; Orallo, F.; Leiro, J. *Veterinary Immunology and Immunopathology* **2008**, 126, 9.
- Kabeya L.M.; de Marchi, A. A.; Kanashiro, A.; Lopes, N. P.; da Silva, C. H. T. P.; Pupo, M. T.; Lucisano-Valim, Y. M. *Bioorg. & Med. Chem. Lett.* **2007**, 15, 1516.

POSTER COMMUNICATIONS – PC.086

EFFECTS OF COUMARIN-RESVERATROL HYBRID DERIVATIVES ON LEUKOCYTE RESPIRATORY BURST RESPONSE.

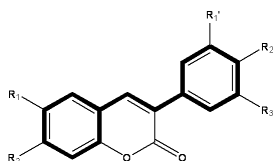
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Resveratrol [3,4',5-trihydroxystilbene] is a phytoalexin of natural origin presents in spermatophytes species such as vines, in response to damage. Resveratrol was already studied as cardioprotective, anticancer, enzymatic inhibitor, antioxidant and anti-inflammatory proving to be very efficient in a large group of in vitro, ex-vivo and/or in vivo experiments. [1]

On the other hand, coumarins consisting of fused benzene and α -pyrone rings, represent a vast family of compounds naturally found in plants that have been reported to possess a wide range of biological activities, including antioxidant and anti-inflammatory abilities without evidence of toxicity [2].

We have synthesized some coumarin/resveratrol hybrid derivatives in which the position of the hydroxy groups has been modified or the hydroxyl groups have been protected, fixing 3,4-double bond of the coumarin nucleus the trans disposition of the *t*-RESV. [3]



Effects of these compounds on the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during the respiratory burst and on the expression of inflammatory genes such as inducible nitric oxide synthase (NOS)-2 have been studied and compared with those obtained for *t*-resveratrol.

Obtained results indicate that some of these coumarin/resveratrol hybrid derivatives at micromolar concentrations have a significant antioxidant activity on the leukocyte response to proinflammatory stimuli and thus suggests that these compounds may have potential applications in the treatment of diseases involving leukocyte hyperresponsiveness.

REFERENCES

1. Leiro, J.; Alvarez, E.; García, D.; Orallo, F. *International Immunopharmacology* **2002**, 2, 767.
2. Bissonnette, E. Y.; Tremblay, G. M.; Turmel, V.; Pirotte, B.; Reboud-Ravaux, M. *International Immunopharmacology*, **2009**, 9, 49.
3. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yáñez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. & Med. Chem. Lett.*, **2006**, 16, 257.

POSTER COMMUNICATIONS – PC.087

DESIGN AND SYNTHESIS OF HETERO ANALOGS OF THE SUBTYPE SELECTIVE EAAT1 INHIBITOR, UCPH-101

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Excitatory amino acid transporters (EAAT) play a pivotal role in regulating the synaptic concentration of glutamate in the central nervous system (CNS). Up-to-date five different subtypes

have been identified, named EAAT1-5, in humans. In rodents their naming is for historic reasons different: GLAST, GLT-1, EAAC1, EAAT4, and EAAT5, respectively. Recently, we have published the first subtype selective inhibitor of EAAT1 (GLAST), UCPH-101. As a part of our medicinal chemistry studies of this novel EAAT1 inhibitor, we have designed and synthesized 6-heteroatom analogs for improved solubility and investigation of the structure-activity-relation (SAR). In details, two new analogs were prepared 6-amino-UCPH-101 and 6-oxy-UCPH-101 by means of two distinct synthetic routes. However both analogs failed to inhibit the EAAT1 subtype as well as EAAT2 and EAAT3 ($EC_{50} > 300 \mu M$). Although this result was surprising to us it contributes to the SAR of UCPH-101 and will serve as important information in our future research work within this field.

POSTER COMMUNICATIONS – PC.088

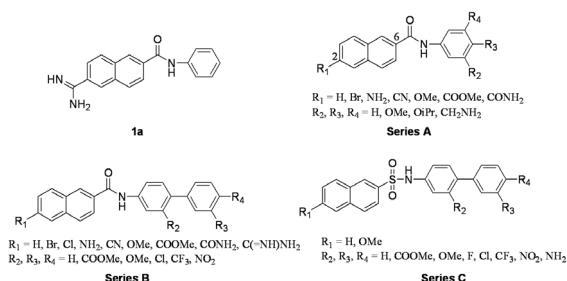
NON-BASIC UROKINASE PLASMINOGEN ACTIVATOR INHIBITORS AS POTENTIAL LEAD COMPOUNDS FOR AN ANTI-METASTATIC THERAPY

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Urokinase-type plasminogen activator (uPA) plays a key role in extracellular proteolytic events associated with tumor cell growth, migration, angiogenesis and metastasis.¹ Multiple independent groups have shown that uPA is a potent prognostic factor in breast cancer. Recently, this prognostic impact was validated in both a prospective randomized trial and a pooled analysis using LOE-1 evidence studies. As a result, uPA is an attractive target for the development of small molecule active site inhibitors. Recent drug development programs have focussed at nonpeptidic inhibitors that target at uPA have amidine or guanidine functional groups attached to aromatic or heterocyclic scaffolds.² There is a general problem of poor oral bioavailability of these charged inhibitors. We report the synthesis and evaluation of a series of uPA inhibitors having non-basic groups attached at 2-position of 6-substituted naphthamide or 6-substituted naphthalene sulfonamide. As a starting point we replaced amidine group of **1a**² by an amide (**Series A**, $R_1 = \text{CONH}_2$, $R_2 = R_3 = R_4 = \text{H}$) that resulted in same range of K_i value. With the help of molecular modeling a series of compounds (**Series B** and **C**) were synthesized for uPA inhibitory activity. The selective compounds were screened against a panel of other physiologically important trypsin-like serine proteases such as tissue plasminogen activator (tPA), plasmin and thrombin. It was noticed that four compounds have an enzymatic inhibition profile comparable to clinical uPA inhibitor **WX-UK1**. We propose that 2,6-disub-

stituted naphthamide template could be used further to design and optimize good clinical candidates.



REFERENCES

1. Duffy, M. J. The urokinase plasminogen activator system: Role in malignancy. *Curr. Pharm. Des.* **2004**, 10, 39–49.
2. Wendt M, Rockway T, Geyer A, McClellan W, Weitzberg M, Zhao X, Mantei R, Nienaber V, Stewart K, Klinghofer V.; Giranda, V. L. Identification of novel binding interactions in the development of potent, selective 2-naphthamide inhibitors of urokinase. Synthesis, structural analysis, and SAR of N-phenyl amide 6-substitution. *J. Med. Chem.* **2004**, 47, 303–324.

POSTER COMMUNICATIONS – PC.089

NOVEL CDC25 PHOSPHATASES SMALL COUMARIN-BASED INHIBITORS: DESIGN, SYNTHESIS AND BIOLOGICAL VALIDATION

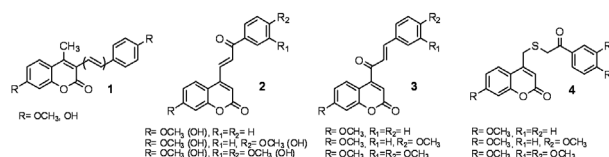
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Cell division cycle 25 (Cdc25) phosphatases are key actors in eukaryotic cell cycle control. The three human Cdc25 phosphatases (Cdc25A, Cdc25B, and Cdc25C) are responsible for dephosphorylating Cdk/cyclins on pThr14 and/or pTyr15 residues, this dephosphorylation triggers the final activation of Cdk/cyclin activity during normal cell cycle progression¹. Cdc25 phosphatases are also central targets and regulators of the G2/M checkpoint mechanisms activated in response to DNA injury. Cdc25A controls both the G1-to-S and G2-to-M transitions, whereas Cdc25B and Cdc25C are regulators of the G2-to-M transition². So far, several lines of experimental evidence have been provided for the involvement of Cdc25 phosphatases in oncogenic transformations and various human cancers³. Altered expression of these phosphatases is associated with checkpoint bypass and genetic instability. Accordingly, increased expression of Cdc25A and Cdc25B is found in many high-grade tumors and is correlated with poor prognosis in human cancers⁴. To date several Cdc25A and Cdc25B class of inhibitors are reviewed in

literature⁵ like potential agents for cancer therapy, mostly such as quinone-based compounds reported active *in vivo*⁶, nevertheless recently a new class of Cdc25B inhibitors were described displayed cytotoxic properties against HeLa cells and against prostatic LNCaP cells⁷. Moreover several Sylibin derivatives showed a strong cell cycle arrest in PC3 cells and differential effects of these compounds on the levels of some cell cycle regulators-cyclins, among these Cdc25A, Cdc25B and Cdc25C⁸, and several flavones were described able to inhibit Cdc-25.1 Phosphatase activity in *C. elegans*⁹. These findings prompted us to project the design, synthesis and biological validation of novel Coumarin-based (like flavon isomer) derivatives (**1-4**). Tested against the three Cdc25 isoform some of such molecules showed inhibiting activity at micromolar concentrations in highly repeatable fluorescence screening assays. The reversibility of fixation of those molecules on Cdc25 isoforms is currently assessed by fluorimetry. These molecules are also tested on commonly used breast cancer cell lines (MCF7 sensitive and MCF-7 resistant to chemotherapeutic drugs), in order to show a correlation between Cdc25 inhibition and a cytostatic potential (dephosphorylation of CDK and cell cycle blocking).



REFERENCES

1. Boutros, R.; Dozier, C.; and Ducommun, B. *Curr. Opin. Cell Biol.* **2006**, *18*, 185-191.
2. Rudolph, J. *Biochemistry* **2007**, *46*, 3595.
3. Wang, P.; Zou, F.; Zhang, X.; Li, H.; Dulak, A.; Tomko, R.J.; Jr, Lazo J.S.; Wang, Z.; Zhang, L.; Yu, J. *Cancer Res.* **2009**, *69*, 8157.
4. Aressy, B.; Ducommun, B. *Anticancer Agents Med Chem.* **2008**, *8*, 818.
5. Lavecchia, A.; Di Giovanni, C.; Novellino, E.; *Curr Med Chem.* **2009**, *16*, 1831.
6. Boutros, R.; Lobjois, V. and Ducommun, B. *Nature Reviews Cancer*, **2007**, *7*, 495-507.
7. Kolb, S. ; Mondésert, O.; Goddard, M.L. Jullien, D.; Villoutreix, B.O.; Ducommun, B.; Garbay, C.; Braud, E. *Chem Med Chem.* **2009**, *4*, 633.
8. Deep, G.; Oberlies, N.H.; Kroll, D.J.; Agarwal, R. *Int J Cancer.* **2008**, *123*, 41.
9. Kim, K.S.; Kawasaki, I.; Chong, Y. and Shim, Y.H. *Mol. Cells* **2009**, *27*, 345-350.

POSTER COMMUNICATIONS – PC.090

ANTI-*LEISHMANIA INFANTUM* ACTIVITY OF IMIDAZOQUINES

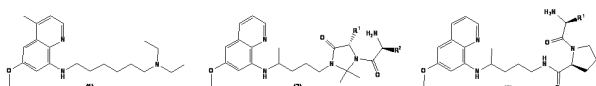
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Leishmanias are tropical and subtropical diseases caused by protozoal parasites of the genus *Leishmania*. The clinical expressions found in humans depend on the *Leishmania* species involved: cutaneous, mucosal and visceral leishmaniasis. This infection is endemic in 88 countries in the Horn of Africa, South Asia, and Latin America.¹

Sitamaquine (**1**), an 8-aminoquinoline (8-AQ), is a new anti-leishmanial oral drug, currently in Phase IIb clinical trials by GlaxoSmithKline. It has been well-tolerated in the most recent Phase II studies, but there are still some unanswered questions concerning its potential toxicities.^{2,3}

Our group developed imidazoquinones with general structure **2**, as peptidomimetic imidazolidin-4-one derivatives of the 8-aminoquinoline anti-malarial primaquine (PQ). Imidazoquinones **2** are stable compounds that preserve the overall bioactivity pattern of the parent drug and are not vulnerable to either oxidative deamination or proteolytic degradation by action of amino- or endopeptidases.⁴ Given their 8-aminoquinolinic nature and anti-protozoal activity, imidazoquinones were seen as possibly useful leishmanicidal agents. Their activity against *Leishmania infantum* was tested using the resazurin dye to measure parasite viability. This study also included dipeptide derivatives of PQ with general structures PQ-Proline-AA (**3**) that are mimetized by the imidazoquinones. We wish to report the results obtained in this study, i.e., on the leishmanicidal activities of compounds **2** and **3** against *L. infantum*.



REFERENCES

- Bern, C.; Maguire, J. H.; Alvar, J. Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS Neglected Trop. Dis.* 2008, 2, No. e313.
- Jha, T. K.; Sundar, S.; Thakur, C. P.; Felton, J. M.; Sabin, A. J.; Horton, J. A phase II dose-ranging study of sitamaquine for the treatment of

visceral leishmaniasis in India. *Am. J. Trop. Med. Hyg.* 2005, 73, 1005-1011.

- Wasunna, M. K.; Rashid, J. R.; Mbui, J.; Kirigi, G.; Kinoti, D.; Lodenyo, H.; Felton, J. M.; Sabin, A. J.; Albert, M. J.; Horton, J. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. *Am. J. Trop. Med. Hyg.* 2005, 73, 871-876.
- Vale, N.; Prudêncio, M.; Marques, C.A.; Collins, M.S.; Gut, J.; Nogueira, F.; Matos, J.; Rosenthal, P.; Cushion, M.T.; Rosário, V.E.; Mota, M.M.; Moreira, R.; Gomes, P. Imidazoquinones as antimalarial and antipneumocystis agents. *J. Med. Chem.* 2009, 52, 7800-7807.

POSTER COMMUNICATIONS – PC.091

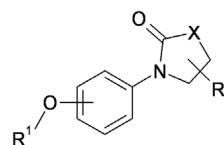
DISCOVERY OF LACTAMS AS A NOVEL CLASS OF HIGHLY POTENT H₃R INVERSE AGONISTS FOR THE TREATMENT OF COGNITIVE DISORDERS

Anne Valade, Sylvain Célanire, Bernard Christophe, Philippe Collart, Sabine Defays, Christel Delaunoy, Marie-Laure Delporte, Frédéric Denonne, Véronique Durieu, Michel Gillard, Delphine Hubert, Bénédicte Lallemant, Yves Lamberty, Geneviève Lorent, Jean-Marie Nicolas, Luc Quéré, Alain Vanbellinghen, Nathalie Vanhoutvin, Valérie Verbois and Laurent Provins

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Since the discovery and cloning of the human histamine H₃ receptor and the identification of the numerous potential therapeutic indications in which it may play a key role, pharmaceutical companies have put tremendous efforts in the identification of potent and selective H₃ ligands. Several of them successfully came up with drug candidates, currently in clinical trials for the treatment of cognitive disorders in Alzheimer's disease, ADHD or schizophrenia but also targeting sleep disorders such as narcolepsy.

In this poster, we would like to report the identification of highly potent H₃R inverse agonists bearing a lactam moiety. Lead generation and optimization efforts around those scaffolds with high ligand efficiency and low lipophilicity will be presented and will include the description of *in vitro* pharmacology, physico-chemical and pharmacokinetic properties as well as the evaluation of selected lead candidates in animal models of memory deficits.



The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5650)

POSTER COMMUNICATIONS – PC.092

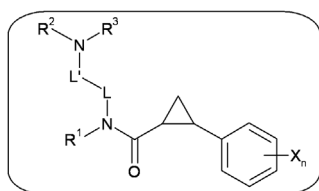
DISCOVERY OF NOVEL SELECTIVE SIGMA-1 LIGANDS AS COGNITIVE ENHANCERS

Anne Valade¹, Sophie Binet², Claire Brown², Eric Detrait¹, Doina Ene², Michel Gillard¹, Michel Guyaux¹, Yves Lamberty¹, Martin Maguire², Nivedita Namdev², Laurent Provins¹, Eric Schwartz², Céline Vermeiren¹

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The sigma receptor is a non-opioid transmembrane receptor which does not share any homology with other mammalian proteins. Since the 1970s, pharmaceutical companies have put tremendous efforts in the identification of potent Sigma-1 ligands. Several of them came up with non-selective pre-clinical candidates (e.g. (+)SKF-10,047). These compounds showed some improvement of cognitive impairment induced by Scopolamine, age or β -amyloid peptide in rodents. Despite promising preclinical data in several therapeutic areas, no clinical proof of concept has been reported to date.

In this poster, we would like to report the identification of phenyl-cyclopropylcarboxamide compounds as novel, selective and potent Sigma 1 ligands. Description of *in vitro* pharmacology, physicochemical and pharmacokinetic properties as well as the evaluation of selected compounds in animal models of memory deficit will be presented.



POSTER COMMUNICATIONS – PC.093

SYNTHESIS AND OPTIMIZATION OF PHARMACOLOGICAL PROFILE OF HEXAHYDRO- AND OCTAHYDRO-AZEPINO[4,3-b]INDOLES

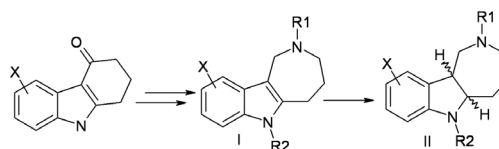
Sergey Tkachenko, Volodymir Kysil, Eugene Frolov, Alex Khvat, Ilya Okun, Oleg Mitkin, Alexander Ivachtchenko

ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, CA 92121 USA

Possibly due to their unique intricacy, therapeutics for CNS disorders have not benefited from rational single targeted drug design. For major feature of many effective CNS drugs is their affinity for a large number of various biological targets involved

in the multifaceted pathogenesis. Design of multi-target-directed CNS-ligands has to be based on the concept of “magic shotgun”, where several key signalling elements are targeted simultaneously, which is opposite to the classic concept of single-target “magic bullet”.

We report the synthesis, biological evaluation and optimization of new CNS drug-like series of hexahydro- and octahydro-azepino[4,3-b]indoles (series I and II respectively).



X = H, Me, MeO, Hal, etc. R1 = Alkyl; R2 = Ar, Het, substituted Alkyl, Aralkyl, Alkynyl, etc.

Compounds of the series I were synthesized in a few steps starting from known tetrahydrocarbazol-4-ones. Series II was obtained by selective reduction of hexahydro-derivatives and included E and Z-isomers.

We have performed a molecular pharmacology profiling of a few representatives of series I and II on a panel of 70 therapeutic targets (GPCRs, ion channels, transporters, and enzymes) to characterize a spectrum of their activities. Selected compounds exhibited high affinity to constellations of histaminergic, serotonergic, alpha-adrenergic, dopaminergic receptors, Ca channels, transporters and cholinesterases. The pharmacological profiles of the compounds tested were sensitive to the nature of substituents X and R2. The decrease of compound lipophilicity by variation of X and R2 could result in diminishing of unwanted affinity toward hERG channel and H₁ receptors. The predominant activity toward 5-HT_{2A} receptors was shown for compounds I and II with R2 = Aryl. With R2 as Aralkyls, the compounds showed very high activity toward 5-HT_{2C}, 5-HT₆ and 5-HT₇ receptors.

Directed optimization of “pharmacological fingerprints” has led to the discovery of new series of chemotypes, which could be a reliable source of advanced drug candidates for the treatment of mood and cognition disorders.

POSTER COMMUNICATIONS – PC.094

IDENTIFICATION OF NOVEL ARYLOXYPROPANOLAMINES AS LIGANDS OF β -ADRENOCEPTORS: A vHTS APPROACH

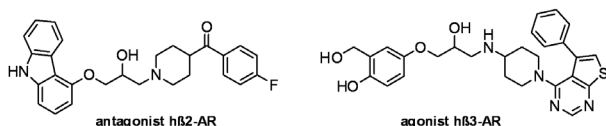
Stefan Tasler, Roland Baumgartner, Astrid Ammendola, Andrea Aschenbrenner, Kristina Wolf

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Based on a distribution of the human β 3-adrenergic receptor (h β 3-AR) in different tissues like white and brown adipocytes, urinary bladder detrusor, gastrointestinal tract, near-term myometrium, brain and the human heart, several indications

emerged for a potential treatment with $\text{h}\beta 3\text{-AR}$ ligands, especially agonists: obesity, type II diabetes, overactive bladder, irritable bowel syndrome (IBS), preterm labor, anxiety and major depression disorder.¹ Antagonists have recently been under evaluation for the treatment of chronic heart failure.² The inhalation of $\text{h}\beta 2\text{-adrenoceptor}$ selective agonists has long been established as therapy of asthma and other bronchospastic conditions.³ Furthermore, systemic $\text{h}\beta 2\text{-agonists}$ are used as tocolytics in preterm labor,³ and antagonists have just been described to have a beneficial effect in wound healing.⁴

Aiming for an *in silico* approach for the identification of new β -adrenoceptor ligands (with an initial focus on $\text{h}\beta 3\text{-AR}$), a direct docking procedure using a homology model for $\text{h}\beta 3\text{-AR}$ was not selected as method of choice, as – at the given point in time – only the crystal structure of the bovine rhodopsin receptor was available as a representative of the target class of GPCRs, with only around 40% sequence homology to $\text{h}\beta 3\text{-AR}$. For an alignment approach, five literature-known $\text{h}\beta 3\text{-AR}$ agonists were selected as templates based on their advancement in clinical development, their activity on $\beta 3\text{-receptors}$ and/or for structural reasons. Each of these templates was used for a vHTS run of a virtual library comprising 4.6 Mio commercially available compounds using the 4Scan® technology,⁵ resulting in the identification of two hit classes of the aryloxypropanolamine type. Optimization programs for both of them resulted in the generation of two potent lead compounds, one as a selective antagonist at $\text{h}\beta 2\text{-AR}$,⁶ the other as a selective agonist at the $\text{h}\beta 3\text{-AR}$.⁷ Details of the hit-to-lead optimization will be presented, including tissue data for the latter (relaxation of pre-contracted human urinary bladder detrusor strips).



REFERENCES

1. Ursino, M. G.; Vasina, V.; Raschi, E.; Crema, F.; De Ponti, F. *Pharmacol. Res.* **2009**, 59, 221.
2. Lee, S.; Schwinger, R. H. G.; Brixius, K. *Pflugers Arch. Eur. J. Physiol.* **2008**, 455, 767.
3. Kolb, P.; Rosenbaum, D. M.; Irwin, J. J.; Fung, J. J.; Kobilka, B. K.; Shoichet, B. K. *Proc. Natl. Acad. Sci. USA* **2009**, 106, 6843.
4. Sivamani, R. K.; Pullar, C. E.; Manabat-Hidalgo, C. G.; Rocke, D. M.; Carlsen, R. C.; Greenhalgh, D. G.; Isseroff, R. R. *PLoS Med.* **2009**, 6, 105.
5. Seifert, M. H. J.; Wolf, K.; Vitt, D. *Biosilico* **2003**, 1, 143.
6. Tasler, S.; Baumgartner, R.; Aschenbrenner, A.; Ammendola, A.; Wolf, K.; Wieber, T.; Schachtner, J.; Blisse, M.; Quotschalla, U.; Ney, P. *Bioorg. Med. Chem. Lett.*, submitted.
7. Tasler, S.; Baumgartner, R.; Ammendola, A.; Schachtner, J.; Wieber, T.; Blisse, M.; Rath, S.; Zaja, M.; Klahn, P.; Quotschalla, U.; Ney, P. *Bioorg. Med. Chem. Lett.*, in preparation.

POSTER COMMUNICATIONS – PC.095

LABELLING STRATEGIES TO STUDY LARGE PROTEIN-LIGAND INTERACTIONS BY NMR

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The application of Nuclear magnetic Resonance (NMR) spectroscopy to large proteins is still a challenge. In spite of technical improvements in recent years, signal overlap continues to be a serious handicap when working with high molecular weight proteins. Therefore, simplification of the spectra of these macromolecules by selective labeling is a useful technique to facilitate their study. In this presentation, we report on two cost-effective labeling strategies for the NMR study of prolyl oligopeptidase (POP). In the first approach, a ¹⁵N-label is selectively incorporated into the tryptophan side chains of the protein and high quality simplified [1H15N]-TROSY HSQC spectra are obtained without the need for deuteration. The assignment of the indole resonances by site-directed mutagenesis and the interaction with a classical covalent POP inhibitor, Z-prolyl-prolinal, is described. In the second strategy, a ¹³C-label is selectively incorporated into the methyl groups of Methionine side chains and high resolution [1H13C]-HMQC spectra are recorded rapidly in a straightforward manner. After assignment of the methyl-¹³C-Met resonances, structural information about POP interaction with Z-prolyl-prolinal, as well as with new POP inhibitors, is obtained. The main advantage of these two methodologies is that they allow the study of POP-ligand interactions and POP conformational changes in solution.

REFERENCES

1. Tarragó, T.; Claasen, B.; Kichik, N.; Rodriguez-Mias, R.A.; Gairí, M.; Giralt, E. A cost-effective labeling strategy for the NMR study of large proteins: selective ¹⁵N-labeling of the tryptophan side chains of prolyl oligopeptidase. *Chembiochem.* 2009, 10, 2736-9.
2. Tarragó, T.; Martín-Benito, J.; Sabidó, E.; Claasen, B.; Madurga, S.; Gairí, M.; Valpuesta, J.M.; Giralt, E. A new side opening on prolyl oligopeptidase revealed by electron microscopy. *FEBS Lett.* 2009, 583, 3344-8.

POSTER COMMUNICATIONS – PC.096

INVESTIGATION OF AGENTS TARGETING NUCLEAR RECEPTOR CO-REPRESSOR PROTEIN

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Acute promyelocytic leukemia (APL), resulting from the fusion of promyelocytic leukemia (PML) protein and retinoic acid receptor (RAR), is a subtype of acute myelocytic leukemic. APL displays resistance to programme cell death and is characterized by an accumulation of blast cells in the bone marrow and peripheral blood. Previous reports by Ng *et al* illustrated the significant role of Nuclear receptor Co-Repressor (NCoR) protein in the resistance to apoptosis in APL cells. Accumulation of misfolded NCoR protein as insoluble aggregates in APL cells was shown to induce endoplasmic reticulum (ER) stress and to activate unfolded protein response (UPR) –induced apoptosis, ultimately contributing to differentiation arrest of APL cells. This establishes the misfolded NCoR protein as an attractive molecular target in APL. Curcumin, an active component in turmeric was shown to selectively promote apoptosis in APL cells containing elevated levels of misfolded NCoR protein over non-APL cells. While curcumin has been known to rescue the native conformation of misfolded proteins such as the amyloid β , its mechanism of action on APL cells appears to involve the inhibition of proteasome mediated degradation of misfolded NCoR protein. Accumulation of misfolded NCoR therefore aggravates ER stress and results in the selective sensitization of APL cells to UPR-induced apoptosis. This finding illustrates the potential of curcumin in targeting misfolded NCoR and thus APL. We aim to develop more potent curcumin analogues with high fidelity for NCoR protein in APL cells. A series of 32 analogues of curcumin have been designed and synthesized with the objective of interrogating structure-activity relationships and providing greater insight into the role of misfolded NCoR as a drug target in APL. Activity and selectivity profiles have been established on the APL cell line NB4-R1 (retinoic acid resistant) and non-APL cell lines HL60 and K562. Herein we report several scaffolds with up to 18-fold improved potency on NB4-R1 while maintaining at least 3-fold selectivity between APL and non-APL cell lines. In particular, one compound exhibited over 200-fold selectivity for NB4-R1 with a 3-fold improved potency over curcumin. With analogues of improved potency and selectivity to NCoR protein, we aim to study the influence of the analogue on the conformational status of NCoR in APL and non-APL cells and its implication in the response of cells to UPR-induced apoptosis.

REFERENCES

1. Ng APP; Fong J.H.; Nin D.S.; Hirpara J.L.; Asou N.; Chen C-S.; Pervaiz S.; Khan M., *Cancer Res.*, **2006**, 66, 9903-9912
2. Ng A.P-P; Khan M., Submitted
3. Hafner-Bratkovic I.; Gaspersic J.; Smid L., *J Neurochem.*, **2008**, 104, 1553-1564

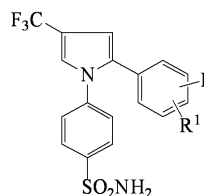
POSTER COMMUNICATIONS – PC.097

ANTITUMOR PROPERTIES OF DERIVATIVES 1,2-DIPHENYLPYRROLE AND THEIR PREPARATION PROCESS

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The development of antitumoral therapies of general applicability is one of the main goals in medicinal chemistry¹. Cancer is a heterogeneous disease characterized by the accumulation of tumor cells, which can cause the death of both animals and humans. Among the strategies envisaged for developing new antitumoral treatments, the promotion of cell death, in particular the induction of apoptosis by the inhibition of signaling through focal adhesions, a mechanism barely explored so far, is especially attractive². The reason for this interest lies in the anti-tumor and antimetastatic activity induced by the blockade of the constitutive signaling through focal adhesions. Herein is described the synthesis and bio-evaluation of 1,2-diphenylpyrrole derivatives as potential drug candidates for treatment or prevention of tumours³.



The synthetic procedure is based in the heterocyclic ring construction, followed by the introduction of arylsubstituted nucleus on position two of pyrrole by a Pd(0) catalyzed cross-coupling reaction.

Discussion of structure-activity relationships will be done in the presentation.

REFERENCES

1. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57-70.
Ashkenazi, A. *Nature Reviews Cancer* **2002**, *2*, 420-430.
2. Schimmer, A. D. *Cancer Research* **2004**, *64*, 7183-7190.
Schimmer, A. D.; Welsh, K.; Pinilla, C.; Wang, Z.; Krajewska, M.; Bonneau, M.-J.; Pedersen, I. M.; Kitada, S.; Scott, F. L.; Bailly-Maitre, B.; Glinsky, G.; Scudiero, D.; Sausville, E.; Salvesen, G.; Nefzi, A.; Ostresh, J. M.; Houghten, R. A.; Reed, J. C. *Cancer Cell* **2004**, *5*, 25-35.
3. Mangues, R.; Casanova, I.; Albericio, F.; Álvarez, M.; Savina, S. EP Patent 09166668.

POSTER COMMUNICATIONS – PC.098

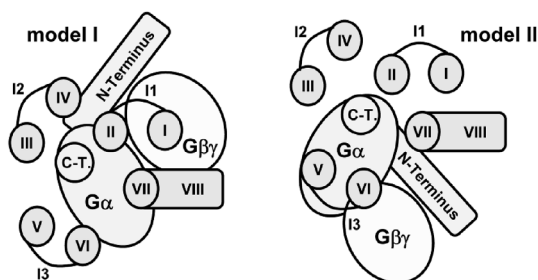
TWO MODELS FOR DISTINCT INTERACTIONS BETWEEN THE HUMAN ADRENERGIC β_2 RECEPTOR AND $G\alpha_s$ – AN IN SILICO STUDY

Straßer, A., Wittmann, H.-J.

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G protein-coupled receptors (GPCR), important targets for several drugs, couple intracellular to heterotrimeric G proteins. These G proteins are switches on molecular level in order to turn on intracellular signal cascades, induced by activation of the corresponding GPCR. Some new crystallographic studies [1] give information about the interaction between receptor and a part of the C-terminus of the $G\alpha$ -subunit of the G protein. But still, there is no crystal structure about a complete GPCR G-protein complex.

The aim of this study was an in silico analysis of the interaction of the human adrenergic β_2 receptor ($h\beta_2R$) with the $G\alpha_s$ subunit. A systematic potential energy surface scan between $h\beta_2R$ and $G\alpha_s$ revealed two energetically preferred $h\beta_2R$ - $G\alpha_s$ complexes. These two complexes were embedded into a lipid bilayer and surrounded by intra- and extracellular water. Subsequently, molecular dynamic simulations were performed in order to get knowledge about distinct hydrogen bond interactions between $h\beta_2R$ and $G\alpha_s$. Based on the modelling results, two stable $h\beta_2R$ - $G\alpha_s$ complexes with completely different orientation between $h\beta_2R$ and $G\alpha_s$ were obtained.



In general, these two models are in good accordance to experimental results, available in literature. However, all experimental results found for several GPCRs, can not be explained by one distinct orientation between receptor and $G\alpha$ subunit at one time. Thus, based on the results of this in silico study, it may be hypothesized, that there exist two different interaction states between receptor and G protein, in dependence of the length of I3-loop or C-terminus. Further experimental mutagenesis studies have to be performed to proof this hypothesis. However, this in silico study gives distinct suggestions for interaction between GPCR and G proteins, which is important for a better understanding on molecular level [2].

REFERENCES

1. Scheerer P, et al., *Nature*, 455:497-503
2. Strasser A, Wittmann HJ, *J Mol Model*, DOI:10.1007/s00894-010-0646-3

POSTER COMMUNICATIONS – PC.099

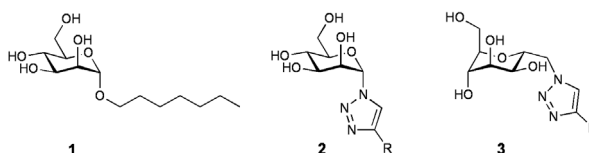
BIOLOGICAL EVALUATION OF α -D-MANNOSYL TRIAZOLES AS LIGANDS FOR FimH

Oliver Schwardt, Said Rabbani, Brian Cutting, and Beat Ernst

Institute of Molecular Pharmacy, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Uropathogenic strains of *Escherichia coli* (UPEC) are accounting for more than 70% of all urinary tract infections (UTIs).¹ These infections are initiated by the adhesion of the mannose-binding lectin FimH, located at the tips of bacterial type 1 pili, to uroplakin receptors in the uroepithelium. Since bacterial resistance to antibiotics is the major problem of recurrent infections, blocking of bacterial adhesion with mannose derivatives like *n*-heptyl α -D-mannoside **1**² is a promising therapeutic approach to prevent infection.

In our search for metabolically stable and pharmacokinetically improved FimH antagonists, a library of mannosyl triazoles of types **2** and **3** was synthesized and tested in target-based and cell-based binding assays. Compared to the reference compound **1**, the affinity of the triazoles **2** and **3** was decreased about 2- to 10-fold. A thorough analysis by NMR spectroscopy (NOESY, COSY, HSQC) revealed that the C-mannosides **3** adopt an unusual ¹C₄ conformation.



REFERENCES

1. T. M. Hooton, W. E. Stamm, *Infect Dis. Clin. North. Am.* **1997**, *11*, 551.
2. J. Bouckaert, J. Berglund, M. Schembri, E. De Genst, L. Cools, M. Wuhrer, C.-S. Hung, J. Pinkner, R. Slättegård, A. Zavialov, D. Choudhury, S. Langermann, S. J. Hultgren, L. Wyns, P. Klemm, S. Oscarson, S. D. Knight, H. De Greve, *Mol. Microbiol.* **2005**, *55*, 441.

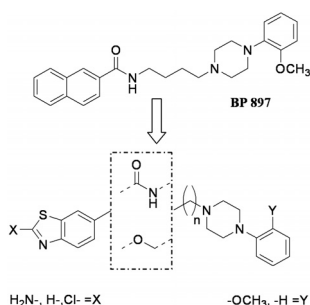
POSTER COMMUNICATIONS – PC.100

POTENT AND SELECTIVE DOPAMINE D_{2/3} LIGANDS WITH BENZTHIAZOLE ELEMENTS

Schübler, M., Schwed, S., Weizel, L., Kottke, T., Stark, H.

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Dopamine D₂ and D₃ receptor subtypes belong to the dopamine D₂-like receptor family, a group of class A G-protein coupled receptors (GPCRs). They have become highly interesting drug targets of intensive research over the past decades. The both receptor subtypes offer valuable points of action for the development of medications against neuropsychiatric disorders like Parkinson's disease, schizophrenia and drug addiction.¹ A former clinical candidate BP 897, (K_i hD₂: 67 nM, hD₃: 0,92 nM, ratio hD₂/hD₃: 66) with an in vitro partial agonist functionality on the hD₃ receptor, has been used as prominent point of beginning in the development of potent dopamine D₂/D₃ receptor compounds.²



In our bioisosteric approach we have exchanged the naphth-2-yl aromatic substructure of BP 897 by benz[d]thiazol-6-yl moieties with different connecting groups. Substituents for variations in X and Y as well as different alkyl spacer lengths n on the benz[d]thiazol-6-ylcarboxamides and on the corresponding ether derivatives have been evaluated concerning their influence on hD₂ and hD₃ receptor subtype affinities (cf. Scheme). The compounds showed moderate to high affinities in the nanomolar concentration ranges in which slight changes of the structural parameters led to a change in the receptor profile.

Synthesis, screening assays, pharmacological affinity data, and structure-activity-relationships on these two new classes of compounds will be described.

REFERENCES

1. A.E. Hackling, H.Stark, *ChemBioChem* **2002**, *3*, 946.
2. M. Pilla et al., *Nature* **1999**, *400*, 371.

POSTER COMMUNICATIONS – PC.101

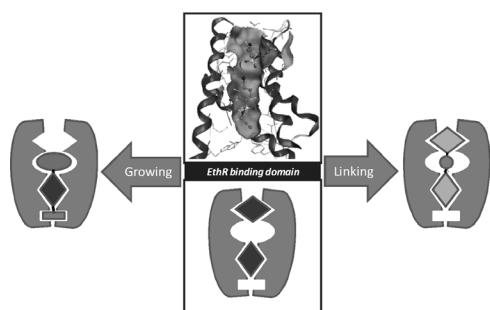
FRAGMENT LINKING AND FRAGMENT GROWING STRATEGIES FOR THE DESIGN AND DISCOVERY OF EthR INHIBITORS AS POTENT ETHIONAMIDE BOOSTERS: A NEW STRATEGY TO FIGHT MDR-TUBERCULOSIS.

Nicolas Willand^{1,6}, Baptiste Villemagne^{1,6}, Sandra Malaquin^{1,6}, Nathalie Guillet^{2,6}, Nicolas Blondiaux^{2,6}, Priscille Brodin⁵, Vincent Villeret^{3,6}, Bruno Villoutreix⁴, Olivier Sperandio⁴, Alain R. Baulard^{2,6} and Benoit Deprez^{1,6}

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Tuberculosis remains a major cause of mortality killing each year more than two million people. Current tuberculosis therapies include a large number of prodrugs that are activated inside of the mycobacteria. The protein EthA is responsible for the activation of Ethionamide, a second-line antibiotic. The negative regulation of *ethA*, exerted by the bacterial transcriptional repressor EthR, limits the effectiveness of the prodrug. [1] Thus, EthR contributes to the innate resistance of mycobacteria to this antibiotic. We recently reported that drug-like EthR inhibitors are able to boost the antimycobacterial efficacy of Ethionamide both *in vitro* and *in vivo*. [2] In the course of identification of new chemical entities, BLAcore assay and X-ray crystallography were used to identify a fragment binder featuring arylsulfonamide framework. Based on this scaffold we developed parallel fragment-based strategies to improve activities. Analysis of the binding mode, design, screening of virtual libraries and hit expansion allowed us to rapidly establish improved molecules. Further elaboration using structure-based drug design resulted in drastic potency improvement while maintaining good physicochemical and pharmacokinetic properties.

This presentation will discuss the design, synthesis and preliminary *in vitro* and *ex vivo* biological evaluation of fragment-based EthR inhibitors.



REFERENCES

1. Engohang-Ndong, J. et al. Mol Microbiol 2004, 51, (1), 175.
2. Willand, N. et al. Nat Med 2009, 15, (5), 537.

POSTER COMMUNICATIONS – PC.102

DISCOVERY AND SAR OF NOVEL FURAN-FREE IMIDAZOPYRIDINE LIGANDS AS SELECTIVE ADENOSINE A2A-ANTAGONISTS FOR PARKINSON DISEASE

Schann Stephan; Mayer Stanislas; Lydie Cardona; Sophie Gardan; Nadia Hubert ; Anne Haberkorn ; Edith Steinberg ; Luc Baron; Franchet Christel; Neuville Pascal

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A2A-receptor antagonism is a novel therapeutic strategy for the treatment of Parkinson's Disease, a neurodegenerative disorder resulting from a gradual loss of dopaminergic neurons. A2A-receptor antagonists have shown efficacy in animal models and promising results in clinical evaluations. Whilst a number of different ligands were described as A2A-receptor antagonists, they can be broadly divided into two families: the xanthine and the non-xanthine, the majority of the latter containing a furan moiety that can be subject to an oxidative metabolism leading to reactive species.

At Domain Therapeutics, we have identified a novel series of imidazopyridine compounds as A2A-receptor ligands. These compounds are completely distinct from the known non-xanthine A2A-receptor antagonists. Their optimization led to compounds with low-nanomolar affinity and efficacy for the A2A-receptor, good selectivity profile and no flag in early ADME-Tox. The best representatives were further evaluated in two rodent models of Parkinson's Disease and showed efficacies after oral administration.

POSTER COMMUNICATIONS – PC.103

A JOURNEY IN PHYSICO-CHEMICAL PROPERTY SPACE, FROM LEAD TO DRUG CANDIDATES: METRICS, CHALLENGES AND PROGRESS IN PEPTIDERGIC GPCRS MODULATORS: OPTIMIZATION OF A V1a ANTAGONIST

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The development of small-molecule ligands of peptidergic receptors (with the exception of opioid receptor ligands) is still considered a challenge by medicinal chemists. The average molecular weight, total polar surface area (TPSA) and cLogP of peptidergic ligands are significantly higher than those of ligands of other targets such as aminergic GPCRs, ion channels and transporters. Since high molecular weight, high TPSA and high cLogP are associated with poor drug-like properties, increased risk of off-target toxicity and poor CNS penetration, small-molecule peptidergic GPCR ligands have suffered from high attrition between the bench and the clinic. It is therefore not surprising that despite significant efforts from the pharmaceutical industry, a relatively small number of peptidergic GPCRs antagonists have been approved for marketing. We will present a range of tools to measure progress along lead optimization, as applied to the specific example of small-molecule, drug-like V1a antagonist.

The talk will include

- A brief outlook of the pharmacology of V1a
- The properties of peptidergic GPCR ligands (in terms of high cLogP, H-Bond donor/acceptor profile, MW) and the challenges associated with these properties, especially regarding absorption, polypharmacology and toxicity.
- The use of in-silico models beyond the "rule-of-five"
- The use of calculated parameters to assess progress within a series (LipE)
- Early results, and several rounds of optimization using library design software among other techniques
- The structure and properties of final compound
- Key learnings

POSTER COMMUNICATIONS – PC.104

NOVEL [1]BENZOTHIENO[3,2-*d*]PYRIMIDIN-4(3*H*)-ONES. LIGANDS FOR THE 5-HT₇ RECEPTOR

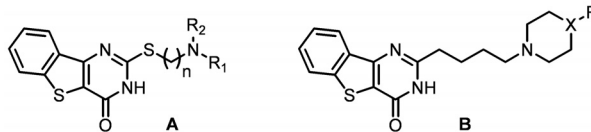
*Giuseppe Romeo*¹, *Valeria Pittalà*¹, *Maria N. Modica*¹,
*Loredana Salerno*¹, *Francesco Guerrera*¹, *Maria A. Siracusa*¹,
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The 5-HT₇ receptor (5-HT₇R) is the last added member of the serotonin (5-HT) receptors family. This receptor, characterized in 1993, belongs to the G protein-coupled receptor family and is positively coupled with adenylyl cyclase. Alternative splicing of the 5-HT₇R gene has been reported to generate different isoforms (5-HT_{7(a), (b), (c)} in rat and 5-HT_{7(a), (b), (d)} in human) which differ only in the length and amino acid composition of their carboxyl-terminal tail. In human, the most abundant isoform is the 5-HT_{7(a)}. These isoforms do not show significant differences in their pharmacological profile, signal transduction or tissue distribution.

Since their discovery, 5-HT₇R has been the subject of intense research due to their widespread distribution in the brain. It has been proposed to be involved in learning and memory, circadian rhythms, locomotor activity, thermoregulation, nociception, as well as in depression, anxiety, psychiatric disorders, epilepsy and some inflammatory processes [1].

In recent years our research group has been involved in the development of selective serotonin receptor ligands [2] and recently our studies have been focused on the design and synthesis of selective 5-HT₇R ligands. In particular, tricyclic [1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-one derivatives bearing an aminoalkylthio side chain in 2-position of the tricyclic system (general formula **A**) had shown affinity for the human cloned 5-HT₇R [3]. We now report the synthesis of a new series of [1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-ones of general formula **B**. These compounds bear in the 2-position of the heterocyclic system an aminobutyl side chain in which the basic moiety is represented by 4-phenyl or 4-benzyl piperazines or piperidines (**B**, X = N, CH). Title compounds were prepared following two different synthetic pathways (each consisting of four steps) starting from methyl 3-aminobenzo[b]thiophene-2-carboxylate. As a general trend, B compounds (having a connecting butyl chain between the tricyclic system and the basic moiety) showed better affinity values for the human cloned 5-HT₇R than those displayed by their corresponding analogues of the A series (with a propylthio chain). Moreover, some of them showed high selectivity over the serotonin 5-HT_{1A} receptor. Complete synthetic pathways to title compounds along with binding data will be reported at the symposium.



REFERENCES

1. Pittalà, V; Salerno, L; Modica, M; Siracusa, M A; Romeo, G. 5-HT₇ receptor ligands: recent developments and potential therapeutic applications. *Mini-Rev. Med. Chem.* 7, 945-960 (2007).
2. Siracusa, M A; Salerno, L; Modica, M; Pittalà, V; Romeo, G; Amato, M E; Nowak, M; Bojarski, A J; Mereghetti, I; Cagnotto, A; Mennini, T. Synthesis of new arylpiperazinylalkylthiobenzimidazole, benzothiazole, or benzoxazole derivatives as potent and selective 5-HT_{1A} serotonin receptor ligands. *J. Med. Chem.* 51, 4529-4538 (2008).
3. Romeo, G; Modica, M; Pittalà, V; Salerno, L; Siracusa, M A; Cagnotto, A; Mennini, T. New Thienopyrimidinone derivatives as potential 5-HT₇ receptor ligands. *XIX National Meeting on Medicinal Chemistry*, 14-18 September 2008, Verona (Italy).

POSTER COMMUNICATIONS – PC.105

DESIGN AND SYNTHESIS OF PROTEIN-PROTEIN INTERACTION MIMICS AS *PLASMODIUM FALCIPARUM* CYSTEINE PROTEASE FALCIPAIN-2 INHIBITORS

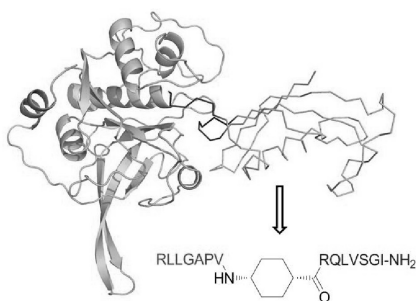
Sergio Romeo^{1,3}, *Luca Rizzi*¹, *Srividhya Sundararaman*²,
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Cysteine proteases play a significant role in the growth and the development of several human parasites such as *Plasmodium*, *Leishmania*, and *Trypanosoma* which affect more than 3.5 billion people worldwide. Falcipain-2 (FP-2) is an important cysteine protease of the human malaria parasite, *P. falciparum* (Pf), and is one of the most promising target for the development of new Pf inhibitors.¹ FP-2 plays roles in haemoglobin degradation and merozoites egression at the asexual blood stages of *Plasmodium* development. Endogenous cysteine protease inhibitors have been described in a number of eukaryotic systems and they represent an important platform for new drug design. Cystatin and its homologues are natural inhibitors of Papain and others cysteine proteases, but the high molecular weight and the peptidic structure raise difficulties in their exploitation in the drug design process. In the present study, we designed a series of small peptides that mimic the protein-protein interaction between falcipain-2 and Chicken Egg White Cystatin and analyzed their effects on falcipain-2 activity.

These peptide mimics were designed keeping in mind the distance of about 4-5 Å between the carbonyl of Pro¹¹ or Val¹² of cystatin. Three different linkers, γ -aminobutyric acid (GABA), *cis*-4-aminocyclohexane carboxylic acid and a macrocycle formed by GABA and two cysteines joined by a disulfide bridge were used to link peptides. Some of these compounds showed considerable inhibition ($\geq 50\%$) at 5 μM (K_i 2-7.5 μM). When these peptide mimics were tested against the mature parasite, they also produced morphological abnormalities especially in the food vacuole, similar to what have been observed with others cysteine protease inhibitors. We believe this approach could be an excellent starting point for the development of new cysteine protease inhibitors and in general for the development of new drugs against *P. falciparum*.



REFERENCES

1. Sijwali, P. S.; Rosenthal, P. J. Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (13), 4384-4389.

POSTER COMMUNICATIONS – PC.106

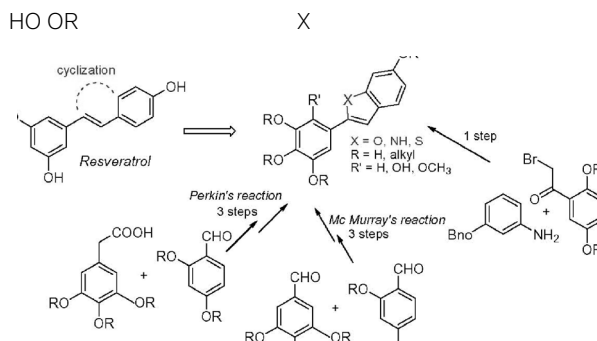
ANTITUMOR AGENTS. SYNTHESIS AND CANCER CELL GROWTH INHIBITION OF NEW RESVERATROL DERIVATIVES

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Pharmaceutical studies suggested that the polyphenol resveratrol (*trans*-3,4',5-trihydroxystilbene) is one of the main wine grape components that protect from vascular and neurodegenerative diseases and cancers. The ability of resveratrol to prevent the occurrence of carcinomas was related to the inhibition of tumor cell cycle and induction of tumor cell death.¹⁻² We focused on the synthesis of new structural resveratrol analogues obtained by cyclization of stilbene (Scheme 1). The double bond was replaced by an unsaturated ring. This modification should allow us to assess the importance of the double bond of natural product.

Scheme 1 OR



OR Three different synthetic pathways for the preparation of the resveratrol analogues have been proposed. A series of these analogues was synthesized via McMurry reaction between two substituted aldehydes or by condensation of the substituted arylacetic acid with the corresponding aldehyde under Perkin's conditions. An analog containing a nitrogen in the aromatic ring was synthesized in one step from the substituted aniline and the corresponding 2-bromo-1-(2,5dimethoxyphenyl) ethanone via Bischler reaction. The dimethoxy compounds were deprotected using BBr₃ giving the desired compounds in high overall yields. The synthesis and biological data will be reported.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Ministry of Science and Technology (CTQ2007-60614/BQU) for the financial support.

REFERENCES

1. G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J. M. Schmidt, F. Hogan, *J. Med. Chem.* **1995**, *38*, 1666-1672.
2. G. R. Pettit, A. Thornhill, N. Melody, J. C. Knight. *J. Nat. Prod.* **2009**, *72*, 380-388.

POSTER COMMUNICATIONS – PC.107

MUTUALLY COMPLEMENTARY LIGAND AND STRUCTURE-BASED COMPUTATIONAL STUDIES ON A NOVEL SERIES OF POTENT AND SELECTIVE A₃ ADENOSINE RECEPTOR ANTAGONISTS

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Adenosine receptors (ARs) are a family of proteins belonging to the superfamily of G-Protein Coupled Receptors (GPCRs), which is the main pharmacological target nowadays. ARs are divided into 4 subtypes (A_1 , A_{2A} , A_{2B} and A_3), and are considered potential drug targets in disorders such as schizophrenia, Parkinson, asthma or cardiovascular diseases. [1] We have recently reported the synthesis of a diarylpyrimidine library as selective A_3 AR antagonists. [2] In a further step, we have grown and experimentally tested against the four human AR subtypes a collection of 150 members of these chemical libraries. [3]

In the present work we determine the binding mode of the family of diarylpyrimidine compounds, and establish structure-activity relationships, all of which will be used as a guide the design of the new libraries herein described. On the basis of the generated model, we also pursue to obtain structural information of the ARs that may account for both for potency and selectivity of AR antagonists.

A homology model of A_3 AR receptor was built with MODELLER using the recently crystallized A_{2A} structure as template. We then carried out an automated docking exploration with the set of 63 compounds for which an experimental value of K_i was obtained on the A_3 AR, with the software GOLD v3.2. This protocol was based on our experience on the GPCR Dock 2008 competition. [4] The molecular alignment generated on the docking step was used as input for a 3D-QSAR study, by means of the second generation of Grid-INdependent Descriptors (GRIND-2), [5] with the software PENTACLE v10.4.

Our systematic docking exploration consistently identifies a conserved binding mode for the series of 2 and 4-amidopyrimidines. The main anchoring point is a double hydrogen bond between the ligand and residue Asn6.55, together with a π -stacking interaction with Phe5.29. This binding mode is consistent with that experimentally observed for the A_{2A} antagonist ZM241385. Regarding the 3D-QSAR study, we obtained a model using the ligands aligned by their docking poses as input, yielding statistical values of good quality ($r^2=0.88$, $q^2=0.70$). With this model we can relate the size and disposition of the different substituents of the ligands, with their location and interactions within receptor sub-sites. Importantly, the conservation of the binding site residues, and their role in affinity and selectivity of antagonists binding is addressed in this work.

REFERENCES

1. Jacobson K.A. et al. Nat. Rev. Drug Discov. 2006; 5(3):247-64
2. Yaziji V. et al. J. Comb. Chem. 2009; 11:519-522
3. Manuscript in preparation.
4. Michino M. et al. Nat. Rev. Drug Discov. 2009; 8(6):455-63
5. Durán A. et al. J. Chem. Inf. Mod. 2009; 49: 2129-38

POSTER COMMUNICATIONS – PC.108

POSITIVE ALLOSTERIC MODULATION OF THE mGluR5 RECEPTOR: SAR, SPR AND IN VIVO ACTIVITY OF ADX47273 DERIVATIVES

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Glutamate is the major excitatory neurotransmitter in the brain. It mediates its actions via the activation of both ligand gated ion channels (iGluRs) and a family of class C G-Protein Coupled Receptors known as metabotropic Glutamate Receptors (mGluRs). mGluRs play important roles in various neurological disorders.

The allosteric binding sites are topographically distinct and are less conserved within the 8 mGluR subtypes compared to the glutamate orthosteric binding site. Consequently, allosteric ligands of mGluR₁₋₈ have higher pharmacophore diversity than orthosteric ligands. Also, allosteric modulators of mGluR₁₋₈ have been shown to offer a valid strategy to develop non amino-acid like therapeutics that can be administered orally and that readily cross the blood-brain barrier. In addition, positive allosteric modulators (PAMs) are unlikely to induce a rapid desensitization such as the currently available agonists.

mGluR₅ receptors are located mainly on post-synaptic elements in the brain (cortex, thalamus, hippocampus and cerebellum), from where they regulate the activity of NMDA & AMPA, as well as the excitability of the post-synaptic neurons.

The aryl-oxadiazole ADX47273 has been the topic of intense interest for the pharmacological evaluation of the mGluR₅ PAM approach; this compound demonstrated promising antipsychotic activity in preclinical rodent models for positive symptoms of schizophrenia and it was also active in cognition models. ADX47273 has opened an avenue to a novel class of drug-like molecules in this field. We will present the multiparametric lead optimization program which led to the identification of several chemical series of highly selective mGluR₅ receptor PAM. Compared to ADX47273 which suffered of a poor bioavailability; the new molecules are systematically active in rodent behavioral models.

POSTER COMMUNICATIONS – PC.109

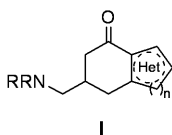
SYNTHESIS, BINDING AFFINITY AND SAR OF NEW HETEROCYCLIC ANALOGUES OF HALOPERIDOL AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

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The newer generation of treatments for schizophrenia, referred to as atypical antipsychotics, add to the blockade of dopamine receptors, a potent activity at serotonin receptors. It is thought that 5-HT_{2A} antagonism together with relatively weaker dopamine antagonism are principal features that differentiate the side-effect profile of atypical antipsychotics, such as clozapine, from the first generation of treatments [1]. Although the novel atypical antipsychotics olanzapine, risperidone and quetiapine have brought about improvements in toleration and negative symptomatology, chronic treatment may lead to unwanted weight gain, blood dyscrasias, and motor dysfunctions, such as extra-pyramidal side effects (EPS) and tardive dyskinesia (TD). These side effects may be linked to drug-dependent affinity for other receptors. The search in our group continues for new atypical antipsychotics that would be more efficacious and would have fewer side effects than currently available treatments [2].

Since antidopaminergic therapies continue in the first line of schizophrenia's treatment, in this communication, we will describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D₂ antagonists for potential use as treatments for schizophrenia. In these compounds a typical bioisosteric replacement of the benzene in the aminobutyrophe none pharmacophore by a five- or six-membered heterocycle (I) containing at least one heteroatom has been applied.



ACKNOWLEDGMENTS

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REFERENCES

1. Meltzer, H. Y.; Matsubara, S.; Lee, J. J. *Pharm. Exp. Ther.* **1989**, 251, 238.
2. (a) Dezi, C.; Brea, J.; Alvarado, M.; Raviña, E.; Masaguer, C. F.; Loza, M. I.; Sanz, F.; Pastor, M. J. *Med. Chem.* **2007**, 50, 3242; (b) Aranda,

R.; Villalba, K.; Raviña, E.; Masaguer, C. F. Brea, J.; Areias, F.; Dominguez, E.; Selent, J.; Lopez, L.; Sanz, F.; Pastor, M.; Loza, M. I. *J. Med. Chem.* **2008**, 51, 6085; (c) Carro, L.; Raviña, E.; Domínguez, E.; Brea, J.; Loza, M.I.; Masaguer, C.F. *Bioorg. Med. Chem. Lett.* **2009**, 19, 6059

POSTER COMMUNICATIONS – PC.111

NATURAL PRODUCT FRAGMENTS AS A NOVEL DIVERSITY SET FOR FRAGMENT-BASED SCREENING

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Our objective is to provide a number of interconnecting approaches to allow natural products to be a competitive source of new leads in modern drug discovery.

We use these new approaches to address the timelines for mining natural products for pharmacologically interesting compounds. In recent years we have -

1. published our work in developing a drug-like natural product library with properties that comply with Lipinski rules for orally active drugs. This demonstrates that many natural products display the physicochemical properties required in orally acting pharmaceuticals.¹
2. demonstrated, using X-ray crystal structure data, that a similarity exists between the recognition of compounds to three biosynthetic enzymes in the flavonoid biosynthetic pathway and the recognition of protein kinases. This demonstrates natural products retain an imprint of biology space and explains why many natural products recognise the ligand binding domain in many therapeutically important proteins.^{2,3}
3. demonstrated that it is possible to take a complex natural product extract and identify those constituents that bind reversibly to proteins using a FTMS method. We have subsequently isolated the active constituent using mass directed fractionation, demonstrated that the compound has functional activity and obtained an X-ray structure of the complex. This mass spectrometry based approach can be used to discover naturally occurring chemicals that bind to native unmodified proteins.⁴

We now present a chemo-informatics study that shows we can capture the diversity of natural products by using naturally occurring chemical fragments. Results from fragment-based drug discovery based on natural product fragments will be presented.

In combination these technological approaches contribute to addressing druggable natural products within normal lead identification timelines.

REFERENCES

1. Quinn, R. J.; Carroll, A. R.; Pham, N. B.; Baron, P.; Palframan, M. E.; Suraweera, L.; Pierens, G. K.; Muresan, S. Developing a drug-like natural product library. *J. Nat. Prod.* **2008**, *71*, 464-468.
2. McArdle, B. M.; Campitelli, M. R.; Quinn, R. J. A Common Protein Fold Topology Shared by Flavonoid Biosynthetic Enzymes and Therapeutic Targets. *J. Nat. Prod.* **2006**, *69*, 14-17.
3. Maresca, A.; Temperini, C.; Vu, H.; Pham, N. B.; Poulsen, S.-A.; Scozzafava, A.; Quinn, R. J.; Supuran, C. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide Inhibitors. *J. Am. Chem. Soc.* **2009**, *131*, 3057-3062.
4. Vu, H.; Pham, N. B.; Quinn, R. J. Direct screening of natural product extracts using mass spectrometry *J. Biomol. Screen.* **2008**, *13*, 265-275.

POSTER COMMUNICATIONS – PC.112

SYNTHESIS AND VASORELAXANT ACTIVITY OF PHTHALAZINONES

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Hypertension is one of the most common cardiovascular diseases that can cause coronary disease, myocardial infarction, stroke and sudden death and is the major contributor to cardiac failure and renal insufficiency. [1]

Within the drugs in the market, hydralazine, one of the first anti-hypertensive agent, has attracted much attention in the last decade and it is newly considered as a lead for developing new drugs, due to its direct vasodilator action. [2]

Structural modification of hydralazine led to the discovery of some pyridazinones and other phthalazine derivatives with broad spectra on the cardiovascular system, including antihypertensive effects and inhibition of platelet aggregation and of phosphodiesterases. [3][4]

A series of N2-substituted phthalazine-1,4-dione derivatives has been synthesized through condensation of substituted phthalic anhydride either with hydrazine or with substituted hydrazines or through N-alkylation of phthalazine-1,4-dione derivatives previously synthesized. [4]

The vasorelaxant activity of these compounds was evaluated on isolated rat aorta rings either with or without endothelium, previously stimulated with phenylephrine. [5] Some of the synthesized compounds have been characterized as agents with clear vasorelaxant effects and therefore with a promising future as antihypertensive agents.

REFERENCES

1. Oates, J. A. Antihypertensive Agents and the Drug Therapy of Hypertension. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th ed.; Pergamon Press, **2001**; Vol.780, Section V.33.
2. Florez, J.; Armijo, J. A.; Mediavilla A. *Farmacología Humana*, 3rd ed.; Masson S.a., Barcelona, 2000; Vol. 671.
3. Heinisch, G.; Kopelent-Frank, H. *Prog. Med. Chem.* **1992**, *29*, 141.
4. del Olmo, E.; Barboza, B.; Ybarra M. I.; López-Pérez, J. L.; Carrón, R.; Sevilla M. A.; Boselli, C.; San Feliciano, A. *Bioorg. & Med. Chem. Lett.* **2006**, 2786.
5. Quezada E., Delogu G., Picciau C., Santana L., Podda G., Borges F., García-Morales V., Viña D., Orallo F. *Molecules* **2010**, *15* (1), 270-9.

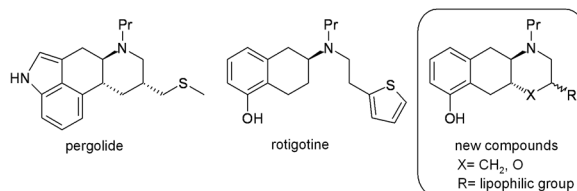
POSTER COMMUNICATIONS – PC.113

SYNTHESIS, X-RAY CRYSTALLOGRAPHY AND IN VITRO CHARACTERIZATION OF NOVEL DOPAMINE RECEPTOR AGONISTS

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Pergolide and rotigotine are used clinically for the treatment of Parkinson's disease. Rotigotine contains a structural alert in the form of the thiophene ring, while the ergot alkaloid-derived dopamine receptor agonists pergolide and cabergolide have been associated with severe multivalvular heart disease.¹ Computational analysis of the presumed binding conformations of rotigotine and pergolide suggested to us that the methyl thioether and thiophene ring of the two drugs occupy the same space in the dopamine receptor. Herein we report the synthesis and structure-activity relationships at dopamine D1 and D2 receptors of a 'hybrid' series in which conformationally restricted rotigotine scaffolds have been combined with R side chains, which were chosen in part based on previously reported pergolide analogs.² The compounds (R = lipophilic group) were prepared by a novel method using (2R, 3R)-8-Methoxy-3-propylamino-1,2,3,4-tetrahydro-naphthalen-2-ol and (R)-(-)-Epichlorohydrin or (S)-(+)-Epichlorohydrin, or (R = H) from an intermediate described by Nozulak *et al.*³ The absolute conformations of these novel compounds were elucidated by X-ray crystallography.



REFERENCES

- Horvath, J.; Fross, R.D.; Kleiner-Fisman, G.; Lerch, R.; Stalder, H.; Liaudat, S.; Raskoff, W.J.; Flachsbarth, K.D.; Rakowski, H.; Pache, J.-P.; Burkhard, P.R.; Lang, A.E. *Movement Disorders* (2004), **19**, 656-662. Pergolide was withdrawn from the US market in 2007 by due to the possibility of heart valve damage.
- Ohno, S.; Koumori, M.; Adachi, Y.; Mizukoshi, K.; Nagasaka, M.; Ichihara, K. *Chem. Pharm. Bull.* (1994), **42**(10), 2042-2048.
- Nozulak, J.; Vigouret, J. M.; Jaton, A. L.; Hofmann, A.; Dravid, A. R.; Weber, H. P.; Kalkman, H. O.; Walkinshaw, M. D. *J. Med. Chem.* (1992) **35**, 480-489.

POSTER COMMUNICATIONS – PC.115

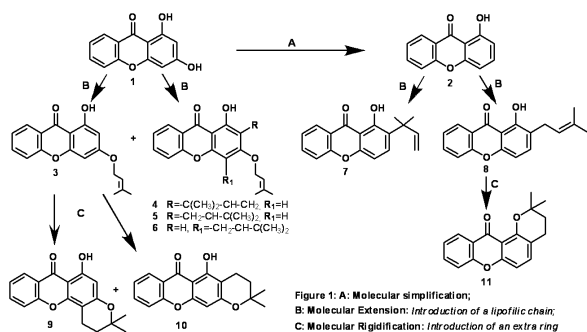
MOLECULAR MODIFICATIONS ON XANTHONIC SCAFFOLD: STRUCTURE-ACTIVITY RELATIONSHIP APPROACHES

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Xanthonic compounds show interesting biological activities associated with their tricyclic scaffold depending on the nature and/or position of the different substituents¹⁻². A relationship between activity and the presence of prenyl groups in key positions on the xanthone nucleus was associated with some biological activities, such as antibacterial, PKC modulation and antitumor¹.

Therefore, prenylated xanthenes could represent an excellent model for the development of new and more effective drugs, particularly in the improvement of antitumor activity, and for this reason the introduction of prenyl groups in "hit" compounds, has been one of the strategies used in our research group³⁻⁴. Based on previous results concerning the effect of several xanthonic derivatives on the *in vitro* growth of some human tumor cell lines³, we have selected 1,3-dihydroxyxanthone (**1**), as starting material to carry out some molecular modifications in order to improve its antitumor activity. The strategy used is showed in **Figure 1**:



Compounds **3-10** were evaluated for their effect on the *in vitro* growth of human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung) and SF-268 (central nervous system). Compound **11** is under study. Xanthenes **4** and **5** exhibited a selective and potent growth inhibitory effect against the MCF-7 cell line, comparing with respective building block **1**. On the other hand, it was observed for compounds **7** and **8**, the appearance of a moderate effect on the activity against the MCF-7 cell line, when compared with their synthetic precursor **2**. Considering prenylated xanthenes **4, 5, 7** and **8**, all with a prenyl group at C2 position of the xanthone scaffold, it was found that compounds **4** and **5** were significantly more active than **7** and **8**, which may indicate that the existence of an additional prenyloxy group at C3 in xanthenes **4** and **5**, could be an important feature for this activity.

ACKNOWLEDGMENTS

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REFERENCES

- Pinto, M., Castanheiro, R., "Natural Prenylated Xanthenes: Chemistry and Biological Activities", Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, Índia, **2009**, Chap. 17, pp.520-676.
- Pinto, M., *et al. Curr. Med. Chem.* **2005**, **12**, 2517.
- Castanheiro, R., *et al. Bioorg. Med. Chem.* **2007**, **15**, 6080.
- Castanheiro, R., *et al. Pharmaceuticals* **2009**, **2**(2), 33.

POSTER COMMUNICATIONS – PC.116

ANTIMITOTIC AGENTS BEARING PYRIDINE N-OXIDE MOIETIES

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Antimitotic agents act by binding to tubulin and thus altering its normal polymerisation / depolymerisation dynamics. This causes inhibition of mitotic spindle formation, which leads to cell cycle arrest and, subsequently, to cell death. Combretastatins and phenstatins are potent antimitotic agents that bind to tubulin at the colchicine site¹. Several combretastatins have entered phase II/III clinical trials as their prodrugs (Fig 1).

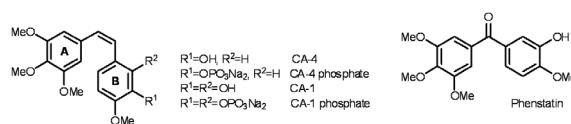


Fig 1. Structure of combretastatin A-4, combretastatin A-1, their prodrugs and phenstatin.

Several modifications have been carried out on the basic structure of combretastatins and phenstatins,² most of them addressed at the so-called B ring and at the bridge between both aromatic systems. Modifications of the A ring (3,4,5-trimethoxyphenyl) are much scarcer, and usually lead to compounds displaying decreased potency.

Recently, we proposed the replacement of ring A by 2,6-dimethoxy-4-pyridyl and its *N*-oxide,³ in order to investigate whether this system can effectively replace such A ring and thus produce potent antitubulin compounds (Fig 2). The *N*-oxide Oxygen could replace the 4-methoxy group of the model compounds and thus contribute to the binding to tubulin by hydrogen bonding, as it has been proposed as binding mode for these agents.

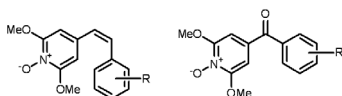


Fig 2. Structure pyridine *N*-oxide analogues of combretastatins and phenstatins.

Oxidation of pyridines to their *N*-oxides is usually described as an easy process, using for example MCPBA. However, for 2,5-disubstituted pyridines with electron-withdrawing groups such oxidation is more difficult to perform. A variety of reagents and conditions failed, although finally the desired pyridine *N*-oxides were obtained. Preliminary activity results indicate that these analogues are less potent than the parent compounds. Synthetic and biological results will be described in detail.

ACKNOWLEDGMENTS

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REFERENCES

1. (a) Lin, C.M., Ho, H.H., Pettit, G.R., Hamel, E. *Biochemistry* **1989**, *28*, 6984. (b) Pettit, G.R., Toki, B., Herald, D.L., Verdier-Pinard, P. Boyd, M.R., Hamel, E., Pettit, R.K. *J. Med. Chem.* **1998**, *41*, 1688.
2. Tron, G.C., Pirali, T., Sorba, G., Pagliai, F., Busacca, S., Genazzani, A.A. *J. Med. Chem.* **2006**, *49*, 3033.
3. Lozano, M. Aramburu, L. Degree Dissertations. University of Salamanca, 2007, 2009.

According to the World Health Organization, about 1.5-2% of the world's population living with epilepsy or will experience it at some point in their life span¹. About (20-30%) of epilepsy patients are resistant to the currently available therapeutic agents. More or less 70% of patients with epilepsy are well controlled by monotherapy with currently available antiepileptic drugs, and another 5-10% of patients could be stabilized by add-on therapies. In the remaining 20% of patients seizures are not efficiently controlled². A series of novel schiff bases of 3-aminomethyl pyridine were synthesized by condensation reaction with substituted aryl aldehydes/ ketones and / cyclic ketones. These schiff bases were screened for anticonvulsant activity³⁻⁵. The chemical structures of synthesized compounds were confirmed by FT-IR, ¹H-NMR, spectra and elemental analysis. A number of compounds were observed to exhibit protection after intraperitoneal administration at the dose 30 & 100mg kg⁻¹ in various models employed. In MES screen five compounds (1_c) N-(2-chlorobenzylidene) (pyridin-3-yl) methanamine, (1_f) 2-methoxy-4-[(pyridine-3-ylmethylimino)methyl]phenol, (2_a) N-(3-phenylallylidene) (pyridin-3-yl) methanamine, (3_b) 3-[1-(pyridin-3-ylmethylimino) ethyl]benzenamine, and (4_a) N-(diphenyl-methylene) (pyridin-3-yl) methanamine, emerged out as most active compounds in series (ED₅₀) 11.70, 6.39, 11.70, 8.64 and 9.13mg kg⁻¹ with high protective index (PI) > 10. Whereas four compounds (1_e) 4-[(Pyridine-3-ylmethylimino) methyl]benzene-1,3-diol, (1_g) N-(3,4-dimethoxybenzylidene) (pyridin-3-yl)methanamine, (2_b) N-[(1H-indol-3-yl)methylene] (pyridin-3-yl) methanamine, and (5) N-cyclohexylidene (pyridine-3-yl) methanamine showed remarkable protection over clinically used drugs in Sc.-PTZ screen (ED₅₀) 6.44, 11.70, 6.47, and 14.16 with (PI > 10). Compound (4_b) showed good anticonvulsant activity (ED₅₀) 20.79 with higher neurotoxicity (PI = 0.57). Compound (1_e) was active in both Sc.-PTZ and in Sc.-STR seizures. Some selected compounds were subjected to oral MES screen (30mg kg⁻¹) in rats, most of the compounds showed peak activity after 0.5h of oral administration. The new series of schiff bases can significantly be used for future development of novel anticonvulsant agent.

REFERENCES

1. Brown TR, Holmes GL. Epilapsy *N. Engl. J. Med.*; 344:1145-1151, (2001).
2. Swann, A.C. *J. Clin. Psychiatry*, 62(14), 16-21, (2001).
3. Krall R. L., Penry J. K., White B. G., Kupferberg H. J., Swinyard E. A., *Epilepsia*, 19, 409-428, (1978).
4. [20] Kupferberg H. J., *Epilepsia*, 30, 51-56, (1989).
5. [21] Dunham M. S., Miya T. A., *Amer. J. Pharm. Ass. Sci.*, 46, 208, (1957).

POSTER COMMUNICATIONS – PC.118

SOME NOVEL HETEROCYCLIC SCHIFF BASES: AS POTENTIAL ANTICONVULSANT AGENTS

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POSTER COMMUNICATIONS – PC.120

NON-COVALENT MASS SPECTROMETRY :
A POWERFUL, INNOVATIVE TOOL FOR EARLY-STAGE DRUG DISCOVERY

Jean-Paul Renaud

NovAliX. Bioparc, boulevard Sébastien Brant, 67400 Illkirch, France.
www.novalix-pharma.com

Non-covalent mass spectrometry is emerging as a powerful tool that can be used at different stages of early-stage drug discovery: (i) as an efficient secondary screening to remove false positives from a primary high-throughput screening or a virtual screening by assessing the physical binding of the primary hits to the target, and (ii) as a high-content technique for the detailed characterization of ligand-target interaction in terms of stoichiometry, reversibility, specificity, and affinity. Collision-induced dissociation experiments can serve to evaluate the enthalpic part of the binding energy, and thus to guide the hit-to-lead process.

This label-free technique consumes low protein amounts, it can be automated and it is suitable for fragment-based drug discovery. The use of non-covalent mass spectrometry in ligand screening and drug design will be illustrated by literature and internal examples.

REFERENCES

1. Vivat et al. (2010) "Native MS: an 'ESI' way to support structure- and fragment-based drug discovery" *Future Med. Chem.* 1 : 35-50
2. Renaud and Delsuc (2009) "Biophysical techniques for ligand screening and drug design" *Curr. Op. Pharmacol.* 9 : 622-628

POSTER COMMUNICATIONS – PC.121

5-BENZYLIDENERHODANINE AND
5-BENZYLIDENETHIAZOLIDINE-2,4-DIONE-
BASED INHIBITORS OF MurD LIGASE

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The escalating rate of bacterial resistance to currently available antibiotics is a well-known problem, and the need for novel anti-

bacterial drugs is increasingly important clinically. Peptidoglycan is an essential bacterial cell-wall polymer, unique to prokaryotic cells and therefore optimal for the selective targeting of microbial vital pathways. The formation of a cytoplasmic peptidoglycan precursor is catalysed by a group of enzymes, called Mur ligases (MurC-MurF), that catalyze a series of reactions, starting from UDP-*N*-acetylmuramic acid (UDP-MurNAc), to yield UDP-*N*-acetylmuramoyl-pentapeptide (UDP-MurNAc-pentapeptide) by sequentially adding L-Ala (MurC), D-Glu (MurD), meso-diaminopimelic acid or L-Lys (MurE), and the D-Ala-D-Ala dipeptide (MurF).^{1,2,3}

We synthesized and biologically evaluated a series of glutamic acid- and 5-benzylidenerhodanine- or 5-benzylidenethiazolidine-2,4-dione-based derivatives as inhibitors of MurD ligase. The series was designed based on the 2,4-diaminoquinazoline-containing highest ranked compound from virtual screening, performed with FlexX docking program using ZINC database.^{4,5} The best compounds were found to inhibit MurD from *E. coli* with IC₅₀ values in the range of 45 to 206 μM. Details of the binding mode of derivatives within the active site were established with three high-resolution cocrystal structures. In the MurD-inhibitor complex, the D-Glu moiety occupies the same site as the D-Glu residue of the product UMAG of the enzymatic reaction catalyzed by MurD. Rhodanine or thiazolidine-2,4-dione rings of the inhibitor occupy the uracyl-binding pocket. The ring nitrogen forms a hydrogen bond with the O^γ of Thr36, and the carbonyl oxygen at position 2 interacts with the Thr36 backbone NH group. NH Group on the *para*-position of benzylidene ring interacts with the enzyme through active site crystal water molecules. The details of the binding mode of compounds in the MurD active site will help to understand the structure-activity relationships and provide a good foundation for structure-based design of a novel generation of MurD inhibitors.

REFERENCES

1. Barreateau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* **2008**, 32, 168-207.
2. Kotnik, M.; Štefanič Anderluh, P.; Preželj, A. *Curr. Pharm. Design* **2007**, 13, 2283-2309.
3. Vollmer, W.; Blanot, D.; de Pedro, M. A. *FEMS Microbiol. Rev.*, **2008**, 32, 149-167.
4. Tomašić, T.; Zidar, N.; Rupnik, V.; Kovač, A.; Blanot, D.; Gobec, S.; Kikelj, D.; Peterlin Mašič, L. *Bioorg. Med. Chem. Lett.* **2009**, 19, 153-157.
5. Zidar, N.; Tomašić, T.; Šink, R.; Rupnik, V.; Kovač, A.; Turk, S.; Patin, D.; Blanot, D.; Contreras Martel, C.; Dessen, A.; Zega, A.; Gobec, S.; Peterlin Mašič, L.; Kikelj, D. *Submitted*

POSTER COMMUNICATIONS – PC.122

IMPROVING ENRICHMENT AND HIT RATE IN VIRTUAL SCREENING

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In the drug discovery process, accurate methods of computing the affinity of small molecules with a desired biological target are strongly needed. Even if, in the last years, the accuracy and efficiency of the available virtual screening algorithms have been improved, many drawbacks and limitations still exist. For example, docking methods lack a reliable simulation of both ligand and receptor flexibilities, as well as good scoring functions able to estimate ligand binding energies in reasonable agreement with experimental data. These limitations often lead to a high level of false positives or false negatives in the hit list. For that reason, it is generally agreed that docking results need to be post-processed with more accurate tools.

To this end, we developed Binding Estimation After Refinement (BEAR), a new and automated post-docking procedure for the conformational refinement of docking poses through molecular dynamics (MD) followed by accurate prediction of binding free energies using MM-PBSA and MM-GBSA¹ (Figure1).

The BEAR performance in virtual screening was evaluated on several macromolecular targets and related sets of known ligands, determining the enrichment factors and assessing the correlation between predicted and experimental binding affinities. These analyses suggested critical improvements with respect to standard docking softwares^{2,3}.

Moreover, when applied in virtual screening campaigns, BEAR was able to discover novel and potent inhibitors of Plasmodium falciparum plasmepsin II with an impressive hit rate⁴, and has been successful in identifying promising scaffolds for the design of irreversible protein kinase inhibitors⁵. Therefore, taken as a whole, the results obtained so far prospect that BEAR may become a prominent tool in the drug discovery pipeline.

The BEAR virtual screening procedure is reliable and strongly automated, and can be tailored to the needs of the end-user in terms of computational time and the desired accuracy of the results. BEAR is under constant development and validation on additional biological targets in order to further improve accuracy, automation and calculation speed.

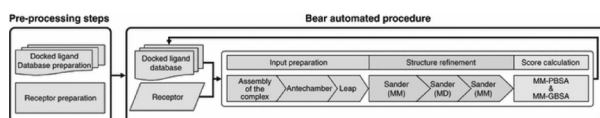


Figure 1 : The BEAR workflow

REFERENCES

1. Rastelli G, Degliesposti G, Del Rio A, Sgobba M (2009) *Chem Biol Drug Des* 73:283–28
2. Ferrari AM, Degliesposti G, Sgobba M, Rastelli G (2007) *Bioorg Med Chem* 15:7865–787
3. Rastelli G, Del Rio A, Degliesposti G, Sgobba M (2010) *J Comput Chem* 31:797–81
4. Degliesposti G, Kasam V, Da Costa A, Kang HK, Kim N, Kim DW, Breton V, Kim D, Rastelli G. (2009) *ChemMedChem* 7:1164–73.
5. Del Rio A, Sgobba M, Parenti MD, Degliesposti G, Forestiero R, Percivalle C, Conte PF, Freccero M, Rastelli G. (2010) *J Comput Aided Mol Des*. Doi: 10.1007/s10822-010-9324-x.

POSTER COMMUNICATIONS – PC.123

SYNTHESIS OF MYCOBACTERIUM TUBERCULOSIS DXR INHIBITORS BY PALLADIUM(II)-CATALYZED HECK ARYLATION OF ACROLEIN

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Tuberculosis remains one of the big infectious diseases around the world. Furthermore, the occurrence of multidrug resistant and extensively drug resistant *Mycobacterium tuberculosis* (*Mtb*) strains makes it even more urgent to develop new drugs to treat tuberculosis infected patients. The enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is involved in the second step of the non-mevalonate pathway for the synthesis of isoprenoids. This biochemical pathway is found in many bacteria and parasites, but in humans isoprenoids are synthesized by the mevalonate pathway. Thus, DXR is a very promising antibacterial drug target.

Fosmidomycin (3-(*N*-hydroxyfomamido)propylphosphonic acid) is known to inhibit *Mtb* DXR with an IC₅₀ of 80 nM. However, this compound is not active at the *Mtb* whole cell level, possibly due to the lack of uptake. In the search for more potent and cell wall permeable compounds for the inhibition of *Mtb* DXR, we have synthesized analogues of fosmidomycin by the introduction of an aromatic substituent in the 1-position of the propylphosphonic acid scaffold. The use of an oxidative Heck reaction facilitated the selective preparation of the essential terminally arylated starting materials directly from acrolein and a number of selected boronic acids. Scope and limitations of the reaction will be discussed as well as the biological activity and suggested binding mode of the target compounds.

POSTER COMMUNICATIONS – PC.124

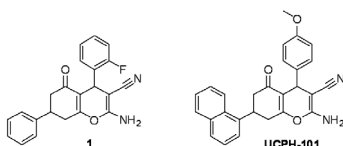
DISCOVERY OF UCPH-101 - STRUCTURE-ACTIVITY RELATIONSHIPS FOR NOVEL CLASS OF EAAT1 INHIBITORS

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L-Glutamate (Glu) is believed to play a key role in a range of neurodegenerative diseases, and psychiatric disorders. Therefore the glutamatergic neurotransmitter system is a highly attractive target from a therapeutic perspective. The excitatory amino acid transporters (EAATs) are transmembrane proteins responsible for the uptake of Glu from the synaptic cleft, thereby terminating the glutamatergic neurotransmitter signal. To date five human subtypes have been identified, termed EAAT1-EAAT5. We believe that valuable information about the physiological functions and thus therapeutic potentials of the specific EAAT subtypes may be addressed by studying the actions of selective ligands. (1,2)

A small commercially available compound library consisting of 3040 compounds was screened for inhibitory activity at EAAT1, EAAT2- and EAAT3-expressing HEK293 cell lines in a [^{3} H]-D-Asp uptake assay.(3) Interestingly, we found **1** to be a selective inhibitor at the EAAT1 (IC_{50} : 4.7 μ M). The discovery of **1** prompted us to commence building a SAR for this class of EAAT1 inhibitors. Analogues which bear one aromatic substituent in both the 4- and 7-position show differential inhibitory potencies at EAAT1 with **UCPH-101** being the most potent inhibitor (IC_{50} : 0.66 μ M). (2)



Furthermore analogues with varying substituent (-H, -COOH, -COOMe, -COOEt, -COO^tBu, -CONH₂) instead of the cyano group have been synthesized. We found that the cyano group is of great importance in retaining the inhibitory activity for this class of EAAT1 inhibitors as none of these analogues showed any inhibitory activity at EAAT1-3. Analogues with variations addressing the importance of the amino group have also been synthesized.

REFERENCES

1. Bunch L, Erichsen MN, Jensen AA, *Expert Opin. Ther. Targets* 2009, 13(6), 719-731
2. Jensen AA, Erichsen MN, Nielsen CW, Stensbøl TB, Kehler J, Bunch L, *J. Med. Chem.* 2009, 53, 912-915
3. Jensen AA, Bräuner-Osborne H, *Biochem. Pharmacol.* 2004, 67, 2115-2127

POSTER COMMUNICATIONS – PC.125

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL ANALOGUES OF OLANZAPINE

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Olanzapine is an atypical antipsychotic and currently one of the most effective treatments to date being able to reduce the symptoms of schizophrenia, whilst also having reduced propensity to cause adverse side effects. Its therapeutic effect arises from its mixed D₂/5-HT_{2A} receptor antagonism, which acts to stabilise the dopamine imbalance commonly observed in sufferers of schizophrenia.¹ Olanzapine, however, has strong binding affinity for a number of other receptors including muscarinic (M₁), histaminergic (H₁) and adrenergic (α_1), which contribute to its side effect profile. There still remains a need for new antipsychotic treatments with improved efficacy as well as reduced side effect potential.

Using olanzapine as a starting point, a series of analogues were synthesised based on a pharmacophore model of currently available antipsychotics (Figure 1).² The analogues comprise various aromatic functionalities linked to the olanzapine core by diverse aliphatic linkers. The pharmacological potential of the analogues were determined through *in vitro* receptor binding assays, with promising analogues further screened in *in vivo* animal models for schizophrenia.

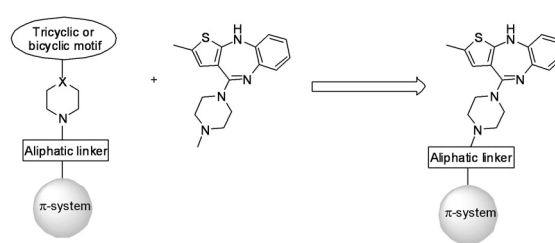


Figure 1. Olanzapine analogues based on a proposed antipsychotic pharmacophore model.

REFERENCES

1. Littrell, K. H.; Petty, R. G.; Wolf, N. M. Olanzapine: a 5-year perspective. *Expert Review of Neurotherapeutics* **2006**
2. Podloucka, A., Synthesis and Preliminary Pharmacological Evaluation of Novel Antipsychotics for the Treatment of Schizophrenia. *Honours Thesis, Monash University* **2002**

POSTER COMMUNICATIONS – PC.127

MEDICINAL PLANTS FROM ANGOLA: FROM CITOTOXICITY TO BACTERICIDE ACTIVITY

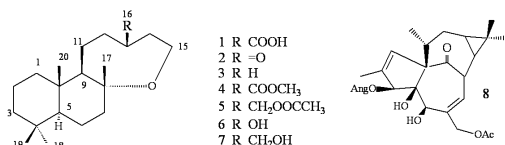
D.I. Mendonça¹, L. Vieira², N. Fernandes¹, A.J. Mendonça², F. Domingues², L. Granadeiro², C. Martins³, J. Gaspar³, J. Rueff³, A. Sant'Ana⁴

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The majority of the Angola population, as in other African countries, uses medicinal plants and goes to traditional healers in his search for medical care. However little has been done to study these medicinal plants. [1] As part of our ongoing study of the plants of Angola [2,3,4], we report here on the study of *Adenodolichos huillensis*, *Hymenodictyon floribundum*, *Parinari pumila*, *Peucedano angolense*, *Eragrostis viscosa*, *Euphorbia conspicua*, *Boscia microphylla* and *Tinnea antiscorbutica*.

Antiradicalar and antioxidant capacity of the extracts in different solvents were evaluated by DPPH and ABTS methods. The disk diffusion method was used to evaluate antimicrobial activity, while the broth microdilution method was used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). The Ames assay and the micronucleus assay were used to determine genotoxicity and evaluate the mutagenic activity of the isolated compounds. Cell viability was evaluated by MTT assay. Molluscicidal activity was determined for adults and egg masses of *Biomphalaria glabrata*.

From *Euphorbia conspicua* some molluscicide compounds with ingenane and ingol skeleton were isolated. *E. viscosa* presents a low antiradicalar capacity, no antifungal activity, inhibit Gram positive bacteria and present bacteriostatic activity against *Bacillus cereus* and *Staphylococcus aureus*. *E. viscosa* afforded citotoxic and bactericide compounds with a new 8 α ,15-epoxy-labdane skeleton.



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REFERENCES

1. Bossard, E.; *Medicine traditionnelle au Centre et à l'Ouest de l'Angola*, Vol. 1, Ministério da Ciência e Tecnologia, Instituto de Investigação Científica Tropical, Lisbon, 1996.

2. dos Santos, A.F.; Lopes, L.A.; Mata, R.C.S.; de Mendonça, D.I.M.D.; Sant'Ana, A.E.G. 2007. *Bioresour. Technol.* 98, 135-139.
3. Borges, C.M.P.; Diakanawma, C.; de Mendonça, D.I.M.D. *J. Braz. Chem. Soc.* advance online publication 22 March 2010.
4. Sebastião, N'S.N.; Cordeiro, I.J.S.; dos Santos, A.F.; Gaspar, J.F.; Martins, C.; Rueff, J.; Diakanawma, C.; Sant'Ana, A.E.G.; de Mendonça, D.I.M.D. *Phytochemistry*; doi: 10.1016/j.phytochem.2010.02.003.

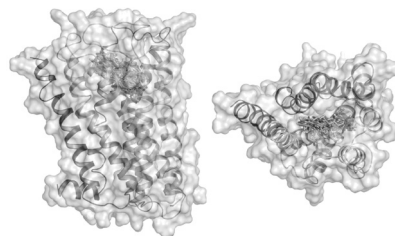
POSTER COMMUNICATIONS – PC.128

AMINERGIC G PROTEIN-COUPLED RECEPTORS; HOMOLOGY MODELING AND EVALUATION BY VIRTUAL SCREENING

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G protein-coupled receptors (GPCRs) are therapeutically significant proteins, targeted by a wide range of drugs. Until recently, the only GPCR crystal structure available was that of rhodopsin, which greatly limited structure-based drug design. The recent release of high-resolution crystal structures of several class A GPCRs has allowed the development of homology models, based on templates other than rhodopsin.



We report the development of homology models of nine therapeutically significant GPCRs (such as the serotonin 5HT_{2A} receptor, pictured).¹ The models were generated and refined in Prime, using the β_2 AR crystal structure (PDB: 2RH1) as the template. The orthosteric sites of the models were optimized using Induced Fit Docking, to allow for sidechain flexibility and the resulting models have been assessed using protein validation tools. Virtual screening was employed to evaluate the models by docking a database of active compounds and drug-like decoys, using Glide. Of the nine homology models developed, six showed moderate to good enrichment in virtual screening experiments (5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, D₂, D₃ and M₁). It is hoped that these models, based on the recent β_2 AR crystal structure, will provide a better starting point for structure-based drug design.

REFERENCES

1. McRobb, F. M. et al. *Journal of Chemical Information and Modeling* 2010. DOI: 10.1021/ci900444q

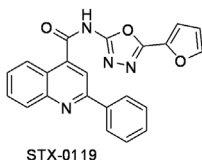
POSTER COMMUNICATIONS – PC.129

DISCOVERY OF NOVEL STAT3 DIMERIZATION INHIBITORS

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Signal Transducer and Activator of Transcription-3 (STAT3) is considered to be a good therapeutic target in oncology. Using virtual screening against the STAT3-SH2 domain and in vitro assays (luciferase reporter gene assay and fluorescence resonance energy transfer-based STAT3 dimerization assay in cells), we identified STX-0119 as a novel STAT3 dimerization inhibitor. STX-0119 abrogated both the subsequent DNA binding of STAT3 selectively and the expression of its target proteins in MDA-MB-468 cells. Structure activity relationships of STX-0119 analogues were investigated and reconciling this with the docking model of the STAT3-SH2 domain/STX-0119. Furthermore, STX-0119 induced selective apoptosis of a cancer cell line with constitutively active STAT3 and suppressed SCC-3 growth in the nude mouse xenograft model by oral administration. This is the first demonstration of in vivo efficacy following oral administration of a STAT3 dimerization inhibitor.



POSTER COMMUNICATIONS – PC.130

PRIMAQUINE-FERROCENE CONJUGATES: ON THE WAY TO SAFER AGENTS AGAINST PNEUMOCYSTIS PNEUMONIA

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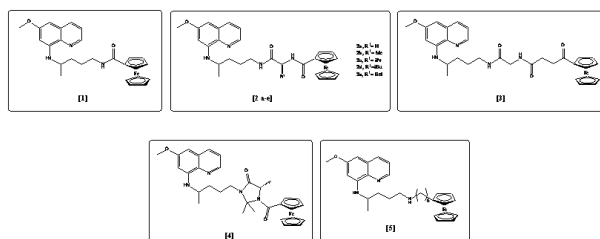
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Ferrocene (Fc) has been used in the preparation of bioactive organometallic structures, such as Fc-conjugates of: peptides and nucleotides¹, clinically relevant drugs as dopamine receptor ligands² and organometallic anti-malarials derived from chloroquine (Ferroquine).³ This chloroquine isostere with Fc-based side chain was 22 times more potent than parental drug and is now under clinical trials.^{3,4}

Our research group has been focused on imidazoquinones, a new class of imidazolidin-4-one peptidomimetic derivatives of Primaquine (PQ) that display anti-malarial and anti-pneumocystis activity while being resistant to premature metabolic inactivation by oxidative deamination or proteolysis.⁵⁻⁸ We hypothesized that conjugation of ferrocene with PQ-based structures (**1-4**) or with PQ's heteroaromatic core 8-amino-6-methoxyquinoline (**5**), could enhance both their anti-plasmodial and anti-pneumocystis activities.⁹



Interestingly, while organometallic conjugates were inactive against *Plasmodium*, they turned out to be moderately to markedly active against *Pneumocystis carinii*, while devoid of cytotoxicity. Compound **2e** was the best of the set, with higher activity than the parent drug (IC₅₀ of 1.1 μM against 1.8 μM for PQ) and dramatically reduced cytotoxicity against A549 human lung fibroblast carcinoma (IC₅₀ >100 μg/mL against 22 μg/mL for PQ). This is an unparalleled finding on PQ-based anti-*Pneumocystis* agents.

REFERENCES

1. H.-B. Kraatz, J. Inorg. Organometal. Polym. Mat., 2005, **15**, 83.
2. D. Huber et al., J. Med. Chem., 2009, **52**, 6860.
3. M. A. L. Blackie et al., J. Organometal. Chem., 2003, **688**, 144.
4. W. Daher et al., Malaria J., 2006, **5**, 11, and references therein.
5. P. Gomes et al., Tetrahedron, 2004, **60**, 5551.
6. M. J. Araújo et al., J. Med. Chem., 2005, **48**, 888.
7. N. Vale et al., Bioorg. Med. Chem. Lett., 2008, **18**, 485.
8. N. Vale et al., J. Med. Chem., 2009, **52**, 7800.
9. J. Matos et al., MedChemComm, 2010, submitted for publication.

POSTER COMMUNICATIONS – PC.131

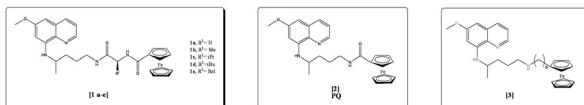
ANTI-PLASMODIUM ACTIVITY OF PRIMACENES

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Primacenes (**1a-e**), our first generation of ferrocene-primaquine conjugates, were recently prepared by us through coupling ferrocenecarboxylic acid to amino groups present in primaquine (PQ, **2**) amino acid derivatives, and tested for their *in vitro* blood-schizontocidal activity against *P. falciparum* and *in vivo* gametocytocidal activity against *P. berghei*.

Ferrocene (Fc) has been used in the preparation of bioactive organometallic structures of clinically relevant drugs.² Insertion of the ferrocene core in well known anti-malarial drugs as Chloroquine (CQ) showed an improvement in the anti-malarial activity. Ferroquine (FQ), a chloroquine isostere with an Fc-based side chain is 22 times more potent than CQ itself.^{3,4} Given the excellent results reported for FQ, we hypothesized that acylation of PQ itself or of its amino acid derivatives might enhance their blood-schizontocidal activity. However, primacenes **1a-e** were practically devoid of any type of anti-malarial activity. Believing that the deleterious effect of ferrocenylation on PQ anti-malarial activity was due to the consequent abolition of a basic aliphatic amine group, earlier proposed as a key feature for activity,⁵ we prepared and tested compounds **1f** and **3**, both possessing such a group. Full recovery of activity was observed, confirming beyond any doubt that PQ derivatives must possess an aliphatic amine in order to display anti-malarial activity of any kind.



REFERENCES

1. J. Matos et al., *MedChemComm*, 2010, submitted for publication.
2. H.-B. Kraatz, *J. Inorg. Organometal. Polym. Mat.*, **2005**, 15, 83.
3. W. Daher et al., *Malaria J.*, **2006**, 5, 11, and references therein.
4. F. Dubar et al., *Molecules*, **2008**, 13, 2900, and references therein.
5. M. J. Portela et al., *Pharm. Res.*, **1999**, 16, 949.

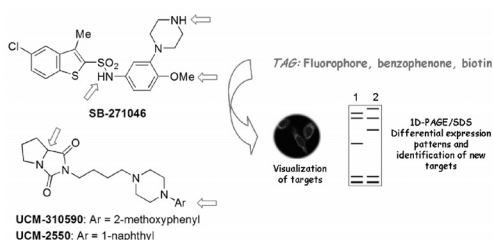
POSTER COMMUNICATIONS – PC.132

SYNTHESIS OF CHEMICAL PROBES FOR THE STUDY OF SEROTONIN 5-HT_{1A} AND 5-HT₆ RECEPTORS

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Development of compounds that enable direct observation of sub-fractions of the proteome has become of paramount importance in the quest of understanding biological processes at the systems level. In this context, activity-based protein profiling (ABPP) can be considered as one of the major contributions. This approach has been successfully applied to the study of different enzyme families.¹ However, the superfamily of G protein-coupled receptors (GPCRs), which account for more than the 50% of the druggable genome,² remains to be addressed. Among the different families of GPCRs, we have been focused on the serotonin receptors, particularly 5-HT_{1A} and 5-HT₆ subtypes, due to their therapeutic potential for the treatment of neuropsychiatric disorders. Herein we present our results concerning the synthesis and evaluation of novel labeled ligands for the study of both 5-HT_{1A} and 5-HT₆ receptors. Our strategy encompasses the selection and tagging of known potent ligands of these receptors with a fluorescent, photoactivatable and/or affinity tags. Fluorescent ligands were synthesized by attachment of the corresponding fluorophore (dansyl, lissamine or NBD) to different positions of known 5-HT_{1A} (UCM-310590 and UCM-2550)³ and 5-HT₆ (SB-271046)⁴ ligands (Figure below). Synthesized probes have shown both good fluorescent properties and high binding affinities, which enable direct observation of the h5-HT_{1A} and h5-HT₆ receptors in cells.^{5,6} These results have provided the basis for the introduction of benzophenone and biotin affording a new series of high affinity probes for covalent binding and affinity pull-down of receptors. These strategies should contribute to optimize the therapeutic exploitation of known or new members of the GPCR superfamily by providing valuable information about their location or level of expression.



REFERENCES

1. Cravatt, B.F. *et al. Annu. Rev. Biochem.* **2008**, *77*, 383.
2. Lagerström, M.C. *et al. Nat. Rev. Drug Discov.* **2008**, *7*, 339.
3. López-Rodríguez, M.L. *et al. J. Med. Chem.* **1996**, *39*, 4439; *J. Med. Chem.* **2005**, *48*, 2548.
4. Bromidge, S.M. *et al. J. Med. Chem.* **1999**, *42*, 202.
5. Alonso, D. *et al. ACS Med. Chem. Lett.* Submitted. 6) Vázquez-Villa, H. *et al. Chem. Eur. J.* Submitted.

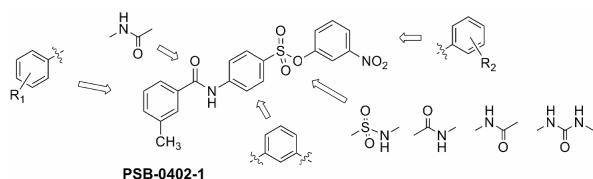
POSTER COMMUNICATIONS – PC.133

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A NEW CLASS OF P2Y₂ RECEPTOR ANTAGONISTS

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P2Y₂ receptors, which are activated by UTP, ATP, and dinucleotides, belong to the family of G protein-coupled nucleotide (P2) receptors.¹ Antagonists for this P2Y receptor subtype have been proposed as anti-inflammatory, neuroprotective and anti-hypertensive agents. However, potent and selective P2Y₂ antagonists are currently not available.² In a screening campaign, we identified 3-nitrophenyl 4-(3-methylbenzamido)benzenesulfonate (PSB-0402-1) as a relatively potent antagonist at human P2Y₂ receptors with an IC₅₀ value of 3.64 µM. In the present study we investigated the properties and structure-activity relationships of this new class of P2Y₂ receptor antagonists.



The stability of PSB-0402-1 was assessed by LCMS. A series of phenyl 3- and 4-benzamidobenzenesulfonates and -sulfonamides was synthesized. Furthermore, the sulfonic acid ester was replaced by amide and urea groups. The compounds were investigated in calcium assays at the G_q-coupled human P2Y₂, P2Y₄ and rat P2Y₆ receptors stably expressed in 1321N1 astrocytoma cells.

The ester function of the lead structure was shown to be highly stable at different pH values. Depending on the substitution of the benzamido moiety, the synthesized phenyl sulfonates showed antagonistic activity at P2Y₂ receptors mostly in the very low micromolar range and selectivity versus P2Y₄ and P2Y₆ receptors. In contrast to the sulfonates the corresponding sulfonamides were inactive. Further optimized compounds, includ-

ing carbamides and diamides, are currently investigated and preliminary test results look promising.

In conclusion, a novel class of relatively potent and selective P2Y₂ receptor antagonists has been identified. They may be useful as pharmacological tools to elucidate the (patho)-physiological roles of the P2Y₂ receptor and have potential as lead structures for drug development.

REFERENCES

1. Abbraccio, M. P.; Burnstock, G. *et al. Pharmacol. Rev.* **2006**, *58*, 281-341
2. (a) Brunschweiler, A.; Müller, C. E. *Curr. Med. Chem.* **2006**, *13*, 289; (b) Müller, C. E. *Curr. Pharm. Des.* **2002**, *8*, 99-110

POSTER COMMUNICATIONS – PC.134

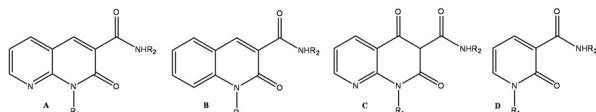
CB2 SELECTIVE CANNABINOID RECEPTOR LIGANDS: PHARMACOMODULATION AROUND THE 1,8-NAPHTHYRIDIN-2(1H)-ON-3-CARBOXAMIDES

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The endocannabinoid signaling system is composed of cannabinoid (CB) receptors, their endogenous ligands and the enzymes that produce and inactivate these ligands. To date, two distinct cannabinoid receptors, CB1 and CB2, have been identified in mammalian tissues and cloned. The psychotropic effects associated with nonselective cannabinoid agonists are thought to be mediated through the CB1 receptor, which is present in the central and peripheral nervous system as well as the periphery. While there is some evidence of the CB2 receptor in the central nervous system, the CB2 receptor is found primarily in the immune system. Over the last few years, the interest for CB2 selective agonists has been growing with proposed indications in the control of both neuropathic and inflammatory pain and more recently in liver pathophysiology, osteoporosis, growth of malignant gliomas, tumors of immune origin, and immunological disorders such as multiple sclerosis. Furthermore, given the presence of the CB₂ receptors in the brain microglial cells, it has recently been shown that CB₂ agonists might provide neuroprotection by blockade of microglial activation. On the basis of docking studies developed using three-dimensional models of

the two CB receptors [1,2], recently we described the synthesis, pharmacological characterization, and molecular modeling studies of a novel series of 1,8-naphthyridin-2(1*H*)-on-3-carboxamide (**A**) acting as selective and potent CB2 receptor agonists and further studies revealed that one of these compounds induced a concentration-dependent decrease of cell viability in Jurkat cells [3].



In order to specify structure-affinity relationships, the derivatives of general structure **B**, **C** and **D** were synthesized using compounds **A** as a template. The binding results indicate that most of these compounds exhibited a remarkable CB2 affinity, with a K_i value in the nanomolar range. This affinity was accompanied by a high selectivity with respect to CB1 receptor. The newly synthesized CB₂ receptor ligands, showing the better binding profiles in the series, were evaluated *in vitro* for their antiproliferative activity.

REFERENCES

1. Manera, C. *et al.*, J. Med. Chem., **2006**, 49, 5947-5957.
2. Tuccinardi, T *et al.*, J. Med. Chem., **2006**, 49, 984-994
3. Manera, C. *et al.*, J. Med. Chem., **2009**, 52, 3644-3651

POSTER COMMUNICATIONS – PC.135

N-ALKYLATED DIKETOPIPERAZINES: EASILY AVAILABLE CSA FOR DETERMINATION OF ENANTIOMERIC PURITY

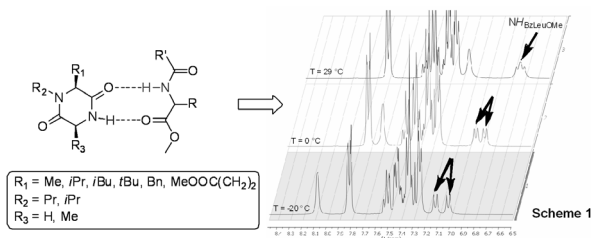
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Diketopiperazines, the smallest cyclic peptides known, show wide spectrum of biological activities.¹ These compounds display some interesting chemical properties regarding medicinal chemistry, among them substituent group stereochemistry, conformational rigidity and donor and acceptor groups for hydrogen bonding.¹

A series of novel chiral nonracemic *N*-alkylated piperazine-2,5-diones were prepared and their properties were investigated by X-ray analysis and NMR spectroscopy. Chiral solvating properties of *N*-alkylated diketopiperazines arise from their ability of hydrogen bonding with *N*-acylated amino acid esters in CDCl₃ solution (Scheme 1).²⁻⁴ Recently, we have shown that diketopiperazines are easily available chiral solvating agents (CSA) for racemic *N*-acylated α -amino acid esters. They were applied for fast and easy determination of enantiomer composition of

fully protected α -amino acid ester derivatives by ¹H NMR devoiding peak broadening which is otherwise a typical disadvantage of chiral lanthanide shift reagents.^{3,4} Besides, these CSA can be used for determination of absolute configuration as well. In summary, these CSA might find use in determination of optical purity of pharmaceutically interesting compounds.



REFERENCES

1. Martins, M. B.; Carvalho, I. *Tetrahedron* **2007**, 63, 9923-9932.
2. Malavašič, Č.; Waggar, J.; Stanovnik, B.; Svete, J. *Tetrahedron: Asymmetry* **2008**, 19, 1557-1567.
3. Waggar, J.; Golič Grdadolnik, S.; Grošelj, U.; Meden, A.; Stanovnik, B.; Svete, J. *Tetrahedron: Asymmetry* **2007**, 18, 464-475.
4. Waggar, J.; Svete, J.; Stanovnik, B. *Synthesis* **2008**, 1436-1442.

POSTER COMMUNICATIONS – PC.136

DISCOVERY OF SELECTIVE INHIBITORS OF UBIQUITIN SPECIFIC PROTEASES

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Regulated protein degradation is primarily controlled by the ubiquitin-proteasome system. This system was established as a valid oncology target since the approval of the proteasome inhibitor Velcade®. Targeting upstream on the ubiquitin conjugation/deconjugation system opens the way to anticancer agents with increased specificity and selectivity.

Ubiquitin-specific proteases (USP) are involved in the deubiquitination of specific target substrates regulating their stability, subcellular localization and/or activation status. Those USP represent druggable targets due to their cysteine protease activity amenable to pharmacological inhibition by small molecules. Genome-wide RNAi screening of the catalytically active human USPs in cancer-relevant cellular models and phenotypic assays allowed us to identify USP7/HAUSP and USP8/UBPY as promising cancer targets. After successful assay development, HTS performed on our chemically diverse library resulted in the discovery of novel USP inhibitors. From these series, hit to lead and

lead optimization programs were performed in order to improve potency, selectivity, cellular activity and pharmacokinetic profile.

Our progress made from the first generation series towards the specificity issue which culminated with the discovery of the first USP8- and USP7-specific series, will be presented.

POSTER COMMUNICATIONS – PC.137

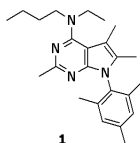
SYNTHESES AND BIOLOGICAL EVALUATION OF A NEW NAPHTHYRIDIN DERIVATIVES AS POTENTIAL CRF₁ ANTAGONIST

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Depression and anxiety are a complex psychiatric disorder that affects an enormous number of people in society, and constitute a major health concern worldwide. Despite the number of treatments available, significant unmet needs still remain in terms of efficacy, onset of effect and tolerability. Corticotropin releasing factor (CRF) is a 41-amino acid peptide that plays a central role in mediating the behavioral, neuroendocrine, and autonomic responses to stress. CRF is synthesized in the hypothalamus and liberated into the hypophyseal-portal system triggering the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. Clinical data indicate that patients with depression and post-traumatic stress disorder show significantly elevated concentrations of CRF in cerebrospinal fluid when compared to normal controls. The CRF receptor has two well-characterized subtypes CRF₁ and CRF₂, which are widely distributed throughout the central nervous system (CNS) and peripheral tissues. A selective CRF₁ antagonist may represent a novel class of compounds for the treatment of anxiety, depression and stress-related diseases.

Several companies have disclosed potent CRF₁ receptor antagonists based on monocyclic and bicyclic cores. The extensively researched antalarmin **1** (1) (2) falls within this broad structural class, and has proven to be functional antagonists, both in vitro and in vivo with exquisite selectivity for CRF₁ receptor, but it has been suggested that the clinical development of this early CRF₁ antagonist was limited (3).



Our efforts have been focused on the modifications in the pyrrolo-pyrimidine core template and peripheral side chains. A series of new [1,8]naphthyridin with amine functionality have been designed and synthesized as possible antagonist CRF₁.

Quite a few compounds have been synthesized and evaluated. CRF receptor binding affinities have been determined by displacement of [¹²⁵I].Tyr⁰-CRF from rat frontal cortex. The results of these studies will be reported and discussed.

This work was co-supported by the Secretariat General for Science and Technology Policy of the Ministry of Science and Innovation and by the INNOTEK Program of the Department of Industry, Commerce and Tourism of the Basque Government.

REFERENCES

1. Webster, E. L.; Lewis, D. B; Torpy, D. J.; Zachman, E. K.; Rice, K. C.; Chrousos, G. P. *Endocrinology* **1996**, 137, 5747-5750.
2. Chen, Y. WO 9413676.
3. Hsin, L. W.; Tian, X. : Webster, E. L.; Coop, A.; Caldwell, T. M. *Bioorg. Med. Chem.*, **2002**, 10, 175-183.

POSTER COMMUNICATIONS – PC.138

DESIGN SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION TOWARDS AROMATASE OF C-4 DERIVATIVES OF FORMESTANE AND 5 α -ANDROST-3-EN-17-ONE

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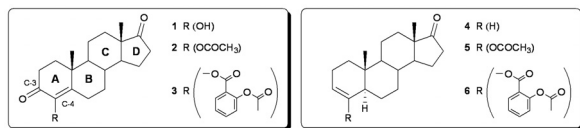
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Hormone-dependent breast tumors require estrogens for their growth.¹ One main approach to block estrogen action targets aromatase, the cytochrome P-450 enzyme responsible for estrogen biosynthesis. In breast cancer, intratumoral aromatase is the source for local estrogen production and its inhibition by aromatase inhibitors (AIs) is an important approach for reducing tumor growth.

Recently, the active site of aromatase has been elucidated, and the molecular basis for enzyme-substrate (androstenedione) interaction was established.² It was found that the volume of the binding pocket is relatively short (< 400 Å) allowing to enter into the cleft only molecules with appropriate dimensions such as androstenedione derivatives with small substituents.

Numerous recent studies have associated cyclooxygenase-2 (COX-2) with breast cancer pathology. Inhibition of COX-2 activity is described to be protective against tumorigenesis in animal models of breast cancer and epidemiologic studies suggest that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid, protects against breast cancer development.³

Formestane **1** is the C-4 hydroxy analogue of the enzyme substrate androstenedione and was the first steroidal AI clinically used. Its C-4 acetoxy derivative **2**, also revealed to have strong anti-aromatase activity.⁴ The 3-deoxy steroid **5 α** -androst-3-en-17-one **4** is also a potent AI recently prepared and evaluated by our team.⁵



In this work, we present a structure-activity study, based on the synthesis and aromatase inhibitory activity evaluation of C-4 acetoxy- and acetylsalicyloxy-derivatives of formestane (**2** and **3**) and of **5 α** -androst-3-en-17-one (**5** and **6**). The results obtained so far suggest that the presence of bulky substituents in C-4 diminishes the aromatase inhibitory activity which is consistent with the short volume of the binding pocket of aromatase.

REFERENCES

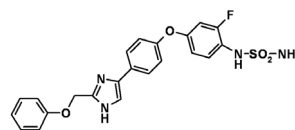
1. Chen, S. *Frontiers of Bioscience* **1998**, 3(9), 22.
2. Glosch, D.; Griswold, J.; Erman, M.; Pangborn, W. *Nature* **2009**, 457, 219.
3. Takkouche, B.; Regueira-Méndez, C.; Etminan, M. *J Natl Cancer Inst* **2008**, 100, 1439.
4. Marsh, D.A.; Brodie, H.J.; Garrett, W.; Tsai-Morris, C.; Brodie, A.M.H. *J Med Chem* **1985**, 28, 788.
5. a) Cepa, M.D.S.; Tavares-da-Silva, E.J.; Correia-da-Silva, G.; Roleira, F.M.F.; Teixeira, N.A.A. *J Med Chem* **2005**, 48, 6379. b) Cepa, M.; Correia-da-Silva, G.; Tavares-da-Silva, E.J.; Roleira, F.M.F.; Teixeira, N. *Steroids*, **2008**, 73, 1409.

POSTER COMMUNICATIONS – PC.139

IRC-083927: A NOVEL TUBULIN POLYMERIZATION INHIBITOR ACTIVE AGAINST CELL LINES RESISTANT TO STANDARD TUBULIN BINDING AGENTS

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IRC-083927

Microtubules are a validated target for antitumor drugs. However, the effectiveness of these microtubule-interacting agents is limited by the fact that they are substrates for drug efflux pumps (PgP) or/and by the acquisition of point mutations in tubulin residues important for drug-tubulin binding.

In order to bypass these resistance systems, we have identified and characterized a novel synthetic imidazole derivative IRC-083927 which prevents polymerization of purified tubulin in vitro by binding to the colchicine site. IRC-083927 inhibits in vitro cell growth of human cancer cell lines displaying resistance to microtubule interacting agents due to the presence of efflux pumps (NCI-H69/LX4 resistant to Navelbine and Paclitaxel) and or due to the presence of mutations on beta tubulin (A549EpoB40 resistant to Epothilone B or Paclitaxel) and on alpha- and beta tubulin (A549EpoB480 resistant to Epothilone B or Paclitaxel). As expected for numerous tubulin binding agents, IRC-083927 also displayed antiangiogenic properties by inhibiting the endothelial cell proliferation and in vitro vessel formation in the low nanomolar range. The high potency and efficacy of IRC-083927 has been shown using chronic oral treatment (2.5 or 5 mg/kg during 28 day period) in all tested models. In addition, IRC-083927 is more efficient than: 1) Velcade against the human myeloma RPMI-8226, 2) ABT-751 against androgen independent prostate cancer PC3 and 3) Combretastin CA4P or ABT-751 against human hormone-independent breast cancer MDA-MB-231. Finally, no signs of toxicity have been observed in mice with this regimen. Chronic exposure of rats to IRC83927 at the maximum tolerated dose induced reduction of the gastric emptying and a slight decrease of white blood cells.

Together, the biological effects induced by IRC-083927 on tubulin interacting agent resistant tumor models and its synthetic nature support further investigations to fully evaluate its significant potential for the treatment of advanced cancers resistant to current clinically available drugs.

REFERENCES

1. WO2007/125197
2. *Molecular Cancer Therapeutics* (2008), 7(8), 2426-2434
Erratum *Molecular Cancer Therapeutics* January 1, 2009 8:274

POSTER COMMUNICATIONS – PC.140

FRAGMENT-BASED APPROACHES TO GPCRS: EFFICIENT DEVELOPMENT OF HISTAMINE H₄ RECEPTOR LIGANDS WITH ANTI-INFLAMMATORY PROPERTIES

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The human histamine H₄ Receptor (hH₄R) is considered a promising therapeutic target for inflammation and pruritis. Fragment screening resulted in the identification of various fragment hits, including quinazolines, quinoxalines and aminopyrimidines. Guided by a pharmacophore model, and supported by homology models, the fragment hits were efficiently optimized into potent hH₄R ligands with nanomolar affinities. In vivo studies in the rat reveal that some of these ligands show excellent anti-inflammatory properties in the carrageenan induced paw edema model.

tent with antagonism of the histamine H₃ receptor. The synthesis and *in vitro* characterization of compound **4** with respect to affinity, functional antagonism and pharmaceutical properties will be discussed along with pre-clinical pharmacokinetic data in mouse, rat and canine. These data will show that compound **4** is a high affinity histamine H₃ antagonist that is orally bioavailable in pre-clinical species. Data demonstrating robust histamine H₃ receptor occupancy in rat (i.e. Figure 1), significant increases in histamine levels in the frontal cortex of rats and the effects of compound **4** on total wake time in rats will also be presented. Based on this pre-clinical data compound **4** was chosen as a clinical candidate.

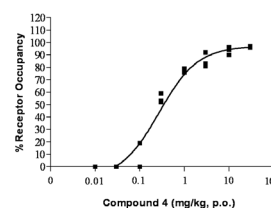
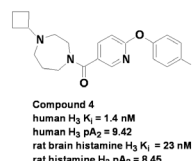


Figure 1. Receptor Occupancy vs Dose in a Rat.

POSTER COMMUNICATIONS – PC.142

COMPUTATIONAL PERSPECTIVE ON THE OPTIMIZATION OF CYP450 2D6 LIABILITIES IN DRUG DESIGN: SELECTIVE ALPHA-2C AGONISTS CASE STUDIES

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Human cytochrome P450 2D6 (CYP2D6) is a heme containing enzyme which plays a critical role in the metabolism of many xenobiotic compounds. CYP2D6 liabilities in drug discovery can result in poor pharmacokinetic properties and a potential for drug-drug interactions associated with severe side effects.

To address CYP2D6 liabilities in lead generation and optimization stages of projects, we use a 3D comparative model structure of the enzyme for which we developed docking and post-processing protocols. CYP2D6 inhibition issues are addressed using 2D-QSAR & 3D-QSAR methods in order to develop predictive models which help medicinal chemists in the decision making process of the projects.

In this poster we will describe the development of these methodologies and show how the models were used to support the design of selective alpha-2C agonists.

The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5601)

POSTER COMMUNICATIONS – PC.141

THE DISCOVERY OF (4-CYCLOBUTYL-[1,4]DIAZEPAN-1-YL)-[6-(4-FLUOROPHENOXY)-PYRIDIN-3-YL]-METHANONE: A NOVEL HISTAMINE H₃ ANTAGONIST SUITABLE FOR CLINICAL DEVELOPMENT

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The discovery and pre-clinical characterization of (4-Cyclobutyl-[1,4]diazepan-1-yl)-[6-(4-fluoro-phenoxy)-pyridin-3-yl]-methanone (**4**) will be discussed. Compound **4** is a high affinity, selective histamine H₃ antagonist that penetrates the central nervous system and shows efficacy in pre-clinical models consist-

POSTER COMMUNICATIONS – PC.143

DESIGN OF MORE POTENT SQUALENE SYNTHASE INHIBITORS COMBINING ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Atherosclerosis is a multifactorial disease with several mechanisms participating in its initial manifestation and progression. These mainly involve hyperlipidemia, oxidative stress and inflammation. Squalene Synthase (SQS), a key enzyme in the cholesterol biosynthetic pathway, occupies the first solely committed step towards the biosynthesis of the sterol nucleus of cholesterol. Because of its unique and strategic location in this pathway, it is an attractive target for the development of novel and improved antidiyslipidaemic agents. These agents are considered to offer a better side-effect profile than inhibitors of (upstream) HMG-CoA reductase (i.e. statins) which are presently extensively used in the management of atherosclerosis.^{1,2} We have previously synthesized and investigated 2-aromatically substituted morpholine derivatives that were shown to reduce cholesterol and triglyceride levels, acting as squalene synthase inhibitors, as well as to combine antioxidant and anti-inflammatory activity^{3,4}.

We hereby evaluated the relationship between the *in vitro* SQS inhibitory action of several of these derivatives and the theoretically derived molecular interactions obtained by docking studies based on the available crystal structure of human SQS and use of *Autodock 4*. Thus, by visualizing preferred binding modes and comparing best fit energy values (ΔG) with experimental binding data of several derivatives, we designed potentially more potent SQS ligands. This design took into consideration structural features that potentially maintained or increased antioxidant and anti-inflammatory activity.

The synthesis of the new derivatives was performed by modification of existing methodology while the new structures were further evaluated *in vitro* (inhibition of SQS and antioxidant activity) and *in vivo* (antidiyslipidemic and anti-inflammatory effect).

The new derivatives were significantly more active than our formerly developed SQS inhibitors, as predicted by our structure-theoretical-activity relationships. Simple regression calculations between $\text{Log}(1/IC_{50})$ (SQS inhibition) and ΔG of derivatives revealed a significant correlation between ΔG and experimental activity ($R^2 = 0.8$, $F = 35$, $p = 0.0002$). Furthermore, the new derivatives had an improved antioxidant profile.

In conclusion, application of findings from docking studies led to the design and development of more potent SQS inhibitors. To

our knowledge, this is the first time that an *in silico* docking study has been performed on the crystal structure of human SQS. Combination of the above approaches may lead to further optimization and development of multi-pharmacophore antiatherosclerosis agents.

REFERENCES

1. Elsayed R et al., Emerging lipid-lowering drugs: squalene synthase inhibitors. *Expert Opin Emerg Drugs*. **2008** 13, 309.
2. Meng CQ, Atherosclerosis is an inflammatory disorder after all, *Cur Top Med Chem*. **2006**, 6, 93.
3. Kourounakis et al., Lipid-lowering (hetero)aromatic tetrahydro-1,4-oxazine derivatives with antioxidant and squalene synthase inhibitory activity. *J. Med. Chem*. **2008** 51 5861.
4. Matralis A. Kourounakis A. Multi-site-directed molecules as a therapeutic approach for atherosclerosis. *20th International Symposium on Medicinal Chemistry*, Vienna, Sept. 2008.

POSTER COMMUNICATIONS – PC.144

EXHAUSTIVE SAMPLING OF DOCKING POSES REVEALS POSSIBLE BINDING MODES OF PROPAFENONES IN P-GLYCOPROTEIN

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The failure of chemotherapies can often be traced back to the acquisition of multidrug resistance (MDR), which can be directly associated with the over expression of P-glycoprotein (P-gp, ABCB1, MDR1). As an ABC-exporter it actively transports a high number of chemically diverse substances out of the cell. This drug translocation process is involved in a catalytic cycle where P-gp undergoes several conformational changes in connection with the binding of substrate and nucleotide as well as ATP hydrolyzation (1).

Propafenones are known for their ABCB1 modulating activity wherefore they have been in focus of several ligand based analyses. In this study the binding of five propafenone derivatives to the human ABCB1 transporter was investigated. Since no X-ray structure of the human protein is available, homology models were built on the basis of the mouse ABCB1 exporter (PDB code: 3G5U, resolution: 3.80 Å, apo) (2) and the bacterial homolog Sav1866 (PDB code: 2HYD, resolution: 3.00 Å, ADP-bound) (3). As these templates represented two different states of the catalytic cycle the docking results were expected to explain the affinity differences caused by ATP binding. As the conformational change of the transporter may lead to a shift in the hydrophobic environment all ligands were docked in a neutral and a protonated form.

As the high poly-specificity of P-gp is connected with its flexibility, we docked into homology models of two different catalytic

states treating selected side chains flexible. To fully explore the pose space, we allowed 500 poses for each docking run, which were subsequently rescored using the external scoring function XScore. With the assumption that propafenone derivatives bind in a similar manner to P-gp the poses were clustered according to the RMSD of the common scaffold. In addition, the protein ligand interaction fingerprint, which is implemented in the MOE software package, also served as clustering principle. Prioritisation of different clusters was further guided by information from structure-activity relationships of the five selected compounds.

With this exhaustive approach it was possible to explore all potential poses inside the large poly-specific binding pocket of P-gp. However, a high number of poses generated could be sorted out by the hypothesis of a common binding mode of all compounds used. The results suggest that interactions with transmembrane helices 5, 6, 7, 8 are present in both states of the catalytic cycle, whereas interactions with helix 12 get lost with the conformational change of the protein.

We acknowledge financial support provided by the Austrian Science Fund, grant F03502.

REFERENCES

- Seeger MA, van Veen HW. Molecular basis of multidrug transport by ABC transporters. *Biochim Biophys Acta*. 2009;1794(5):725-37
- Aller SG et al. Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding. *Science*. 2009;323(5922):1718-22
- Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature*. 2006;443(7108):180-5

POSTER COMMUNICATIONS – PC.145

NOVEL ESTRONE MIMETICS WITH HIGH 17 β -HSD1 INHIBITORY ACTIVITY

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17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) catalyzes the reduction of estrone into estradiol, which is the most potent estrogen in humans. Lowering intracellular estradiol concentration by inhibition of this enzyme is a promising new option for the treatment of estrogen dependent diseases like breast cancer and endometriosis. Besides the already described (hydroxyphenyl)naphthols¹ and bis(hydroxyphenyl)heterocycles², we identified heterocyclic substituted biphenyls and their azanalogues as new class of 17 β -HSD1 inhibitors³. The latter resulted from the combination of ligand- and structure-based design, which was based on mimicking estrone, especially focusing on

the imitation of the D-ring keto group with (substituted) heterocycles. Molecular docking provided insights into plausible protein-ligand interactions for this class of compounds. The most promising compound **12** showed an inhibitory activity in the high nanomolar range and very low affinity for the estrogen receptors α and β . Thus, compound **12** is a novel tool for the elucidation of the pharmacological relevance of 17 β -HSD1 and might be a lead for the treatment of estrogen dependent diseases.

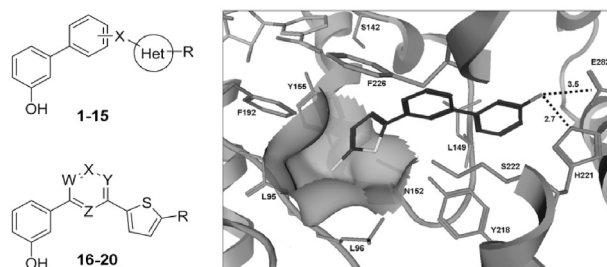


Figure 1: General structures of synthesized compounds are shown on the left. Docking complex between 17 β -HSD1 (PDB-ID: 1EQU) and compound 12 (violet) is depicted on the right.

REFERENCES

- Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. *J. Med. Chem.* **2009**, 52, 6724.
- Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. *Mol Cell Endocrinol.* **2009**, 301, 205-211.
- Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. *Bioorg. Med. Chem.* **2010**, doi: 10.1016/j.bmc.2010.03.065

POSTER COMMUNICATIONS – PC.146

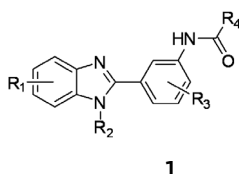
SYNTHESIS AND ANTICHLAMYDIAL ACTIVITY OF 2-ARYLBENZIMIDAZOLE DERIVATIVES

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Chlamydia pneumoniae is a common intracellular bacterium causing mild acute respiratory infections and community acquired pneumonia. However, pathogen can turn chronic, and these infections are associated with atherosclerosis¹, asthma², and Alzheimer's disease³. Due to the biphasic life cycle of *C. pneumoniae* there is an obvious demand to continue developing new type of antibiotics and effective new antichlamydial compounds.

In our previous study certain 2-arylbenzimidazole derivatives were found to have activity against *C. pneumoniae*.⁴ In this present study a new set of 2-arylbenzimidazole derivatives was synthesized, and their inhibitory effect against *C. pneumoniae* was evaluated. Thirty-one 2-arylbenzimidazole derivatives based on the general structure **1**, and two 2-arylimidazoles, were synthesized. Structural modifications of the compounds were designed based on the activity results. The best compounds inhibit the growth of the bacterium at micromolar concentration. Synthesis of the library and the structure-activity relationships will be described.



REFERENCES

1. Watson C, Alp NJ. *Clin. Science* **2008**, 114: 509-531.
2. Hahn DL, Peeling RW, Dillon E, McDonald R, Saikku P. *Ann. Allergy Asthma Immunol.* **2000**, 84: 227-233.
3. Gérard HC, Dreses-Werringloer U, Wildt KS, Deka S, Oszust C, Balin BJ, Frey II WH, Bordayo EZ, Whittum-Hudson JA, Hudson AP. *FEMS Immun. & Med. Microb.* **2006**, 48: 355-366.
4. Alvesalo JKO, Siiskonen A, Vainio MJ, Tammela PSM, Vuorela PM. *J. Med. Chem.* **2006**, 49: 2353-2356.

POSTER COMMUNICATIONS – PC.147

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL COMT INHIBITORS

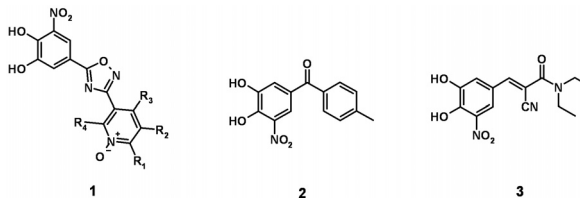
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Catechol-O-methyltransferase (COMT) is a magnesium-dependent enzyme that catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the phenolic group of substrates, inactivating endogenous catechol neurotransmitters and xenobiotic catechols such as L-DOPA. In mammals, COMT is distributed throughout various organs such as liver, kidney, lung, blood cells and skeletal muscles. Inhibitors of COMT are used in the treatment of central and peripheral nervous system disorders such as Parkinson's disease [1] (PD). In the presence of a decarboxylase inhibitor COMT becomes the major metabolizing enzyme for levodopa, catalyzing the metabolism to 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD) both in the brain and periphery.

We have recently discovered a new class of peripherally selective, long acting COMT inhibitors [2] of general formula **1** that

may possess therapeutic advantages over established COMT inhibitors such as tolcapone **2** and entacapone **3**.



Synthesis and biological evaluation will be presented.

REFERENCES

1. (a) Axelrod, J.; Senoh, S.; Witkop, B. *J. Biol. Chem.* **1958**, 233, 697. (b) Guldberg, H.; Marsden, C. *Pharmacol. Rev.* **1975**, 27, 135.
2. Learmonth, D.; Kiss, L.; Leal Palma, P.; Dos Santos Ferreira, H.; Araújo Soares-Da-Silva, P. WO2007013830A1, **2007**.

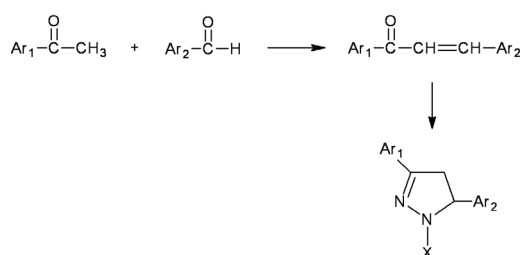
POSTER COMMUNICATIONS – PC.148

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NEW 2-PYRAZOLINE DERIVATIVES

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Chalcones, 1,3-diphenyl-2-propene-1-ones, which are member of the flavonoid family, have been shown to display a various array of pharmacological activities such as antimicrobial, antiinflammatory. Besides their important pharmacological activities, chalcones are also well known intermediates for synthesizing various heterocyclic compounds like flavonoids, flavanone, pyrazoles, oxazoles, pyrimidines and 1,5-benzothiazepines. Among these compounds, 2-pyrazolines are attracting interest of many researchers in medicinal chemistry because of their bioactivity such as antimicrobial [1], antiinflammatory [2], antidepressant and anticonvulsant [3] activities. In an effort to develop new 2-pyrazoline derivatives as anticonvulsant agents, a novel series of chalcones were prepared by Claisen Schmidt condensation. Thus, 1,3,5-trisubstituted-2-pyrazolines were synthesized by the reaction of chalcones with thiosemicarbazide, substituted thiosemicarbazides or substituted semicarbazides. Structures of the compounds have been confirmed by IR, ¹H-NMR spectral data and elemental analysis. 1,3,5-trisubstituted-2-pyrazoline derivatives have been testing for the anticonvulsant activity by pentylenetetrazole induced seizure and maximal electroshock seizure tests.



X: CSNH₂, CONHAr₃, CSNHAr₄

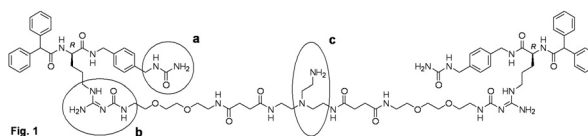
ACKNOWLEDGMENT

The authors wish to thank Marmara University Scientific Research Projects Commission (BAPKO, Project number, SAGA-040609-0140, 2009) to financial support for this study.

REFERENCES

- Özdemir A, Turan-Zitouni G, Kaplancıklı ZA, Revial G, Güven K. Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. *Eur J Med Chem.* 42(3):403-409, 2007.
- Shoman ME, Abdel-Aziz M, Aly OM, Farag HH, Morsy MA. Synthesis and investigation of anti-inflammatory activity and gastric ulcerogenicity of novel nitric oxide-donating pyrazoline derivatives. *Eur J Med Chem.* 44(7):3068-3076, 2009.
- Özdemir Z, Kandilci HB, Gümüşel B, Çaliş Ü, Bilgin AA. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. *Eur J Med Chem.* 42(3):373-379, 2007.

assay [4]: IC₅₀ = 0.71 µM; flow cytometric Y₄R binding assay⁴: K_i = 0.30 µM).



In contrast to the Y₁R, which prefers (R)-configured argininamides, stereochemical discrimination by the Y₄R was not detected. Highest Y₄R antagonistic activity resided in compounds having an H-donor group **a** (urea or hydroxyl) as well as an additional basic moiety **c** at a certain distance from the crucial acylguanidine **b**. This suggested the synthesis of monovalent argininamides combining the putative pharmacophoric groups **b** and **c** by spacers of different lengths.

In summary, the presented class of compounds is considered a promising starting point in the search of more potent and selective Y₄R antagonists, superior to recently identified acylguanidines derived from histamine H₂ receptor ligands [4, 5].

REFERENCES

- Kamiji, M. M. et al., *Curr. Topics Med. Chem.*, **2007**, 7, 1734-1742,
- Balasubramaniam et al., *J. Med. Chem.*, **2006**, 49(8), 2661-2665,
- Nagaaki, S. et al., *Expert Opin. Ther. Patents*, **2009**, 19(10), 1401-1415,
- Ziemek, R. et al., *J. Recept. Signal Transduct.*, **2007**, 27, 217-233.
- Kaske, M. et al., *Frontiers in Medicinal Chemistry*, Heidelberg, March **2009**, Poster No. HMC 24

POSTER COMMUNICATIONS – PC.149

MONOVALENT AND BIVALENT ARGININAMIDE-TYPE ANTAGONISTS OF THE HUMAN NPY Y₄ RECEPTOR

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The neuropeptide Y (NPY) system has emerged as a complex network of peptides and receptors. Among the NPY receptors (Y₁R, Y₂R, Y₄R, Y₅R) the Y₄R is unique due to preferential binding of pancreatic polypeptide (PP) instead of NPY. The biological role of the Y₄R is far from being understood. PP-based peptides are described to inhibit food intake, suggesting the Y₄R to be a potential target for the treatment of obesity. With respect to more detailed investigations, potent *non-peptidic* Y₄R ligands are required as pharmacological tools. However, such compounds are not reported in the literature so far [1, 2, 3].

Surprisingly, the investigation of a library of argininamides, initially designed as Y₁R antagonists, revealed that certain bivalent compounds are Y₄R antagonists with activities in the low micromolar or sub-micromolar range (example cf. **Fig. 1**; Y₄R Ca²⁺

POSTER COMMUNICATIONS – PC.150

MODULATION OF PHYSICO-CHEMICAL PROPERTIES OF PHENOL DERIVATIVES OF 1,3-DIMETHYL-PYRIMIDO[2,1-f]PURINEDIONES – ADENOSINE A_{2A} RECEPTOR LIGANDS.

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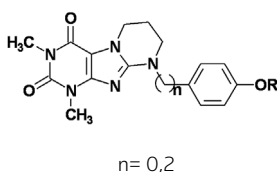
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The adenosine receptor (AR) family consists of four G protein-coupled receptor subtypes: A₁, A_{2A}, A_{2B} and A₃. Adenosine A₁ or A_{2A} receptor antagonists may be useful for the treatment of acute and chronic neurodegenerative disorders including cerebral ischemia, Parkinson's and Huntington's diseases, cognitive deficits and Alzheimer's disease as well as for the treatment of peripheral diseases, such as cardiac failure [1, 2].

In our efforts to develop novel adenosine receptor ligands a series of N⁹-phenyl-substituted 1,3-dimethylpyrimido[2,1-

7]purinediones have previously been obtained and characterized as selective adenosine A_{2A} receptors antagonists [3, 4]. In the present work phenol derivatives of 1,3-dimethyl-6,7,8,9-tetrahydropyrimido[1,2-*f*]purine-2,4(1*H*,3*H*)-dione were considered as scaffolds for further modifications. The main purpose of the designed structures was to introduce polar moieties in order to improve the water solubility of the poorly soluble class of compounds.



Thus, a series of O-substituted hydroxyphenyl(alkyl) derivatives has been synthesized and investigated at adenosine receptor. Affinity data for rat adenosine A₁, A_{2A}, and human adenosine A_{2B} and A₃ receptors were collected as described before [5]. In most cases, tested compounds showed affinity towards adenosine A_{2A} receptor in the micromolar or submicromolar concentration range and lacked significant potency for the other adenosine receptor subtypes. The K_i value for the most potent A_{2A} AR ligand of the present series was 329 nM.

ACKNOWLEDGMENT

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REFERENCES

1. Fredholm, B.B. *et al.* Pharm. Rev.; **2001**; 53(4): 527-552;
2. O. Yuzlenko, K. Kieć-Kononowicz.; Curr. Med. Chem.; **2006**; 13: 247-64;
3. Drabczyńska, A. *et al.* Bioorg. Med. Chem.; **2007**; 15(22):6956-6974;
4. Drabczyńska, A. *et al.* Bioorg. Med. Chem.; **2007**; 15(14):5003-5017;
5. Scheiff, A.B. *et al.* Bioorg. Med. Chem.; **2010**; 18(6):2195-2203.

POSTER COMMUNICATIONS – PC.151

A SIMPLE METHOD FOR SYNTHESIS OF S-PROLINE DERIVATIVES

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An object of the present work is to show simple method to synthesis of 4-cyclohexyl-S-proline as an intermediate product in the preparation of Fosinopril - prodrug used in the treatment of hypertension and acute or chronic congestive heart failure.

Our three-steps method including simple, common known reaction: aldol reaction, Michael reaction, hydrogen reduction.

In the first stage of the suggested synthesis we used aldol condensation. That powerful method was used to form carbon-carbon bonds between cyclohexane carboxaldehyde condensed with molecule containing an acidic hydrogen atom.

In the second step of the 4-cyclohexyl-S-proline synthesis the Michael reaction was used. In this is the nucleophilic addition of a carbanion to an α, β unsaturated carbonyl compound and formation of C-C bonds. The resulting product contains one asymmetric carbon atom. In an extension of those studies, we report amplification of our qualitative reports by determining the enantiomeric excess after second and third step of reaction.

Moreover, the reaction was carried out with the primary and secondary amines as catalyst. The final product was collected and purified by repeated column chromatography on silica gel. The structures were confirmed by spectroscopic methods: UV-vis, IR, ¹H NMR.

POSTER COMMUNICATIONS – PC.152

EXPLORING G-PROTEIN COUPLED RECEPTORS OLIGOMERIZATION INTERFACE WITH ADAPTIVE POISSON-BOLTZMANN SOLVER (APBS)

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The classical idea that G-protein coupled receptors (GPCRs) function as monomeric entities is increasingly giving way to the paradigm of dimerization-dependent GPCRs function (1). Furthermore, experimental and computational efforts have led to the concept that GPCRs exist not only as homodimers and heterodimers, but also that their oligomeric assembly could have important functional roles. However, at present there is only one entry for a GPCRs dimer in the Protein Data Bank, namely the homodimer of rhodopsin (PDB ID 2I37) (2) which is claimed to be stable upon receptor activation. Although the monomers in this dimer interact through the interface formed by the first and second transmembrane (TM1-TM2) helices, most experimental evidence indicate the interface constituted by the fourth and fifth transmembrane regions (TM4-TM5). Atomic Force Microscopy has confirmed this interface for the rhodopsin homodimer, providing experimental support for the construction of a corresponding model that is now widely accepted (3).

However, our recent investigation, using iterative protein-protein docking methods demonstrated the possible existence of addi-

tional interfaces (particularly that of TM1-TM2) besides TM4-TM5 (4). On the basis of these results, we have further explored the oligomerization interface of GPCRs with Adaptive Poisson-Boltzmann solver (APBS) (5).

APBS is a software package for modeling biomolecular solvation through solution of the Poisson-Boltzmann equation (PBE) and is one of the most popular continuum models for describing electrostatic interactions between molecular solutes in a given medium. Using VMD and APBS we built the system to simulate receptor molecules in a membrane placed between water layers. The obtained patterns of electrostatic interactions allowed to rationalize our earlier results obtained with iterative protein-protein docking and to point out key regions of transmembrane bundles and intracellular loops of GPCRs engaged in the oligomerization process.

REFERENCES

1. Filizola, M. *Life Sci.* **2010**, in press.
2. Salom, D.; Lodowski, D.T.; Stenkamp, R.E.; Le Trong, I.; Golczak, M.; Jastrzebska, B.; Harris, T.; Ballesteros, J.A.; Palczewski, K. *Proc. Natl. Acad. Sci.*, **2006**, *103*, 16123.
3. Fotiadis, D.; Liang, Y.; Filipek, S.; Saperstein, D.A.; Engel, A.; Palczewski, K. *FEBS Lett.* **2004**, *564*, 281.
4. Kaczor, A.; Selent, J.; Pastor, M. unpublished results.
5. Baker, N.A.; Sept, D.; Joseph, S.; Holst, M.J.; McCammon, J.A. *Proc. Natl. Acad. Sci.* **2001**, *98*, 10037.

POSTER COMMUNICATIONS – PC.153

ITERATIVE PROTEIN-PROTEIN DOCKING AS A METHOD FOR MODELING G-PROTEIN COUPLED RECEPTORS DIMERS

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Until very recently, structure-based drug design methods using G-protein coupled receptors (GPCRs) as targets focused almost exclusively on the inhibition of a single receptor at the orthosteric binding pocket. However, more and more experimental evidence from AFM, FRET, BRET, cysteine cross-linking and receptor chimeras studies indicate the existence of physiologically relevant GPCR dimers/oligomers. Targeting these complexes selectively or designing small molecules that inhibit receptor-receptor interactions might provide new opportunities for novel drug discovery (1). In order to study the mechanisms and dynamics which rule GPCRs oligomerization, it is essential to understand the dynamic process of receptor-receptor association, and to identify regions that are suitable for selective drug binding. Since nearly no X-ray structure of any GPCRs dimer is currently

available, various computational techniques have been applied for modeling of GPCRs dimers, based on the sequence analysis, protein-protein docking algorithms, or molecular dynamics techniques. As it has recently been indicated in our study (2), commonly used protein-protein docking approach may prove unsuitable for membrane proteins. This drawback lies in the fact that most protein-protein docking algorithms were built considering only globular and water-soluble proteins.

In this scenario, the aim of this work was the elaboration of an efficient protocol for enhancing results of protein-protein docking for modeling GPCRs dimers. Two dimers were studied: rhodopsin-rhodopsin homodimer and the recently published mGluR2-5HT_{2A} receptor heterodimer (3). The method involved the application of Rosetta software (4) to obtain populations of dimers with all the possible interfaces followed by scoring according to (i) surface of dimer interface, (ii) the ratio of hydrophobic versus polar surface and (iii) the surface of selected amino acids in dimer outface. The best models were minimized and the whole cycle was iteratively repeated until the results converged to a consistent dimer formation. In the case of the rhodopsin dimer, a consistent percentage in the highest-scored population of dimers was obtained immediately after the first cycle for dimers which direct their transmembrane helix (TM) TM4 and TM5 into the dimer interface. For the mGluR2-5HT_{2A} receptor dimer, several runs led to TM45 interface for 5-HT_{2A} receptor and TM56 for mGluR2. The proposed protocol might be used as an efficient approach for the modelling of GPCRs dimers.

REFERENCES

1. Filizola, M. *Life Sci.* **2010**, in press.
2. Kaczor, A.; Selent, J.; Pastor, M. unpublished results.
3. Bruno, A.; Guadix, A.E.; Costantino, G. *J. Chem. Inf. Model.* **2009**, *49*, 1602.
4. Daily, M.D.; Masica, D.; Sivasubramanian, A.; Somarouthu, S.; Gray, J.J. *Proteins.* **2005**, *60*, 181.

POSTER COMMUNICATIONS – PC.154

MORACIN O AND MORACIN P AS HIF-1 α INHIBITORS

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Moracins O and P were isolated from the oriental medicine Mori Cortex Radicis. The moracin O (IC₅₀ = 0.14 nM) and P (IC₅₀ = 0.65 nM) exhibited excellent inhibitory activity against hypoxia inducible factor (HIF-1). HIF-1, a key mediator for adaptation process of cancer cells to tumor hypoxia, has emerged as an important molecular target for anticancer drug discovery. Herein we report the synthetic methodology for moracin O and P. We initially have accomplished syntheses of the moracin O and P via 2-prenylated phenol as a common intermediate. The key steps

during the process of synthesis are regiospecific intramolecular cyclization. Using 2-prenylated phenol, benzohydrofuran moiety in moracin O was proceed through epoxidation and subsequent intramolecular cyclization with base and benzohydropyran moiety in moracin P was formed by *in situ* epoxidation following intramolecular cyclization with acid. This synthetic methodology was further utilized for derivatization of moracin O and asymmetric synthesis.

REFERENCES

1. Harris AL. *Nat. Rev.*, **2002**, 2, 38–47.
2. Navneet Kaur, Yan Xia, Yinglan Jin, Nguyen Tien Dat, Kondaji Gajulapati, Yongseok Choi, Young-Soo Hong, Jung Joon Lee and Kyeong Lee, *Chem. Commun.*, **2009**, (14), 1879 – 1881.
3. Amato Giaccia, Bronwyn G. Siim and Randall S. Johnson, *Nature Reviews Drug Discovery*, 2003, 2, 1-9.

POSTER COMMUNICATIONS – PC.155

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 1,4-DITHIOSUBSTITUTED PHTHALAZINES AND 1,2-BIS-(4-(THIOSUBSTITUTED)-PHTHALAZIN-1-YL)-DISULFIDES

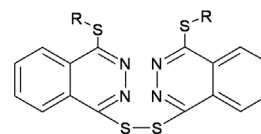
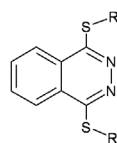
losu Jiménez^{1*}, Elena Ibáñez¹, Iranzu Lamberto¹, Daniel Plano¹, María Font², Ignacio Encío³, Juan Antonio Palop^{1*}, Carmen Sanmartín¹.

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A serie of 1,4-dithiosubstituted phtlazines have been designed in order to combine the biological effects attributed to the heterocycle ring and sulfur derivatives separately. Cytotoxicity, VEGFR-2 inhibition, and DNA bisintercalation are the main therapeutic activities^{1,2} observed for phtlazinic derivatives; while organic and inorganic sulfur compounds have been used in medicine due to their antioxidant, apoptosis induction and chemopreventive properties³⁻⁵.

The synthesis of thioderivatives was performed by aliphatic nucleophilic substitution of different benzyl and alkyl halides with 1,4-dithiophthalazine, or by aromatic electrophilic substitution of 1,4-di-chlorophthalazine with the corresponding benzylthiol.

In order to study the relationship between molecular symmetry and biological activity⁶ a serie of 1,2-bis-(4-(thiosubstituted)-phtlazin-1-yl)-disulfides have been prepared by aliphatic nucleophilic substitution of different benzyl and alkyl halides with 1,2-bis-(4-(thiol)-phtlazin-1-yl)-disulfide.



R= H, CH₃, benzyl, 4-chlorobenzyl, 4-substitutedbenzyl.

All products are being tested as cytotoxic agents against five human cancer cells: CCRF-CEM (leukemia), HT-29 (colon adenocarcinoma), HTB-54 (lung carcinoma), MCF-7 (breast adenocarcinoma) and PC-3 (prostatic adenocarcinoma); and a non-tumoral cell line 184B5 (breast epithelium). The biological activity results will allow us to compare 1,4 symmetrical substitution and dimeric symmetry.

Currently, we are working on a new synthetic derivatives based on the sulfur atoms substitution by selenium in order to study if this modification increases the biological activity of our compounds.

REFERENCES

1. Xin Zhai; Juan Li; Lei He; Su Zheng; Yao Bin Zhang; Ping Gong. *Chinese Chemical Letters*. 19 (2008)29-32.
2. Evgueni L. Piatnitski, Matthew A. J. Duncanson, et al. *Bioorganic & Medicinal Chemistry* 16 (2006) 1579 -1581.
3. Erin, E. Battin; Julia ; *Journal of Inorganic Biochemistry* 102 (2008) 2036–2042.
4. Juli Bai; Ziqiang Meng. *Regulatory Toxicology and Pharmacology* 43 (2005) 272–279
5. Rosa M. Pascale; Maria M. Simile; Maria R. De Miglio; Francesco Feo. *Alcohol* 27 (2002) 193–198
6. Sanmartín, C.; Font, M.; Palop, J.A. *Mini-Reviews in Medicinal Chemistry*, 2006, 6, 639-650.

POSTER COMMUNICATIONS – PC.156

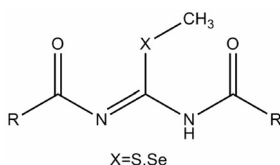
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW IMIDOSELENOCARBAMATES AS POTENTIAL ANTIPROLIFERATIVE AGENTS IN CANCER TREATMENT

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Nowadays, the *in vitro* and *in vivo* activity of a great variety of organic derivatives of Selenium¹ has provided new aims in drug

development of potential antitumoral agents. Setting as the principal aim the search and optimization of new structures with well determined characteristics, the design, synthesis and biological evaluation of new imidoselenocarbamates as potential antitumoral agents have been carried out. Therefore, the design has been based in a structural study of known cytotoxic molecules, which has provided a number of essential features for the activity²⁻⁵, among which the symmetry, a selenomethyl group as central element and an amide group as connection between the central element and the active heteroaromatic groups seem to be the most important. These heteroaromatic groups have been chosen by their known antitumoral activity they have by themselves. Satisfying our aims, the synthesis and biological evaluation of 20 derivatives with the following general structure are presented in this work.



To confirm how atoms with similar chemical properties, as Sulfur and Selenium, have a different biological activity, 10 imidoselenocarbamates and their sulfurated analogues are shown. The results confirm the different activity of the derivatives in all the studied cell lines. MCF-7 (breast) and HT-29 (colon) lines are the most sensible to this kind of compounds, reaching GI_{50} values of nM in some of the derivatives.

REFERENCES

1. Sanmartín, C.; Plano, D.; Palop, J.A. Selenium Compounds and Apoptotic Modulation: A New Perspective in Cancer Therapy. *Mini-Rev. Med. Chem.* 8, 1020-1031, 2008.
2. Plano, D.; Sanmartín, C.; Palop, J.A. Novel potent organoselenium compounds as cytotoxic agents in prostate cancer cells. *Bioorg. Med. Chem. Lett.* 17, 6853-6859, 2007.
3. Sanmartín, C.; Font, M.; Palop, J.A. Molecular Symmetry: A Structural Property Frequently Present in New Cytotoxic and Proapoptotic Drugs. *Mini-Rev. Med. Chem.* 6, 639-650, 2006.
4. Cubedo E, Cordeu L, Bandres E, Rebollo A, Malumbres R, Sanmartín C, Font M, Palop JA, Gacía-Foncillas J. New symmetrical quinazoline derivatives selectively induce apoptosis in human cancer cells. *Cancer Biol Ther.* 5(7), 850-859, 2006.
5. Font M, Ardaiz E, Cordeu L, Cubedo E, García-Foncillas J, Sanmartín C, Palop JA. Structural characteristics of novel symmetrical diaryl derivatives with nitrogenated functions. Requirements for cytotoxic activity. *Bioorg Med. Chem.* 14(6), 1942-1948, 2006.

POSTER COMMUNICATIONS – PC.157

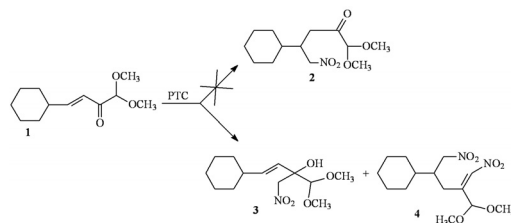
INVESTIGATION OF SYNTHESIS OF 4-CYCLOHEXYL-(S)-PROLINE - CRUCIAL INTERMEDIATE IN FOSINOPRIL PREPARATION

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Looking for an efficient, environment friendly and economical large scale laboratory synthesis of 4-cyclohexyl-(S)-proline – an intermediate in the preparation of Fosinopril – prodrug used in the treatment of hypertension and acute of chronic congestion heart failure, we proposed a three step reaction scheme, crucial step of which consists on Michael reaction between easy available ketoacetal **1** and nitromethane.

It is obvious, that proper selection of the base-solvent system used for reactions induced by bases is of crucial importance. We choosed Phase Transfer Catalysis - the most general, efficient and environment-friendly methodology of performing organic reactions in which organic anions react with organic substrates. According to this methodology reactions are performed in immiscible two-phase systems. One phase (inorganic) is a base for generation of organic anions. The second (organic) phase contains organic reactants, usually neat or sometimes in appropriate solvents. Upon introduction of a catalyst – tetraalkylammonium salt – continuous transfer of reacting anions, produced in the interfacial region, into the organic phase in the form of lipophilic ion pairs with the catalyst cation takes place. All further reactions occur in the organic phase. Earlier we applied this methodology for syntheses of some intermediates in preparation of Anastrozole and Sibutramine. We found, that instead of **2** a mixture of two products is formed in the reaction mentioned – namely **3** and **4**, their ratio being dependent on reaction time and temperature.



3 and **4** are highly functionalized compounds and may be used in syntheses of other products, possessing biological activity.

POSTER COMMUNICATIONS – PC.159

HOW THE RECRUITMENT OF COACTIVATORS REFLECTS THE CONFORMATION OF THE ESTROGEN RECEPTOR

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When it associates to estrogenic ligands, the estrogen receptor (ER) adopts an agonist conformation that allows its dimerization and phosphorylation. In addition, its interaction with estrogens confers ability to recruit coactivators. Briefly, coactivators are proteins required for transcription that engulf, through their LxxLL motif (where L correspond to leucine and x to any amino-acid) in a shallow hydrophobic binding site located at the surface of ER. It is of note that LxxLL motifs (or NR-boxes) adopt a α -helical conformation when associated with ER. The recruitment of LxxLL motifs is closely related to the orientation of the ER helix H12. Indeed, in the presence of antagonists, H12 occupies the LxxLL-binding site, preventing thereby the recruitment of coactivators. In contrast, in presence of agonists, H12 projects in distance of the LxxLL-binding site, allowing the recruitment of coactivators.

However, little is known about inter-relations existing between ligand-binding affinities, transcription and the recruitment of LxxLL motifs. To explore these relationships, we measured the effects of a panel of ligands on transcription as well as on the recruitment of LxxLL motifs. Data were compared to the ability of the ligands to compete with tritiated estradiol.

Transcriptional activities displayed by ligands were strongly associated with their ability to induce the recruitment of LxxLL motifs, particularly in the case of type I agonists (steroids, DES, flavones and isoflavones). In contrast, in the case of type II agonists (triphenylethylenes, cis-diphenylethylenes), transcriptional activities were less dependent upon the recruitment of LxxLL. This feature may explain why such derivatives are considered as partial agonists.

Our study suggests that the pharmacological evaluation of ER ligands of therapeutic interest should be tested not only with respect to transcription but also with respect to their ability to recruit LxxLL motifs. This approach may contribute to elucidate their mode of action as well as the related conformational status of ER. At least, our approach could be relevant to a better understanding of Structure-Activity Relationships associated with estrogens.

REFERENCES

1. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757.

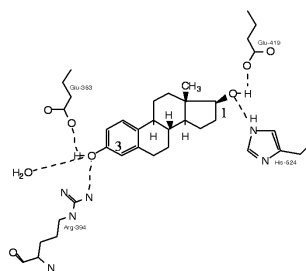
POSTER COMMUNICATIONS – PC.160

MODULATION OF THE STEROID ESTROGEN PHARMACOPHORE THROUGH INTRAMOLECULAR ELECTRONIC EFFECTS: APPLICATIONS TO STRUCTURE / AFFINITY RELATIONSHIPS

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Steroidal estrogens interact with the estrogen receptor (ER) to enhance transcription of target genes. This interaction requires various ER amino-acids among which the Arg-353 (helix H3), the Glu-394 (helix H6) and the His-524 (helix H11). In fact, the His-524 interacts with the hydroxyl in position 17 β of the steroid core structure of ligands whereas the Arg-353 and the Glu-394 interact with their phenolic hydroxyl in position 3 (with the contribution of a structure molecule of water) as shown in Scheme 1 [1]. The latter interaction appears as an absolute requirement for an efficient association with ER. Accordingly, it is usually considered that the presence of a phenolic group in a hydrophobic molecule of similar size than 17 β -estradiol is sufficient for ER recognition.



Scheme 1: Interaction of 17 β -estradiol within the ligand-binding pocket of the estrogen receptor.

A number of estradiol derivatives substituted in 11 β have been synthesized and their pharmacological profile extensively explored with respect to ER. Remarkably, these compounds are of great interest and exert a panel of actions through ER binding.

Remarkably, long hydrophobic chains exert an antagonist activity by engulfing within a pocket encompassing the helix H3 and different from the one accepting the ligand, limiting therefore

the flexibility of H3 through intermolecular hydrophobic contacts as well as the recruitment of coactivators [2]. In the case of short substituents in 11 β , the mechanisms by which ligands exert estrogenic effects are still obscure and do not appear to be related to such behaviour. By using mass spectrometry, we showed that the effects exerted by these short substituents are partially intramolecular [3]. Thus, the nature of the substituents as well as their stereochemistry may exert significant electronic effects that could modulate the acidity of the 3-phenolic hydroxyl. Therefore, we assume that such effects could influence the affinity as well as the ER-dependent transcriptional activity of steroid estrogens.

Our work suggests that Structure / Affinity Relationships studies associated with estrogens should not be restricted to the optimization of the intermolecular interactions by generating new anchoring points. Indeed, the optimisation of the anchoring strengths stabilizing ligand / ER association through indirect electronic effects should be also considered.

REFERENCES

1. Jacquot *et al.* Mini-Rev. Med. Chem. (2003); 3: 333-346;
2. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757;
3. Bourgoïn-Voillard *et al.* J. Am. Soc. Mass Spectrom. (2009); 20: 2318-2333.

POSTER COMMUNICATIONS – PC.161

MOLECULAR MECHANISMS THAT MODULATE ESTROGEN RECEPTOR-MEDIATED TRANSCRIPTION : A DYNAMIC APPROACH

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The estrogen receptor subtype α (ER α) is a nuclear transcription factor of 66 kDa implicated in various ligand-dependent (or independent) physiological and malignant processes such as breast cancer. ER α ligands activate (agonists) or repress (antagonists) transcription by modifying the conformational status of ER α as well as the recruitment of specific protein partners (coactivators and corepressors). With regard to steroidal ligands, both steric and electronic effects provoked by substitutions in position 11 β were found to influence the ER α conformation. Indeed, intramolecular electronic effects modulate the acidity of the 3-phenolic hydroxyl of these ligands [1] and regulate thereby the dynamic of the receptor's helix H3, which encompasses key residues important for the recruitment of co-activators. Indeed, the interaction between H3 and ligands is mediated through

hydrogen contacts between the Glu-353 (helix H3) / Arg-394 (helix H6) side chains and the 3-phenolic hydroxyl [2,3].

Recently, we found that these conformational modifications modify another surface region of ER α containing a type II β -turn [4]. In fact, this type II β -turn is subjected to a spatial reorientation depending upon the nature of the ligand, suggesting that it is a crucial regulatory region of ER α . An extensive study of this region reveals that it is surrounded by a mixed α -helix / polyproline II motif (residues 295 to 311) included within the autonomous regulatory function of the receptor. This motif, which corresponds to a regulatory platform subjected to a number of post-translational modifications (acetylation, phosphorylation, méthylation, SUMOylation, ubiquitylation), encompasses the third NLS of ER α , confers calmodulin-binding ability [5,6] and is sensitive to proteolysis. The corresponding peptide (ER α 17p, P₂₉₅LMIKRSKNSLALS₃₁₁), which interacts with holo-calmodulin in the micromolar range through a entropy-driven mechanism, associates with ER α [7] and the heat-shock protein Hsp70 [8], which is known to modulate receptor conformation and associated transcriptional activity. These properties may explain our finding, i.e that ER α 17p induces estrogenic responses exclusively in ER α -positive breast carcinoma cells and provokes the degradation of ER α through a proteasomal-dependent process. Curiously, this peptide inhibits the recruitment of the p160 co-activator motif LxxLL, suggesting that it may support an additional antagonist activity.

Thus, we have identified a new platform that could be useful for new therapeutic approaches. Functional and biomolecular characteristics of ER α , reported here, may contribute to the design of new estrogen antagonists devoted to the treatment of breast tumors.

REFERENCES

1. Bourgoïn-Voillard *et al.* J. Am. Soc. Mass Spectrom. (2009); 20: 2318-2333;
2. Jacquot & Leclercq *in* Estrogens: Production, Functions and Applications, Ed. Nova Science Publishers, Inc (2009);
3. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757;
4. Jacquot *et al.* J. Steroid Biochem. Mol. Biol. (2007); 104: 1-10;
5. Gallo *et al.* *in* Medicinal Chemistry Research Progress, Ed. Nova Science Publishers, Inc (2009);
6. Gallo *et al.* Mol. Cell. Endocrinol. (2008); 291: 20-26;
7. Gallo *et al.* Lett. Drug Design Discov. (2007); 4: 346-355;
8. Gallo *et al.* J. Steroid. Biochem. Mol. Biol. (2008); 109: 138-149.

POSTER COMMUNICATIONS – PC.162

HYDRATION SITE THERMODYNAMICS
EXPLAIN STRUCTURE-ACTIVITY
RELATIONSHIPS FOR TRIAZOLYPURINE
ANALOGUES BINDING TO THE A2A
ADENOSINE RECEPTOR*Christopher Higgs, Thijs Beuming, Woody Sherman*Schrodinger, Inc., 120 West 45th Street, 17th Floor, New York, New York 10036

A series of triazolypurine analogues shows interesting and uninituitive SAR against the A2A adenosine receptor. As the 2-substituted aliphatic group is initially increased to methyl and isopropyl there is a decrease in potency; however, extending the substituent to n-butyl and n-pentyl results in a significant gain in potency. This trend cannot be readily explained by ligand-receptor interactions, steric effects, or differences in ligand desolvation. Here, we show that a novel method for characterizing solvent thermodynamics in protein binding sites correctly predicts the trend in binding affinity for this series based on the differential water displacement patterns. In brief, small unfavorable substituents occupy a region in the A2A adenosine receptor binding site predicted to contain stable waters, while the longer favorable substituents extend to a region that contains several unstable waters. The predicted binding energies associated with displacing water within these hydration sites correlate well with the experimental activities.

KEYWORDS

A2A adenosine receptor, antagonist, GPCR, WaterMap, binding energy, desolvation

POSTER COMMUNICATIONS – PC.163

NOVEL CALPAIN INHIBITORS WITH
ANTICATARACT PROPERTIES*Rodrigo Alarcón, Adrián Luna, Mónica Ortiz,
Carlos F. Lagos and *Patricio Huenchunir*

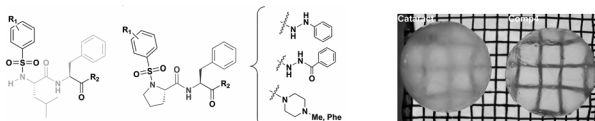
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Cortical cataract is associated with Ca²⁺ overload and protein loss, where an established feature is that there are major structural modifications to the water-soluble crystallins. It has become increasingly clear that the elevated levels of Ca²⁺ associated with some lens insults can induce crystalline proteolysis by calpains.

Calcium activated proteases calpains are known to occur in the lens, and the anterior epithelial layers and cortical regions of human cataract have been shown to contain calpain 2, a key enzyme implicated in the genesis of some forms of cataract. We have used a structure-based approach to design novel potential calpain 2 inhibitors, which might interfere with cataract progression.

To test the effect of test compounds on cataract formation, an in vitro bovine lenses assay was developed. Bovine lenses can be maintained in culture and remain transparent for at least 10 days. Incubation with CaCl₂ causes opacification in these lenses. Control and compound-treated lenses were pre-incubated in culture buffer, and their opacities determined by means of optical densitometry measurements, after cataract induction by addition of CaCl₂ (15 mM) and incubation for 20 h.

The synthesized calpain 2 inhibitors show IC₅₀ values in the micromolar range and prevent opacification of isolated bovine lenses when exposed to a CaCl₂ solution. Molecular modeling experiments show that these compounds assume an extended β -sheet-like structure shown by other calpain inhibitors.



ACKNOWLEDGMENTS

FONDEF Project N° D08I1139 and CONICYT for CFL PhD fellowship.

POSTER COMMUNICATIONS – PC.164

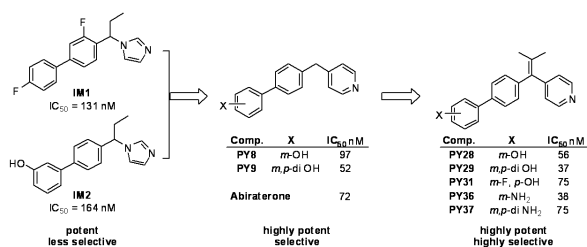
BIPHENYL METHYLENE HETEROCYCLES AS
POTENT CYP17 INHIBITORS FOR THE
TREATMENT OF PROSTATE CANCER: FROM
IMIDAZOLES TO PYRIDINES*Qingzhong Hu, Carsten Jagusch, Ulrike E. Hille,
Rolf W. Hartmann*

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It is well known that the growth of up to 80 % of prostate carcinoma depends on androgen stimulation. Therefore, segregation of tumour cells from androgen will effectively prevent cancer cell proliferation. However, current standard therapy "combined androgen blockade" (CAB) often leads to resistance which can be associated with androgen receptor mutations. The mutated androgen receptor recognizes antagonists or glucocorticoids as agonists leading to the collapse of CAB therapy.

Therefore, inhibition of CYP17, which is the crucial enzyme in androgen biosyntheses, was proposed as a promising therapy because it totally blocks androgens formation in testes and adrenals, as well as inside cancer cells. The recent success in phase II and III clinical trials of steroidal CYP17 inhibitor Abiraterone demonstrates the benefits of CYP17 inhibition as therapy for castration-resistant prostate cancer. However, due to possible side effects associated with steroidal scaffolds, non-steroidal CYP17 inhibitors were designed and synthesized.

By mimicking the natural substrates, several series of biphenyl methylene heterocycles were designed as CYP17 inhibitors. Although potent inhibitors have been identified in imidazoles¹ showing favorable pharmacokinetic properties,² they were not sufficiently selective. After replacing imidazolyl by 4-pyridyl, improvements of activity and selectivity were observed. Inspired by the imidazole SARs that substituents on the A-ring and the methylene bridge showed profound influence on the activity, thorough investigation was performed on the pyridyl scaffold. It has been found that H-bond forming groups furnishing on the A-ring largely elevated the activity. As for the methylene bridge, flexible alkyl groups reduced potency, whereas conformation rigidifying *i*-propylidene groups significantly improved activity and selectivity. These efforts resulted in compounds more potent than or comparable to the drug candidate Abiraterone ($IC_{50} = 72$ nM). Most of these potent compounds also exhibited better selectivity profiles toward CYP11B1, CYP11B2, CYP19 and hepatic CYP3A4 than Abiraterone. They can be considered as promising drug candidates after further validation *in vivo*.



REFERENCES

- Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Hartmann, R. W. et al. *Bioorg. Med. Chem.* **2008**, *16*, 7715–7727.
- Hu, Q.; Negri, M.; Olgen, S.; Hartmann, R. W. *ChemMedChem*. accepted.

POSTER COMMUNICATIONS – PC.166

NC-2500, A NOVEL AND ORALLY ACTIVE XANTHINE OXIDOREDUCTASE INHIBITOR

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Gout, a chronic disease that results from hyperuricemia, is one of the most painful forms of arthritis. Xanthine oxidoreductase (XOR) catalyzes the terminal steps in urate synthesis in humans and animals, thus XOR inhibitors are the mainstay of treatment for hyperuricemia in patients with gout. NC-2500, a thiazolopyrimidine derivative, is a novel, orally active inhibitor of XOR. NC-2500 inhibited XOR activity derived from bovine milk and urate production by rat, dog and human hepatocytes with an IC_{50} of 10^{-9} mol/L. Besides, it exhibited a dose-dependent and highly potent plasma urate-lowering effect after oral administration in a rat model of oxonate-induced hyperuricemia and in normal dogs; the minimum effective dose was 0.3 and 0.1 mg/kg, respectively. The effect of NC-2500 was particularly marked in dogs whose metabolism of NC-2500 in *in vitro* studies was similar to that of humans and it was more than 10 times more potent than that of febuxostat. NC-2500 also exhibited unique pharmacokinetic properties such as enterohepatic circulation, low covalent binding to human liver microsomes and high non-renal elimination.

These characteristics of NC-2500 may offer improved treatment for hyperuricemia over allopurinol and febuxostat, which are the currently available XOR inhibitors, at a very low therapeutic dose and with a potential low incidence of idiosyncratic drug toxicity and serious hepatotoxicity.

POSTER COMMUNICATIONS – PC.167

A COMPREHENSIVE COMPUTATIONAL STUDY OF ASPARTIC PROTEASES IN DRUG DESIGN

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Proteolytic enzymes (also known as proteases) are key regulators of a number of important biological processes in virtually all organisms. The family of aspartic proteases, where the catalytic center is constituted by two aspartic acids, has received much interest from the biopharmaceutical sector. The most successful

case in pharmaceutical research is the HIV-1 protease and the HIV-PI (protease inhibitors of HIV), but several aspartic protease inhibitors are in preclinical research or clinical trials in many diseases, including: malaria (plasmeprin inhibitors), Alzheimer disease (β -secretase inhibitors) or hypertension (renin inhibitors). Other aspartic proteases are discussed as potential drug target.¹ Comparisons of inhibitor binding within the aspartic peptidase family has been a very important approach to improve the structure-based drug design, and this continues to be a fertile area.

This report is part of a project addressed to build a computational platform to describe and compare the binding modes of inhibitors to different aspartic proteases. As a first step, we report a virtual screening protocol for HIV-p on the basis of the LIE method for calculation of ligand affinities.

HIV-p/inhibitor complexes for which there is experimental structural and affinity data have been collected from the PDBbind database (<http://www.pdbbind.org>). The initial set was divided into a training set and a test set. LIE binding affinities were estimated for the test set, according to the general procedures described elsewhere,² on the basis of separate 0.5ns MD run of the ligand-protein complex and ligand-free states. A optimization of the LIE parameters preceded the application of the same procedure to the test set.

The LIE methodology demonstrated its utility in a VS protocol in order to discriminate high and low affinity ligands, with an associated error of less than 1 kcal/mol. Interestingly, for the present system under study (HIV-p), the optimal weighting factors of the corresponding electrostatic and non-electrostatic interactions in the LIE equation differed from the original parameters. In particular, an increase in the weight of the hydrophobic interactions was needed in order to better reproduce experimental results. Due to the relatively large size of the ligands, this results are in agreement with previous observations of LIE calculations on protein-protein interactions.³ The optimized model was consistently applied to the test set, and only a moderate increase up to 1.35 kcal/mol for the mean unassigned error was observed. A deeper investigation of the chemical nature of the ligands revealed that compounds with polyhydroxyl substitutions are most problematic. These observations are currently compared with a similar study on other proteases.

REFERENCES

1. Abbenante, G. and Fairlie, D.P. *Med Chem* 1, 71-104 (2005).
2. Aqvist, J., et al. *Protein Eng* 7, 385-391 (1994).
3. Almlöf M. et al. *Biophysical J* 90, 433-442 (2006).

POSTER COMMUNICATIONS – PC.168

ELECTRON-NUCLEAR QUINTESSANCE OF DRUGS THERAPEUTIC ACTION

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There are many different QSAR methods. All of them can be divided into two groups: the methods which consider molecular exterior and the methods considering molecular interior. For the first group of methods, the enough wide definition of exterior is used which can include not only molecular shape but also a distribution of charges, Van der Waals and electrostatic potentials, centers of potential hydrogen bonds and hydrophobic (or lipophilic) interactions etc. at the molecular surface. The significance of molecular exterior can hardly be underestimated. It is obvious that exterior determines molecular capability for intermolecular interactions. Though, frequently consideration of exterior is not enough for description of properties. The simple example of that is the pair of lists of cardboard and steel having the same form and the same sizes (i.e. the same exterior) but possessing by extremely different properties. Thus, the exterior shows the immediate properties of an object while the interior determines its potential capabilities, abilities for changes under the external influence. Therefore, in this work we suggested to use the combination of the methods – exterior-based BiS/MC [1] and interior-based ConGO [2] algorithms. The first one permits to determine conformational state of a molecule interacting with the target, while the second one is used for detailed study of the electron structure of the selected conformers. The distinguishing feature of the approach is the usage of quantum wave functions for description of molecular interior.

The work of the approach was demonstrated under consideration of DNA-antimetabolites, fXa inhibitors, inhibitors of 5-HT_{1A} and α_1 -AR receptors. The biological activities of the drugs were evaluated with very high quality – in all cases CrossR2 exceeded 0.90. The analysis of the sets permitted to determine pharmacophoric and antipharmacophoric fragments of drugs electronic structures. The consideration of fXa inhibitors showed the high quantum similarity of the most active molecules of the set while less active fXa inhibitors demonstrated considerable differences in their electron structures. Study of the molecules interacting with competing 5-HT_{1A} and α_1 -AR receptors revealed that intercalation of high active molecules is determined by folded conformation allowing their fitting into the receptor cavities without any problem. Interacting with the receptors, the molecules frequently have similar pharmacophoric electron fragments while their antipharmacophoric fragments defer significantly. The analysis of DNA-antimetabolites showed that in the case of the most active molecules the antipharmacophore fragments of the electron structure are, as a rule, located at distances exceeding the Van der Waals radii of atoms. The electron density is extremely low at such distances. Therefore, its negative influ-

ence on the activity level of high active compounds is reduced to the minimum. The points determining the antiparmacophore effect can be mostly attributed to methylene fragments of the structures. Probably these fragments of the molecules increase the structure volume interfering their intercalating into the receptor (DNA).

ACKNOWLEDGMENTS

SKIF GRID supercomputer initiative.

REFERENCES

1. Potemkin V., Grishina M. Principles for 3D/4D QSAR classification of drugs// *Drug Discovery Today*, 2008, V. 13 (21/22), 952 – 959.
2. Potemkin V.A., Grishina M.A. A new paradigm for pattern recognition of drugs// *Journal of Computer Aided Molecular Design*.- 2008.- 22.-P. 489-505

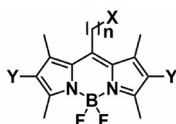
POSTER COMMUNICATIONS – PC.169

SYNTHESIS OF NOVEL FLUORESCENT-LABELED LIGANDS FOR STUDYING ADENOSINE RECEPTORS

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4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-s-indacene (BODIPY) dyes are useful tools to image processes in living cells due to their exceptional spectral properties, i.e. high absorption coefficients, fluorescence quantum yields ($\Phi > 0.5$) and peak intensities, photochemical stability, and their small size.^{1,2}



$n = 1, 2, 3, 4, 5, 10$; $X = Br, I, NH_2, OH, SH$; $Y = H, SO_3H$

Fig. 1: Functionalized BODIPY derivatives

In the present study we synthesized new, functionalized BODIPY derivatives, which fluoresce at approximately 500 nm and therefore do not interfere with biological fluorophores. Various functional groups (Br, I, NH_2 , SH, OH) were attached via alkyl spacers of different lengths to the BODIPY core in order to allow for their attachment to receptor ligands, or their integration into the pharmacophore of adenosine receptor ligands. All new, functionalized BODIPY derivatives showed high fluorescence quantum yields and absorption and emission wavelengths near 500 nm.

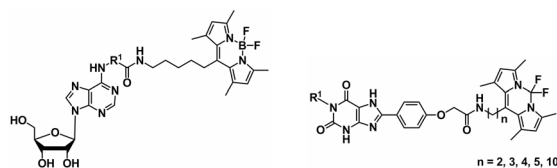


Fig. 2: Fluorescent-labeled adenosine receptor ligands

In a subsequent step we coupled these BODIPY dyes to adenosine and xanthine derivatives, respectively. The affinities of the obtained derivatives were determined in radioligand binding studies at membrane preparations cell lines expressing A_1 , A_{2A} , A_{2B} , or A_3 receptors, respectively.

Fluorescent A_1 -agonists and A_{2B} -antagonists were obtained, some of which show high potency (K_i values at low nanomolar concentrations) combined with receptor subtype-selectivity. The new fluorescent adenosine receptor ligands will be useful tools for studying adenosine receptor binding and function.

REFERENCES

1. Loudet, A.; Burgess, K. BODIPY dyes and their derivatives: synthesis and spectroscopic properties. *Chem. Rev.* **2007**, *107*, 4891-4932.
2. Middleton R.J.; Briddon S.J.; et al. New fluorescent adenosine A_1 -receptor agonists that allow quantification of ligand-receptor interactions in microdomains of single living cells. *J. Med. Chem.* **2007**, *50*, 782-793.

POSTER COMMUNICATIONS – PC.170

DISCOVERY OF POTENT AND SELECTIVE INHIBITORS OF DRUGGABLE CARBONIC ANHYDRASE ISOFORMS

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Carbonic anhydrases (CA, EC 4.2.1.1) are a family of metalloenzymes comprising various isoforms with different tissue and cellular localization. They play an important role in many physiological processes linked to the catalytic hydration of CO_2 to bicarbonate regulating respiration, pH, electrolyte secretion, biosynthetic reactions, bone resorption, calcification, etc. Furthermore, these enzymes are involved in several pathological pathways suggesting the development of carbonic anhydrase inhibitors (CAIs, Scheme 1) as new tools for diuretics, anticonvulsants, antiobesity and antitumor drugs. The most potent CAIs (see Figure) are characterized by the presence of a sulfon-

amide/sulfamate functionality coordinating the zinc ion and interacting with specific amino acid residues in the catalytic site.

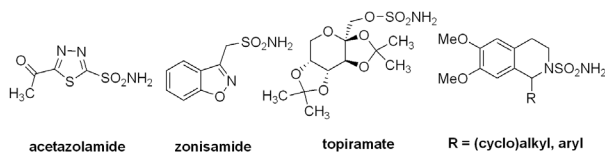


Figure. Carbonic anhydrase inhibitors (CAIs)

In previous works, we rationally designed and tested some isoquinolinesulfonamide derivatives as CAIs showing K_i values at nanomolar concentration as well as significant selectivity towards some druggable hCA isoforms (e.g. hCA VII, hCA IX, and hCA XIV).^[1,2] On this basis we herein report new studies addressed to deepen the structural understanding for carbonic anhydrase inhibition through SAR considerations, target-based computational approaches and crystallographic data of inhibitors in complexes with some selected hCA isoforms.

Research supported by MIUR- 20085HR5JK_002

REFERENCES

1. R. Gitto, S. Ferro, S. Agnello, L. De Luca, G. De Sarro, E. Russo, D. Vullo, C. T. Supuran, A. Chimirri, *Bioorg. Med. Chem.* **2009**, 17, 3659-3664.
2. R. Gitto, S. Agnello, S. Ferro, L. De Luca, D. Vullo, J. Brynda, P. Mader, C. T. Supuran, A. Chimirri, *J. Med. Chem.* **2010**, 53, 2401-2408.

POSTER COMMUNICATIONS – PC.173

ANALYSIS OF HIGH-ENERGY WATER SITES IN PROTEIN-PROTEIN INTERFACES: UNDERSTANDING AFFINITY, SELECTIVITY, AND APPLICATION TO DRUG DESIGN

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PDZ domains have well known binding preferences for distinct C-terminal peptide motifs. For most PDZ domains, these motifs are of the form [S/T]-W-[I/L/V]. Although the preference for S/T has been explained by a specific hydrogen bond interaction with a histidine in the PDZ domain and the (I/L/V) is buried in a hydrophobic pocket, the mechanism for Trp specificity at the second to last position has thus far remained unknown. Here, we apply a method to compute the free energies of explicit water molecules and predict that potency gained by Trp binding is due to a favorable release of high-energy water molecules into bulk. The affinities of a series of peptides for both wild-type and mutant forms of the PDZ domain of Erbin correlate very well with the computed free energy of binding of displaced waters,

suggesting a direct relationship between water displacement and peptide affinity. Finally, we show a correlation between the magnitude of the displaced water free energy and the degree of Trp-sensitivity among subtypes of the HTRA PDZ family, indicating a water-mediated mechanism for specificity of peptide binding. In a subsequent example we apply the same method on the protein-protein interface of Bcl-xl and rationalize the SAR of ABT737 chemical series based on release of high-energy water molecules.

POSTER COMMUNICATIONS – PC.174

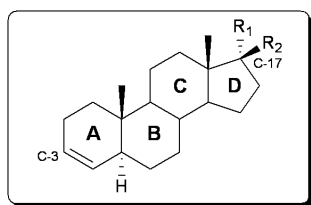
STRUCTURE-ACTIVITY RELATIONSHIPS ON D-RING MODIFIED 3-DEOXY-3-ANDROSTENES AS AROMATASE INHIBITORS

Carla L. Varela¹, Elisiário Tavares-da-Silva¹, Margarida M. D. S. Cepa³, Margarida Azevedo³, Cristina B. Amaral³, Georgina Correia-da-Silva^{2,3}, Natércia A. A. Teixeira^{2,3}, Fernanda Roleira¹

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Aromatase is a cytochrome P-450 enzyme that catalyses the aromatization of androgens, the final step in the biosynthesis of estrogens. Aromatase inhibitors (AIs) reduce the synthesis of estrogens and offer a therapeutic alternative for the treatment of estrogen-dependent cancers such as breast cancer.¹ Recently, the active site of aromatase has been elucidated, and the molecular basis for enzyme-substrate (androstenedione) interaction has been established,² which could lead to a more efficacious intervention at the level of estrogen production inhibition. Keeping this in mind, the design, synthesis and evaluation of new androstenedione-based inhibitors, containing into the A- and D-rings chemical key-features important for the enzyme-drug interaction, would greatly contribute for establishing new structure-activity relationships (SAR). These SAR are valuable tools for understanding the enzyme inhibition mechanism and to find more selective and potent AIs.

From the established SAR for steroidal AIs, it is known that the presence of oxygen functions at C-3 is not mandatory to have activity. On the contrary, some planarity in the A-ring and A/B-ring region seems to be important for aromatase inhibition.³⁻⁵ In this work, we present a structure-activity study, based on the synthesis and aromatase inhibitory activity evaluation, of steroid compounds having double bonds at C-3, which can confer the required planarity in the A/B-ring region, and different substituents at C-17. It was found that none of the 17-substituted 3-androstenes synthesized (**2-5**) is as good aromatase inhibitor as the 5 α -androst-3-en-17-one **1**.



- 1 R₁; R₂ (=O)
- 2 R₁; R₂ (=S)
- 3 R₁; R₂ (=NOH)
- 4 R₁ (H); R₂ (OH)
- 5 R₁ (H); R₂ (OCOCH₃)

REFERENCES

1. Chen, S. *Frontiers of Bioscience* **1998**, 3(9), 22. b) Blickenstaff, R. T. *Antitumor Steroids*, Academic Press (NY); **1992**.
2. Glosch, D.; Griswold, J.; Erman, M; Pangborn, W. *Nature* **2009**, 457, 219.
3. Nagaoka, M.; Watari, Y.; Yajima, H.; Tsukioka, K.; Muroi, Y.; Yamada, K.; Numazawa, M. *Steroids* **2003**, 68(6), 533.
4. Cepa, M.D.S.; Tavares da Silva, E.J.; Correia-da-Silva, G.; Roleira, F.M.F.; Teixeira, N.A.A. *J Med Chem* **2005**, 48, 6379.
5. Cepa, M.; Correia-da-Silva, G.; Tavares da Silva, E.J.; Roleira, F.M.F.; Teixeira, N. *Steroids* **2008**, 73, 1409.

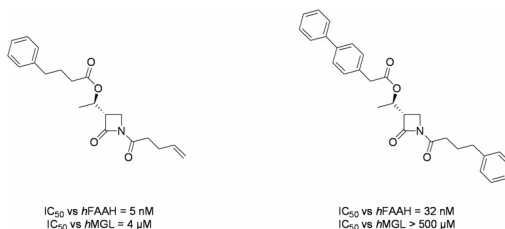
POSTER COMMUNICATIONS – PC.175

SYNTHESIS AND PHARMACOLOGICAL STUDIES OF NOVEL HUMAN FAAH INHIBITORS, BASED ON THE β -LACTAM TEMPLATE

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A library of thirty β -lactams has been prepared from (3*R*,4*R*)-3-[(*R*)-1'-(*t*-butyldimethylsilyloxy)-ethyl]-4-acetoxy-2-azetidinone, and the corresponding de-acetoxy derivative, by sequential *N*- and *O*-functionalizations with various ω -alkenoyl and ω -arylalkanoyl chains. All compounds were selective inhibitors of *h*FAAH versus *h*MGL, and IC₅₀ values in the nanomolar range (5–14 nM) were recorded for the best representatives. From time-dependant preincubation and rapid dilution studies, and from docking analyses in a homology model of the target enzyme, a reversible mechanism of inhibition of *h*FAAH is proposed.¹



REFERENCES

1. FELEDZIAK, M.; MICHAUX, C.; URBACH, A.; LABAR, G.; MUCCIOLI, G. G.; LAMBERT, D. M.; MARCHAND-BRYANERT, J. J. *Med. Chem.*, 2009, 52 (2), 7054-7068.

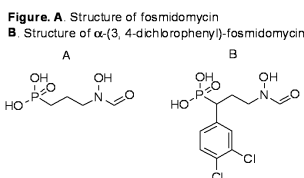
POSTER COMMUNICATIONS – PC.176

NOVEL α -ARYL SUBSTITUTED FOSMIDOMYCIN ANALOGUES AS *MYCOBACTERIUM TUBERCULOSIS* 1-DEOXY-D-XYLULOSE-5-PHOSPHATE REDUCTOISOMERASE INHIBITORS

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According to WHO, *Mycobacterium tuberculosis* (Mtb) the bacteria, which causes tuberculosis, infects one-third of the world's population causing 2 million deaths each year¹. Recently, it has been demonstrated that 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) is essential for *in vitro* growth of Mtb². The DXR enzyme is not present in humans, which makes it an interesting target for new and potentially selective antibacterial agents. Fosmidomycin, a natural antibiotic, which is a known inhibitor of *E. coli* and *P. falciparum* DXR, inhibits Mtb DXR with an IC₅₀ of 80 nM. Still, due to the lack of uptake it does not affect the growth or viability of Mtb. One of the approaches that can be used to improve the Mtb cell wall permeability is to increase the general lipophilicity of the compound. Therefore we identified α -(3, 4-dichlorophenyl)-fosmidomycin, previously reported as a *P. falciparum* DXR inhibitor³, as an interesting starting point for the development of novel Mtb DXR inhibitors. We have resynthesized the compound and demonstrated that it potently inhibits Mtb DXR (IC₅₀ = 150 nM).



However, when tested for activity on Mtb whole cells it lacked activity (MIC>32 μ g/ml).

X-ray crystallographic studies were therefore initiated and the Mtb DXR structure with the inhibitor was solved. Analysis of the structure suggested that an unexploited hydrated cavity near the 3, 4-dichlorophenyl ring could potentially be used for optimizing the binding of this structural class of compounds.

REFERENCES

1. WHO, <http://www.who.org>.
2. Brown, A. C.; Parish, T. *BMC Microbiol.* **2008**, 8, 78.
3. Haemers, T.; Wiesner, J.; Van Poecke, S.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Van Calenbergh, S. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1888-1891.

POSTER COMMUNICATIONS – PC.177

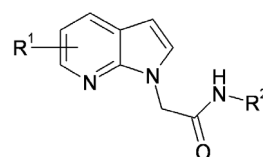
DISCOVERY OF 7-AZAINDOLES AS A NOVEL CLASS OF KCNQ2/KCNQ3 ACTIVATORS

Cécile Pégurier¹, Isabelle Niespodziany¹, Christian Wolff¹, Marc De Ryck¹, Hélène Bruyère², Stéphane Leleu², Gérald Guillaumet² and Sylvain Routier²

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The KCNQ2/KCNQ3 channel (M-channel) is a voltage-gated potassium channel generating an outward rectifying K⁺ current leading to cell membrane hyperpolarization. M-channels are predominantly expressed in the CNS and in dorsal root ganglion (DRG) neurons and their role consist in controlling resting membrane potential, responsiveness to synaptic inputs and spike frequency adaptation. The current generated by the KCNQ2/KCNQ3 channels can be linked to neuronal hyperexcitability, in that their activation may convey anti-epileptic, analgesic and neuroprotective activity. Retigabine (Valeant Pharmaceuticals), a potent M-channel activator, is under approval by FDA and EMEA for the treatment of epilepsy and has also proven effective in animal models of neuropathic pain, anxiety and migraine.

In this poster, we would like to report the identification of a novel class of KCNQ2/KCNQ3 activators: the 7-azaindole acetamide derivatives. Full details of synthesis and structure-activity relationships are given as well as the evaluation of one derivative in an animal model of persistent pain.



POSTER COMMUNICATIONS – PC.178

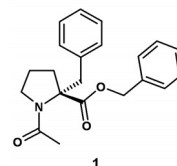
TOWARDS A KCC2 BLOCKER PHARMACOPHORE MODEL

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KCC2 is a cation-chloride co-transporter which acts as a modulator of inhibitory neurotransmission, both in the brain and in the spinal cord, hence its growing interest as a new target for neuronal hyperexcitability disorders such as epilepsy or neuropathic pain. We previously reported¹ the discovery and the optimization of a new family of potent and selective KCC2 blockers which can be represented by the lead compound **1**.

This poster describes the development of the first pharmacophore model for KCC2 blockers. Since no information was available concerning the structural requirement for binding to KCC2, a multi-disciplinary approach was used to identify the bioactive conformation of the lead compound **1**: several physico-chemical studies such as X-ray diffraction and NMR were combined to molecular modeling techniques, SAR analysis and synthesis of constrained analogues in order to determine a subset of potential bioactive conformations. These conformations were further compared to the conformational spaces of different chemical series of KCC2 blockers in order to identify the KCC2 pharmacophore which was validated by the discovery of more potent analogues.



The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5296).

REFERENCES

1. Pégurier, C; et al. *Bioorg. Med. Chem. Lett.* (2010), 2542.

POSTER COMMUNICATIONS – PC.179

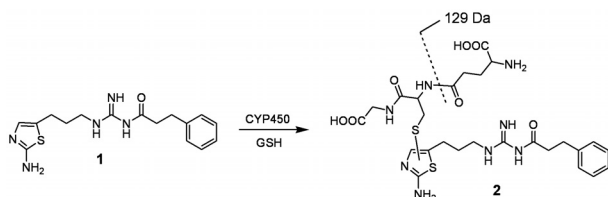
REACTIVE INTERMEDIATES IN THE BIOTRANSFORMATION OF AMINOTHIAZOLE-TYPE HISTAMINE H₂ RECEPTOR AGONISTS: DETECTION BY GLUTATHIONE TRAPPING USING LC/MS

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We previously reported that *N*^G-acylated 2-aminothiazol-5-ylpropylguanidines are potent and highly selective histamine H₂ receptor agonists.¹⁾ There is strong evidence that CYP450-mediated bioactivation of the 2-aminothiazole ring results in highly reactive intermediates, capable of covalent protein binding. Such reactions may be associated with idiosyncratic adverse drug reactions such as drug-induced hepatotoxicity.^{2), 3)} Therefore, the identification of the toxic potential of new chemical entities is mandatory at an early stage of drug discovery and development. In view of future pharmacological *in vivo* investigations, representative *N*^G-acylated 2-aminothiazol-5-ylpropylguanidines were studied with respect to the formation of reactive intermediates. We explored the toxic potential *in vitro*, using phenobarbital-induced rat liver microsomes and glutathione (GSH) as a trapping reagent for electrophilic reactive metabolites. Neutral loss (NL) scanning with a triple quadrupole LC/MS system was applied to detect putative covalent glutathione adducts. The NL of 129 Da corresponding to pyroglutamate is a common fragmentation pathway of glutathione-containing compounds.

As detected by NL scanning, the histamine H₂ receptor agonist AK471 (**1**) is activated by CYP450 enzymes to give a glutathione-containing trapping adduct (**2**), which was further characterized by MS/MS.



REFERENCES

1. Kraus, A., et al., Chem. Med. Chem. **2009**, 4, 232-240.
2. Subramanian, R., et al., Chem. Res. Toxicol. **2010**, 3, 653-663
3. Park, B. K., et al., Toxicology **2000**, 153, 39-60

POSTER COMMUNICATIONS – PC.180

STUDIES ON SYNTHESIS AND *IN VITRO* CYCLOOXYGENASE INHIBITORY ACTIVITIES OF DIARYL HETEROCYCLIC COMPOUNDS

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In this study, twenty-eight original of thirty-two compounds having 2-oxo-5*H*-furan, 2-oxo-3*H*-1,3-oxazole and 1*H*-pyrazole as central ring while 3-methyl-2-oxo-3*H*-benzoxazole and 4-sübstitutedphenyl attached were synthesized. Compounds were differentiated by substitution of phenyl ring with small groups such as OCH₃, Cl, F, SO₂CH₃ at *para* position.

Based on the literature, the compounds synthesized are expected to have COX inhibitory activity. The activities of the final fifteen compounds for possible COX inhibitory activity were performed by *in vitro* Purified Enzyme Assay at 10 µM concentration and the IC₅₀ values of the active compounds were calculated. A 2-oxo-5*H*-furan derivative Compound **7** and 1*H*-pyrazole derivatives Compound **25**, **26**, **27** have COX-2 IC₅₀ values as 0.325, 0.011, 0.011, 1.000 µM; COX-1 IC₅₀ values as 0.061, 0.004, 1.000 and 6.000 µM, respectively.

Molecular modelling studies which were performed on the binding site of COX-1 and COX-2 using the compounds having COX inhibitory activity, support the results of *in vitro* enzyme assay.

POSTER COMMUNICATIONS – PC.181

EFFECT OF CEREBROCRIST ON GLUCOSE AND LIPID METABOLISM, BODY AND ORGAN WEIGHTS, FOOD AND WATER INTAKE OF NORMAL RATS

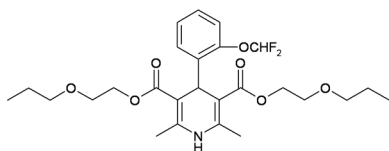
Janina Briede, Mara Stivrina, Dzintra Stoldere, Ilga Dabina, Brigita Vigante and Gunars Duburs

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Impaired carbohydrate and lipid metabolism, and hyperinsulinemia are the early pathogenic factors of obesity and Type 2 diabetes. In subjects with Type 2 diabetes in humans and rodent models there were observed: 1) resistance of adipose tissue,

muscle and liver to insulin; 2) excessive accumulation of lipids in nonadipose tissues as skeletal muscle, liver, heart, pancreatic beta cells that can lead to functional impairment these organs, body weight gain, polyuria, polydipsia etc.

In our previous study we showed that a new antidiabetic agent cerebrocrast (C), derivative of 1,4-dihydropyridine, normalized glucose levels in the organism, intensified and restored the action of insulin [1]. The aim of this work was: to investigate the effect of C on normal rats glucose and lipid metabolism, body weight, food and water intake.



C administration at doses of 0.05 and 0.5 mg/kg b. wt. (p.o.) for 3 consecutive days significantly increased glycogen content in the liver, decreased blood glucose and glycogen level in the kidney and heart. C partially inhibited fatty acid β -oxidation in liver mitochondria, and therefore favoured glucose oxidation and prevented the accumulation of lactate in the cells. C promoted lipolysis, thereby providing energy for tissue and cells, decreasing triacylglycerol content in organs, levels of β -hydroxybutyrate in blood.

C, during the experimental period (from 3 to 27 days after the last administration), decreased body weight by an average of approximately 32.3%, food intake by about 10-15% at the beginning of the experiments and by 22.6% at the end of the experiments. C has long-lasting effects on these parameters. These properties of C are important for treatment of Type 2 diabetes.

ACKNOWLEDGMENTS

This research work was supported by COST D34 and VP-05-07

REFERENCES

1. J. Briede, M. Stivrina, Dz. Stoldere, B. Vigante, G. Duburs Effect of cerebrocrast, a new long-acting compound on blood glucose and insulin levels in rats when administrated before and after STZ-induced diabetes mellitus. *Cell Biochem Funct* 2007; **25**: 673-680.

POSTER COMMUNICATIONS – PC.182

PLASMODIUM FALCIPARUM DNA GYRASE A AND ITS MOLECULAR DOCKING STUDY WITH CIPROFLOXACIN ANALOGUES

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59650 Villeneuve d'Ascq cedex, France. ² Université Lille Nord de France, Inserm U547, Institut Pasteur, 1 Rue du Pr Calmette, B.P. 245, 59019 Lille Cedex, France.

Structure-activity relationship studies were carried out by chemical modification of ciprofloxacin (Fig. 1), a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. The analogues combining bioorganometallic chemistry and the prodrug approach were evaluated for antimalarial activity. The novel compounds exhibited potent and improved in vitro antimalarial activity, being 10- to 100-fold more active than ciprofloxacin against *Plasmodium falciparum* chloroquine-susceptible and chloroquine-resistant strains.¹

As fluoroquinolones are thought to inhibit the plastid gyrase, the mode of binding of ciprofloxacin and its analogues to DNA gyrase was analyzed by means of docking calculations over the surface of the QRDR (quinolone resistance-determining regions) of GyrA.

On the basis of the results presented here, it will be possible to initiate rational drug design efforts around this fluoroquinolone scaffold for the treatment of malaria.

REFERENCES

1. Dubar, F., Anquetin, G., Pradines, B., Dive, D., Khalife, J., Biot, C. *J. Med Chem.* 2009, 52, 7954-7957.

This article has been selected for a highlight in Nature/SciBX.

POSTER COMMUNICATIONS – PC.183

NOVEL INHIBITORS OF ANGIOGENESIS THAT BLOCK THE PRODUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR INDUCED BY HYPOXIA

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Tumour angiogenesis, the formation of new blood vessels from the preexisting vasculature, is stimulated by several growth factors. From these, the main regulator of physiological and pathological angiogenesis is the vascular endothelial growth factor (VEGF), which is highly stimulated by hypoxia-inducible-factor (HIF-1). VEGF is required for tumour growth, invasion and metastasis in many animals models. In this work we searched for molecules with the ability to inhibit the production of VEGF in tumoral cells induced by hypoxia. For this purpose, we analyzed the biological activity of about 200 structurally-related com-

pounds synthesized by the Department of Organic Chemistry and Pharmaceuticals. Specifically, we measured their effects on cell viability, cell proliferation and on the inhibition of hypoxia-induced VEGF secretion in the lung cancer cell line H157. We found that several imidoselenocarbamate derivatives effectively blocked the production of VEGF stimulated by hypoxia in the low micromolar range. These compounds did not inhibit the classical pathways that regulate HIF-1 α expression, such as PI3K-Akt or MAP-kinase pathways, suggesting that they regulate VEGF production by a HIF-1 independent mechanism. We are performing molecular modelling studies to establish structure-activity relationships that may help us to direct the search of more active derivatives. The knowledge of the mechanism of action of these compounds and their structure-activity relationships, would make possible the design of novel and more effective inhibitors of pathological angiogenesis.

POSTER COMMUNICATIONS – PC.184

NOVEL QUINAZOLINE DERIVATIVES AS CYTOTOXIC AGENTS IN SEVERAL CANCER CELL LINES

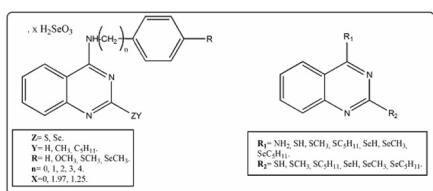
Esther Moreno^{1*}, Enrique Domínguez¹, Ylenia Baquedano¹, Daniel Plano¹, María Font², Ignacio Encío³, Juan Antonio Palop¹, Carmen Sanmartín¹

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Selenium is an essential trace element for human health. There are some indicators that selenium in doses above the physiological requirement has biological effects and can be applied in the prevention and therapy of several diseases^[1]. Epidemiological studies have suggested that low blood selenium levels were associated with an increased incidence and mortality from various kinds of cancer^[2]. Some potential mechanisms have been proposed for organoselenium compounds in cancer chemoprevention: antiandrogen activity, antioxidant functions, DNA repair^[3]...

In order to continue with the researched work of our group^[4,5], the synthesis and biological evaluation of new quinazoline derivatives have been proposed, according to the general structures:



Cytotoxic activity of all the compounds has been evaluated in four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and two non-malignant cell lines (184B5 and BEAS). For all of the cell lines, the following cytotoxic parameters have been determined: LD₅₀, TGI y GI₅₀. Several compounds show some cytotoxic activity, being more potent than some of the drugs currently used in clinical trials, such as: etoposide and cis-platine.

REFERENCES

- Behne, D.; Alber, D.; Journal Trace Elements in Medicine and Biology; in press.
- Chen, Y.; Hall, M.; Graziano, J. H.; Slavkovich, V.; Van Geer, A.; Parvez, F.; Ahsan, H.; Cancer Epidemiology Biomarkers Prevention; 16: 207, **2007**.
- Sanmartín, C.; Plano, D.; Palop, J. A.; Mini Reviews in Medicinal Chemistry; 8: 120-131, **2008**.
- Cubedo, E.; Cordeu, L.; Bandres, E.; Rebollo, A.; Malumbres, R.; Sanmartín, C.; Font, M.; Palop, J. A.; García-Foncillas, J.; Cancer Biology and Therapy; 5:7, 850-859, **2006**.
- Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J. A.; Bioorganic and Medicinal Chemistry Letters; 17, 6853-6859, **2007**.

POSTER COMMUNICATIONS – PC.185

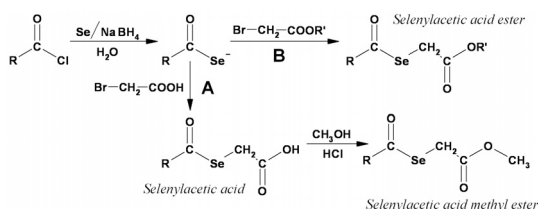
ESTERIFICATION IMPROVES BIOLOGICAL ACTIVITY OF SELENYLACETIC ACIDS

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Selenium and its organic and inorganic derivatives fulfil a lot of biological functions and have been used in cancer prevention and chemotherapy¹. Several selenocompounds, like diphenyldiselenide² and Se-methylselenocysteine³, are under investigation because of their cytotoxic and anticarcinogenic properties. Due to these antecedents, our research group designed, synthesized and determined biological activity parameters of twenty-six novel selenocompounds with the general formula Ar/Het-CO-Se-CH₂-COOH.⁴ The five most active selenylacetic acids obtained had a good cytotoxicity activity in prostate cancer cell line (PC-3), but activity of the remaining ones was below our expectatives.

Consequently, we decided to study esterification, a logical structural modification of the previous compounds, and planned the chemical synthesis of carboxylic esters of selected selenylacetic acids. Methyl, *tert*-butyl and phenyl esters had been isolated and the compounds evaluated in PC-3 cancer cell line had, in general, better biological results than the corresponding acids.



Methyl esters chemical synthesis was carried out following the Fischer-Speier mechanism: the corresponding selenylacetic acid was solved in methanol and heated at boiling temperature in presence of hydrochloric acid (method A). Acids used as reagents are synthesized⁴ by reaction of the appropriate aryl or heteroaryl chloride with sodium hydrogen selenide in a first step and with α -bromoacetic acid in a second one. Non-methyl esters were obtained using a synthetic pathway similar to the method described above: in the alternative method B hydrogen selenide reacted with the pertinent α -bromoacetic acid ester instead of doing it with α -bromoacetic acid.

REFERENCES

1. Boosalis, M.G. *Nutr. Clin. Pract.* **2008**, 23(2): 152-160.
2. Rosa, R.M.; Picada, J.D.; Saffi, J.; Henriques, J.A.P. *Mu. Res.* **2007**, 628: 87-98.
3. Hurst, R.; Elliot, R.M.; Goldson, A.J.; Fainweather-Tait, S.J. *Cancer Lett.* **2008**, 269: 117-126.
4. Sanmartin, C.; Plano, D.; Domínguez, E.; Font, M.; Calvo, A.; Prior, C.; Encío, I.; Palop, J.A. *Molecules*, **2009**(14): 3313-3338.

POSTER COMMUNICATIONS – **PC.186**

A NEW CONCEPT FOR THE CONTROL OF PROTEASE ACTIVITY: SUBSTRATE-DEPENDENT MODULATION.

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IDE (Insulin-Degrading Enzyme) is an enzyme known for decades, yet remaining poorly understood. It is an ubiquitous, highly conserved Zinc metalloprotease that is involved in the clearance of many physiologically important peptides. There is a renewed interest for IDE since the last few years as both functional and genomic studies have placed IDE at the crossroads between type 2 diabetes and Alzheimer's disease. Indeed, amongst other peptides, insulin and β -amyloid are two cognate substrates of IDE. Moreover, the X-ray structure of IDE was solved by Tang et al. (1) in 2006. Initially, our project was to design inhibitors to study the role of IDE in cells and in normal and sick.

In the course of that project, we have discovered a series of drug-like modulators of IDE (2). X-ray analysis of ligand-enzyme complexes, revealed that unexpectedly these compounds are exosite ligands of IDE. Consistently with their peculiar binding mode, these compounds behave as inhibitors or activators of the enzyme in a substrate-dependent manner.

We present Structure-“Substrate-Profile”-Activity Relationships in the light of several X-ray structures.

At last some compounds were optimized for cell-permeation and results on several cell-based assays are presented and discussed.

The mode of action of our compounds opens new avenues both for the study of the function of IDE and for the design of therapeutic interventions.



Binding IDE in the exosite.

REFERENCES

1. Shen, Y., et al., Structures of human insulin degrading enzyme reveal a new substrate recognition mechanism, *Nature* 2006, 443,(7113), 870
2. Deprez-Poulain, R., et al. European Patent Ligands of Insulin Degrading Enzyme and their uses, March 2010.

POSTER COMMUNICATIONS – **PC.187**

ISOMERIC BENZOYLPIRROLYLACETIC ACIDS AS ALDOSE REDUCTASE INHIBITORS. AN IN SILICO STUDY

Vassilis J. Demopoulos

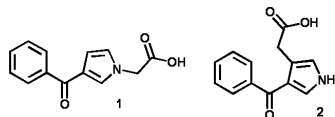
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Diabetes mellitus is a complex metabolic disorder and is one of the world's most common diseases and one of the biggest public health problems. The diabetic individual is prone to late onset complications that are largely responsible for the morbidity and mortality observed in the patients. Aldose reductase enzyme (AR, ALR2, E.C. 1.1.1.21) of the polyol metabolic pathway was first found to be implicated in the etiology of the secondary complications of diabetes. Aldose reductase inhibitors (ARIs) have therefore been noted as possible pharmacotherapeutic agents. Although several ARIs have progressed to the clinical trials' level, only one (Epalrestat) is currently on the market and only in

Japan. However, the inhibition of the polyol pathway is considered to be a promising approach to control diabetic complications.¹ In previous studies we have shown that (3-benzoylpyrrol-1-yl)acetic acid (1) is an active ARI in the micromolar range while its isomer, (4-benzoyl-1H-pyrrol-3-yl)acetic acid (2), is virtually inactive.^{2,3} In the present work the differences of 1 and 2 were studied via in silico methodologies. Thus, it was found that 1, in comparison with 2, exhibits a higher partitioning value (calculated logP), a higher acidity (calculated pKa), a lower molecular polar surface area (calculated TPSA), and a higher calculated van der Waals molecular surface area. It should be noted that previous reports have highlighted the importance of both lipophilicity and molecular surface area for ARI activity, while there is an anion binding pocket located in the active site of ALR2.^{1,4,5} Finally, the low energy structures of both compounds 1 and 2 were superimposed on the crystal structure of the known ARI zoporlestat. It was found that the derived RMS_(n=3) values were 2.33 and 5.01 respectively, indicating a significant better fit for compound 1 with zoporlestat. ALR2 is an enzyme showing pronounced induced-fit adaptation⁶, thus we propose that the results of the present work could comprise a useful tool for designing new and potent ARIs.

The following softwares, alone or in combination, were used in the above work: ClogP, Pallas, Molgen, MacSpartan and Hyperchem.

Scheme



REFERENCES

- Alexiou et. al., *Curr. Med. Chem.*, 16, 734-752 (2009),
- Demopoulos and Rekka, *J. Pharm. Sci.*, 84, 79-82 (1995).
- Nicolaou et. al., *J. Med. Chem.*, 47, 2706-2709 (2004),
- Alexiou et. al., *Bioorg. Med. Chem.*, 16, 3926-3932 (2008),
- Chrysanthakopoulos et. al., *QSAR Comb. Sci.*, 28, 551-560 (2009)
- Zentgraf et. Al., *Angew. Chem. Int. Ed. Engl.*, 46, 3575-3578 (2007).

POSTER COMMUNICATIONS – **PC.190**

ADENOSINE A₁ RECEPTOR: SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW PARTIAL AGONISTS

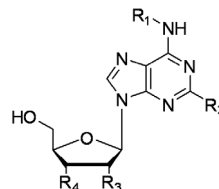
Diego Dal Ben¹, Michela Buccioni¹, Catia Lambertucci¹, Carmen Lammi¹, Gabriella Marucci¹, Sauro Vittori¹, Rosaria Volpini¹, Karl-Norbert Klotz², Gloria Cristalli¹

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Adenosine modulates cellular functions through the interaction with specific plasma membrane receptors classified in four subtypes as A₁, A_{2A}, A_{2B}, and A₃. Adenosine A₁ receptors (AA₁R) are present in cells throughout the body, and they mediate functions like slowing heart activity, reduction of lipolysis in adipose tissue, and inhibition of neuronal activity. Agonists of this subtype are useful in the treatment of acute and chronic disorders of heart rhythm such as atrial fibrillation, supraventricular tachycardia, and atrial flutter. Exposure to AA₁R partial agonists, which may selectively target a tissue and give functional response coupled to high AA₁R density, is preferred versus the use of full agonists due to the possibility to reduce unwanted side effects.¹

It is well known that the mono-substitution of adenosine at N⁶-position led to selective AA₁R ligands, while the lack of adenosine ribose hydroxyl groups produces the shift from full to partial activation of adenosine receptors.^{2,3}

Hence, a new series of N⁶-substituted 2'- or 3'-deoxyadenosine derivatives, bearing or not a chlorine atom in 2-position, have been synthesized. Preliminary biological results indicated that these compounds showed good affinity for AA₁R together with a sub maximal activation of the receptor.



R₁ = substituted (hetero)cycloalkyl
R₂ = H, Cl
R₃ / R₄ = H, OH

REFERENCES

- Dhalla, A. K.; Shryok, J. C.; Shreeniwas, R.; Belardinelli, L. Pharmacology and therapeutic applications of A₁ adenosine receptor ligands. *Curr. Top. Med. Chem.* **2003**, 3, 369-85.
- Vittori, S.; Lorenzen, A.; Stannek, C.; Costanzi, S.; Volpini, R.; Ijzerman, A. P.; von Frijtag Drabbe Kunzel, J. K.; Cristalli, G. N-Cycloalkyl derivatives of adenosine and 1-deazaadenosine as Agonists and Partial Agonists of the A₁ adenosine receptor. *J. Med. Chem.* **2000**, 43, 250-60.
- Cristalli, G.; Palle, V.; Zablocki, J. Partial and full agonists of A₁ adenosine receptors. US Patent 7163928, **2007**.

POSTER COMMUNICATIONS – **PC.191**

ADENOSINE RECEPTOR MODELING: THE IMPACT OF A_{2A} CRYSTAL STRUCTURE

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The evolution of the field of GPCRs modeling, including adenosine receptors (ARs), has depended on the availability of suitable receptor structural templates. For several years, modeling studies of GPCRs were based on the 3D structures of bacteriorhodopsin and bovine rhodopsin.¹ While the early models consisted basically on the transmembrane domains, the following studies led to improvement of the receptors structure by progressive insertion of intra- and extra-cellular domains and through refinement stages time by time more sophisticated. Rhodopsin-based modeling became the routine approach to develop adenosine receptor structural models. SAR and mutagenesis studies were not only target topics but also tools to refine theoretical models. The recent publication of the crystal structures of engineered adrenergic (beta1 and beta2)²⁻⁴ and adenosine A_{2A}R⁵ receptors confirm the helical arrangement similarity among the GPCRs, which can be explained in function of structural similarity of the interacting systems like G proteins or receptor kinases, but they also indicate a high variability in the GPCRs binding site leading to receptor diversity and ligand selectivity.

Comparison of rhodopsin- and adrenoceptor-based AR models with the AA_{2A}R crystal structure highlights deviations of modeling results from the experimental data, in particular in predicting the binding compound position and orientation, receptor extracellular domains fold, and the possible roles of water molecules in receptor-ligand interaction, but also it underlines the correctness of prediction of the role of key binding site residues in interaction with ligands. This data is supported by several docking and 3D-QSAR studies on ARs that allowed to design potent and often AR subtype selective ligands.

Even if it can be discussed the different utility for rational drug design of native and extensively engineered GPCRs, without doubt the AA_{2A}R crystal structure represents a breakthrough in X-ray field and provides a powerful tool to understand structural and functional properties of ARs.

REFERENCES

1. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000**, *289*, 739-45.
2. Warne, T.; Serrano-Vega, M. J.; Baker, J. G.; Moukhametzianov, R.; Edwards, P. C.; Henderson, R.; Leslie, A. G.; Tate, C. G.; Schertler, G. F. Structure of a β_1 -adrenergic G-protein-coupled receptor. *Nature* **2008**, *454*, 486-91.
3. Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258-65.
4. Hanson, M. A.; Cherezov, V.; Griffith, M. T.; Roth, C. B.; Jaakola, V. P.; Chien, E. Y.; Velasquez, J.; Kuhn, P.; Stevens, R. C. A specific cholesterol binding site is established by the 2.8 Å structure of the human β_2 -adrenergic receptor. *Structure* **2008**, *16*, 897-905.
5. Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y.; Lane, J. R.; Uzman, A. P.; Stevens, R. C. The 2.6 angstrom crystal structure of a human A_{2A} adenosine receptor bound to an antagonist. *Science* **2008**, *322*, 1211-7.

POSTER COMMUNICATIONS – PC.193

BIOLOGICAL ACTIVE PRENYLATED XANTHONES: RECENT PROGRESS IN SYNTHETIC METHODOLOGIES

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Xanthonic derivatives, namely prenylated xanthones have been reported to mediate some important biological activities, concerning a large variety of targets with therapeutic value [1-2]. This class of compounds has been found to exhibit interesting activities such as antibacterial, antifungal, PKC modulation and antitumor [1-2]. The presence of the prenyl groups becomes an important structural factor for the interaction of xanthones biological targets, taking into account the influence on the physico-chemical properties, including lipophilicity, and the overall stereochemistry of the molecule, which may create additional interactions [1]. On the other hand, the PKC isoenzymes are related to very important biological actions mainly in tumorigenesis. Clarification of the role of individual PKC isoforms in the cell has been difficult by the lack of selective PKC modulators [3].

In order to optimize the synthetic process to obtain compounds of this series and also to improve the yields, some new methodologies were followed, based on molecular modification strategies applied to 1,3-dihydroxy-2-methylxanthone (**1**) (Fig. 1) [4,5]. This compound was already evaluated as being active against the breast adenocarcinoma cell line (MCF-7) [4] as well as a PKC activator [6]. The experimental conditions involved microwave assisted organic synthesis (**1** via **A** to **2-3**; **3** via **B** to **4**) and heterogeneous catalysis using Amberlyst®15 as ion-exchange resin (**1** via **C** to **5-6**).

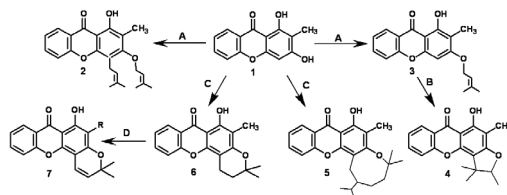


Figure 1:
A: Molecular Extension: Introduction of a lipidic chain
B: Molecular Rigidification: Introduction of an extra ring
C: Molecular Rigidification: Introduction of an extra ring
D: Molecular Rigidification: Inclusion of an insaturation

Compounds **2-4**, **6-7** were evaluated for their effect on the *in vitro* growth of three human tumor cell lines, MCF-7 (breast), NCI-H460 (non-small cell lung), SF-268 (central nervous system). Compound **5** is under study. It was also investigated the modulatory activity of some prenylated xanthones, on isoforms

α , β , δ , η and ζ of PKC. Xanthone **6** was found to exhibit a selective and potent growth inhibitory activity against the MCF-7 cell line, comparing with respective building block **1**, being able to become a new “hit” compound for this series. The results also showed that xanthone **1**, a non-selective PKC activator, gave rise to a new η -isoform-specific PKC inhibitor (**2**).

ACKNOWLEDGMENTS

FCT (I&D 4040/2007), FEDER, POCI for financial support.

REFERENCES

1. Pinto, M., Castanheiro, R., “Natural Prenylated Xanthenes: Chemistry and Biological Activities”, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, Índia, 2009, Chap. 17, pp.520-676.
2. Pinto, M., *et al.* *Curr. Med. Chem.* **2005**, *12*, 2517.
3. Webb, B., *et al.* *British J. Pharmacol.* **2000**, *130*, 1433.
4. Castanheiro, R., *et al.* *Bioorg. Med. Chem.* **2007**, *15*, 6080.
5. Pinto, M., Castanheiro, R. *Curr. Org. Chem.* **2009**, *13*(12), 1215.
6. Saraiva, L., *et al.* *Bioorg. Med. Chem.* **2002**, *10*, 3219.

POSTER COMMUNICATIONS – PC.194

PYRAZOLYLMETHYLCARBAMOYLMETHYL-PIPERAZINES AS CALCIUM CHANNEL BLOCKERS

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Calcium channels exist in nerve, muscle, and endocrine cells, and are responsible for a number of diseases. Of the subtypes of the calcium channels, we have been interested in the T-type channels that are known to be related to learning and memory, pain, epilepsy, etc. The first T-type calcium channel blocker, Mibefradil, was launched as an antihypertensive in 1997. The drug, however, was withdrawn in the next year due to drug-drug interaction.

Of many diseases related to the T-type calcium channels, we focused on neuropathic pain, and referring to the structure of Mibefradil and other CNS acting drugs, we designed pyrazole-containing structures that are expected to block T-type calcium channels selectively. A number of compounds were synthesized and evaluated for their biological properties.

In this presentation, we describe the synthesis of pyrazolyl-methylcarbamoymethylpiperazines and their calcium channel blocking activities, along with other biological properties, as an attempt to find new drug candidates for neuropathic pain.

POSTER COMMUNICATIONS – PC.195

DISCOVERY OF SELECTIVE AND POTENTIAL T-TYPE CALCIUM CHANNEL MODULATORS: SYNTHESIS AND BIOLOGICAL EVALUATION OF PIPERAZINE ANALOGUES

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T-type calcium channels, also known as low-voltage-activated calcium channels play an important role in the regulation of neuronal excitability in the central and peripheral nervous system. Recent studies showed that modulation of T-type calcium channels involves several pathophysiological diseases such as epilepsy, pain, hypertension, congestive heart failure and cancer. Therefore, potent and selective inhibitors are requested to explore the fundamental function of T-type calcium channels in such disease states.

Based on three dimensional ligand-based pharmacophore model, we have designed and synthesized a series of new piperazine derivatives. Their inhibitory activities on T-type calcium channels have been evaluated using *in-vitro* assay against α_1G and α_1H subtypes by FDSS screening system. Furthermore, we have selected the compounds with high potencies and then analyzed their selectivities over hERG and N-type calcium channels as well as their stabilities in both rat plasma and human hepatic microsomes.

REFERENCES

1. S. J. Gu, J. K. Lee, A. N. Pae, H. J. Chung, H. Rhim, S. Y. Han, S.-J. Min, Y. S. Cho. *Bioorganic & Medicinal Chemistry Letters in press*.
2. Y. Oh, Y. J. Kim, S. H. Seo, J. K. Lee, H. Rhim, A. N. Pae, K.-S. Jeong, H. Choo, Y. S. Cho. *Bull. Korean Chem. Soc.* **2008**, *29*(10), 1881-1882.
3. H. S. Kim, Y. Kim, M. R. Doddareddy, S. H. Seo, H. Rhim, J. Tae, A. N. Pae, H. Choo, Y. S. Cho. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 476-481.

POSTER COMMUNICATIONS – PC.196

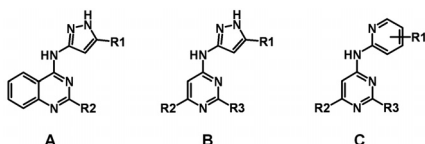
PKNB INHIBITORS AS ANTI-TUBERCULOSIS AGENTS

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Tuberculosis (TB) causes over 2 million deaths worldwide per year, and with the increasing prevalence of multi-drug resistant strains novel therapeutic approaches are urgently needed. PknB is one of 11 serine-threonine protein kinases found in *Mycobacterium tuberculosis* (*Mtb*) and has been shown to be essential for bacterial growth¹: therefore inhibition of PknB represents a potential therapeutic strategy for the treatment of TB.

A high-throughput screen of our compound collection against the PknB enzyme was conducted and hits were identified. The synthesis of analogues was performed and the structure-activity relationships around the template **A** (scheme 1) were investigated, which yielded inhibitors in the nanomolar range but with low efficacy against *Mtb* growth as determined in the Alamar Blue cell-based assay. Template modifications were made and potency against the enzyme was optimized around **B** and **C** (scheme 1) to give inhibitors in the low nanomolar range with improved physical properties. These compounds were shown to have Minimum inhibitory concentrations (MICs) in the low micromolar range against *Mtb*.



Scheme 1 Generalised structures of PknB inhibitors

REFERENCES

1. P. Fernandez et al., *Journal of Bacteriology*, **2006**, 188, 7778.

POSTER COMMUNICATIONS – PC.197

IDENTIFICATION OF ESSENTIAL AMINO ACID RESIDUES FOR PROTEIN-PROTEIN INTERACTION: APPLICATION OF A MICROWAVE PEPTIDE SYNTHESIZER

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In the framework of a collaborative project with different groups of the University of Leuven and the University of Edinburgh, a 13-mer peptide has been found active against the replication of the X (confidential) virus. In order to investigate the relevance of each residue in the protein-protein interaction responsible for the viral proliferation, different strategies have been applied such as Ala-scanning, fragmentation of the mother sequence, replacement with unnatural amino acid, cyclization.

Due to the relatively high number of peptides needed for this study and the complexity of the peptidic sequence, we decided to use a microwave peptide synthesizer, applying the Fmoc-strategy. This choice is due to the recent remarkable progress achieved in MASPOS (Microwave-Assisted Solid-Phase Organic Synthesis) which has led to a dramatic reduction of reaction times as well as to a higher purity of the products compared to conventional solid phase strategy. [1, 2, 3]

REFERENCES

1. Bacsa, B., Horváti, K., Bösze S., Andreae F. and Kappe C. O. *J. Org. Chem.*, **2008**, 73, 7532–7542.
2. Bacsa, B., Bösze S. and Kappe C. O. *J. Org. Chem.*, **2010**, 75 (6), 2103–2106.
3. Fara, M. A.; Diaz-Mochon, J. J.; Bradley, M. *Tetrahedron Lett.* **2006**, 47(6), 1011–1014.

POSTER COMMUNICATIONS – PC.198

SYNTHESIS AND BINDING AFFINITY OF NEW TETRALONE ANALOGUES OF HALOPERIDOL AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

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Schizophrenia is a complex disorder affecting approximately 1% of the population. For the treatment of this disease, classical (typical) neuroleptics such as haloperidol (**Fig. 1**) are currently used, but their use is associated with severe mechanism-related side effects, including induction of acute extrapyramidal symptoms (EPS), and they are ineffective against negative symptoms of schizophrenia [1].

The introduction of clozapine (**Fig. 1**) for treatment-resistant schizophrenia gave rise to a new group of atypical or non-classical antipsychotics which have no EPS and are effective against negative symptoms. These drugs exhibit potent antagonism at multiple receptor subtypes including serotonin and dopamine receptors, suggesting the implication of the serotonergic system in this pathology [2].

As part of our ongoing work to develop multi-target ligands for potential use as treatments for schizophrenia, we have studied the modulation of the butyrophenone system, with the aim of synthesizing a single molecule that can antagonize receptors of 5-HT₂ and D₂ families [3]. Therefore, we have obtained a variety of different butyrophenone analogues in which the rigidity of the haloperidol butyric chain was increased by its inclusion into a tetralone scaffold (**I, Fig.1**).

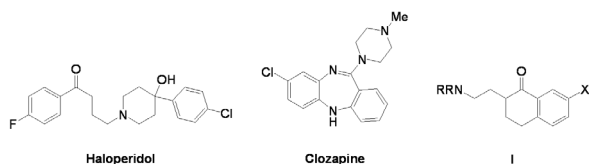


Figure 1

The synthetic route and the binding affinities of these new compounds on dopamine D₂ and serotonin 5-HT_{2A} and 5-HT_{2C} human receptors will be discussed in the communication.

ACKNOWLEDGMENTS

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REFERENCES

- Altar, A.; Martin, A. R.; Thurkauf, A. in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn.; Ed. D. J. Abraham, John Wiley & Sons, New Jersey, 2003, Vol. 6, p. 599.
- Marino, M. J.; Knutsen, L. J. S.; Williams, M. J. *Med. Chem.* **2008**, *51*, 1077.
- (a) Brea, J.; Rodrigo, J.; Carrieri, A.; Sanz, F.; Cadavid, M. I.; Enguix, M. J.; Villazon, M.; Mengod, G.; Caro, Y.; Masaguer, C. F.; Ravina, E.; Centeno, N. B.; Carotti, A.; Loza, M. I. *J. Med. Chem.* **2002**, *45*, 54. (b) Fontenla, J. A.; Osuna, J.; Rosa, E.; Castro, M. E.; Ferreiro, T.; Loza, M. I.; Calleja, J. M.; Sanz, F.; Rodríguez, J.; Ravina, E.; Fueyo, J.; Masaguer, C. F.; Vidal, A.; de Ceballos, M. L. *J. Med. Chem.* **1994**, *37*, 2564. (c) Carro, L.; Ravina, E.; Domínguez, E.; Brea, J.; Loza, M. I.; Masaguer, C. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6059.

POSTER COMMUNICATIONS – PC.200

THE DISCOVERY OF CDP323, A NOVEL AND POTENT INHIBITOR OF THE INTEGRINS $\alpha 4 \beta 1$ (VLA-4) AND $\alpha 4 \beta 7$

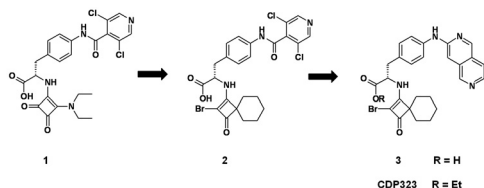
Julien Brown¹, Stuart Bailey¹, Stephen Brand¹, Ben de Candole¹, James Johnson¹, Paul Hale², Martyn Robinson², Tony Shock², Ailsa Webster², Lloyd King³, Ted Parton³, Tim Bourne⁴, Roly Foulkes⁴ and Neil Gozzard⁴

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The integrins are a family of heterodimeric cell-surface adhesion receptors which mediate trafficking of lymphocytes to sites of

inflammation. Substantial pharmacological evidence has implicated the integrins $\alpha 4\beta 1$ (VLA4) and $\alpha 4\beta 7$ in the pathogenesis of disorders including rheumatoid arthritis (RA), inflammatory bowel disease, asthma and multiple sclerosis (MS). As part of our anti-inflammatory programme we will describe the discovery of **CDP323**, a potential small molecule treatment for RA and MS.

The development of orally active $\alpha 4$ integrin antagonists, derived from the early peptide leads has been widely recognised as a key challenge in this field. We will discuss our progress starting from the previously reported¹ phenylalanine based squaric acid derivative **1**. As part of the optimisation process we evaluated the novel 3-amino-cyclobut-2-en-1-one scaffold as a versatile squaramide surrogate. Synthesis, SAR and *in vitro* profiling will be described. Optimisation of potency, *via* introduction of substituents at the 2 and 4 positions of the cyclobutenone, initially led to **2**. However, PK and oral bioavailability were markedly enhanced through replacement of the dichloropyridyl head group with a 2,7-naphthyridine leading to the wholly non-peptidic antagonist **3**. Prodrugs of **3** were designed leading to the ethyl ester, **CDP323**, as a development and clinical candidate. Compounds from this series are potent inhibitors of $\alpha 4\beta 7$ and $\alpha 4\beta 1$ dependent trafficking *in vivo* and significantly reduce clinical scores in a mouse collagen-induced arthritis model. **CDP323** has recently been evaluated in Phase II studies of MS.



REFERENCES

1. J.R. Porter et al, Bioorg. Med. Chem. Lett. **2002**, 12, 1015.

POSTER COMMUNICATIONS – PC.202

1-METHYL-3-PHENYLPYRROLIDINE AND 3-METHYL-1-PHENYL-3-AZABICYCLO[3.1.0]HEXANE AS MONOAMINE OXIDASE B SUBSTRATES

Jacobus J. Bergh,¹ Anél Pretorius,¹ Modupe O. Ogunrombi,¹ Neal Castagnoli, Jr.,² and Jacobus P. Petzer¹

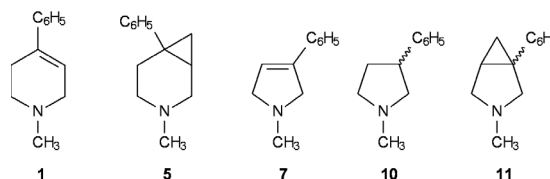
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It is well known that the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (**1**) acts as a substrate for monoamine oxidase B (MAO-B). The MAO-B substrate properties of the cyclic tertiary aminyl class of substrates are thought to be dependent on the presence of the allylic π -bond, the only known

exception being the 3,4-cyclopropyl analogue of MPTP, racemic 3-methyl-6-phenyl-3-azabicyclo[4.1.0]heptane (**5**). The π -bond characteristics of the cyclopropyl group may be responsible for the good substrate properties of **5**.

Another member of the cyclic tertiary allylaminyl class of MAO-B substrates, is the 5-membered ring MPTP analogue, 1-methyl-3-phenyl-3-pyrroline (**7**). In this study, we have synthesised and examined the interactions of the corresponding saturated pyrrolidinyl, [(±)-1-methyl-3-phenylpyrrolidine (**10**)] and 3,4-cyclopropyl, [(±)-3-methyl-1-phenyl-3-azabicyclo[3.1.0]hexane (**11**)], analogues of 1-methyl-3-phenyl-3-pyrroline with MAO-B.

The values of the steady-state kinetic parameters (K_m and V_{max}) for the baboon liver mitochondrial MAO-B catalyzed oxidation of the test substrates **10** and **11** were determined by measuring the initial rates of H_2O_2 production. Substrate **11** ($K_m = 148 \mu M$; $V_{max} = 16.9 \text{ nmol/min-mg protein}$) was shown to act as a good substrate for baboon liver MAO-B. An unexpected outcome was that the saturated pyrrolidinyl analogue **10**, also proved to be a MAO-B substrate ($K_m = 234 \mu M$; $V_{max} = 8.37 \text{ nmol/min-mg protein}$). This result suggests that the presence of an allylic π -bond or 3,4-cyclopropyl-fused system is not a prerequisite for cyclic tertiary amines to display MAO-B substrate properties and provides additional opportunities to map the nature of the interactions of MAO-B with tertiary aminyl substrates.



POSTER COMMUNICATIONS – PC.203

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL, N-BENZYLAMINOETHYL DERIVATIVES OF CHROMANYL IMIDAZOLETHIONE-BASED INHIBITORS OF DOPAMINE β -HYDROXYLASE

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BIA 5-453 (**1**), a peripherally-selective inhibitor of dopamine β -hydroxylase (DBH), is currently under clinical evaluation for the treatment of chronic heart failure and hypertension [1,2]. In the course of a search for peripherally-selective DBH inhibitors, novel N-benzyl derivatives **2** have been prepared and their biological activity has been studied [3].

1. Beliaev, A.; Ferreira, H.; Learmonth, D. A.; Soares-da-Silva, P. Dopamine β -Monooxygenase – Mechanism, Substrates and Inhibitors. *Current Enzyme Inhibition* **2009**, *5*, 27-43.
2. Beliaev, A.; Learmonth, D.A.; Soares-da-Silva, P. Synthesis and biological evaluation of novel, peripherally selective chromanyl imidazolethione-based inhibitors of dopamine β -hydroxylase. *J. Med. Chem.*, **2006**, *49*, 1191-1197.
3. Soares-da-Silva, P.; Learmonth, D.A.; Beliaev, A. 1,3-Dihydroimidazole-2-thione derivatives as inhibitors of dopamine β -hydroxylase. *PCT Int. Appl.* WO2008/136695A1. 13 Nov 2008.

of the compounds were identified by IR, ¹H-NMR, ESI-MS and elemental analysis data. Anticonvulsant activities were examined by maximal electroshock and subcutaneous Metrazol-induced seizure tests. Neurotoxicity was determined by the rotorod test. All these tests were performed in accordance with the procedures of the Antiepileptic Drug Development program⁴. Compound bearing 2,5-difluorobenzyl moiety showed the highest anticonvulsant activity at all doses at 4h against scMet-induced seizure test. Compounds also screened for their *in-vitro* antibacterial and antifungal activities. Standard strains of *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecalis*, and *B. subtilis* with their pathogens from clinical isolates as well as fungi such as *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* were used to ascertain the antimicrobial activity whereas ampicilline, gentamicine, levofloxacin, ketoconazole, and fluconazole was used as the standard positive control for antibacterial and antifungal activities, respectively. Microbroth dilution method recommended by Clinical and Laboratory Standards Institute is used. According to the results, 3- and 4-trifluorobenzyl derivatives showed significant antibacterial activity against Gram-negative (MIC: 8-16 µg/ml) and Gram-positive (MIC: 4-16 µg/ml), respectively. Important outcome of the exhaustive screening of all the new candidates in the present experiment was that the introduction of piperazine containing Mannich groups at the 6-position of chlorokojic acid nucleus significantly improved the biological activity. This study is supported by *H.U. Scientific Research Fond*.

REFERENCES

1. Aytemir MD, Çaliş Ü, Özalp M, Arch Pharm Chem Life Sci, 337, 281-288, 2004.
2. Aytemir MD, Çaliş Ü, Arch Pharm Chem Life Sci, 343, 173-181, 2010.
3. Aytemir MD, Özçelik B, Med Chem Res, DOI 10.1007/s00044-010-9338-x.
4. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Epilepsia 19, 409-428, 1978.

POSTER COMMUNICATIONS – PC.207

BZM055, A PET/SPECT TRACER CANDIDATE FOR STUDYING FTY720 BRAIN DISTRIBUTION AND MYELIN IMAGING

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FTY720 (fingolimod, Gilenia®) is a sphingosine 1-phosphate (S1P) receptor modulator that showed significant improvement in relapse rate and MRI outcomes after oral administration to multiple sclerosis (MS) patients.^[1] This drug prevents lymphocyte egress from lymph nodes,^[2] leading to a reduced infiltration

of lymphocytes into the central nervous system (CNS).^[3] Preclinical evidence also suggests that FTY720 may provide additional neuroprotection to the CNS via modulation of cerebral S1P receptors.^[4]

To further study the therapeutic action of FTY720, it would therefore be useful to quantify its pharmacokinetics and organ distribution in patients. As it has already been shown in rodents that FTY720 accumulates in myelin sheaths,^[5] we reasoned that an imaging agent based on this drug might also allow to image myelin and study disease progression, in addition to following the pharmacokinetics of FTY720 in the brain of MS patients.

FTY720 does not contain any atom whose Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) radioisotope would have a half-life compatible with its pharmacokinetic properties. It is therefore not possible to study this drug with such imaging techniques without modifying it slightly. With this in mind, we defined the criteria that would make a radiolabeled derivative of FTY720 an adequate surrogate of the drug, mimicking its pharmacokinetics and organ distribution, but also its affinity and selectivity for S1P receptors, as well as having similar overall physicochemical properties.

Furthermore, FTY720 itself is inactive at S1P receptors, but is phosphorylated *in vivo* by sphingosine kinase-2 to (S)-FTY720-phosphate, which is the active moiety binding to S1P receptors. Any derivative mimicking FTY720 should hence also be a substrate of SphK2, and be phosphorylated in a similar manner. Besides measuring phosphorylation rates of tracer candidates, we synthesized their phosphate analogues to enable the comparison of their affinity for S1P receptors, and adequately compare their overall properties to (S)-FTY720-phosphate.

From all candidate tracers profiled according to these criteria, we selected BZM055 for PET/SPECT radiolabeling and further characterization, as it is the closest mimic of the drug. Its profile is described in the poster.

REFERENCES

1. L. Kappos et al. N. Engl. J. Med. 2010, 362(5), 387.
2. a) M. Matloubian et al. Nature 2004, 427(6972), 355; b) S. Mandala et al. Science 2002, 296(5566), 346.
3. a) A. Compston et al. Lancet 2008, 372(9648), 1502; b) I. Bartholomaeus et al. Nature 2009, 462 (7269), 94; c) V. Brinkmann. Pharmacol. Ther. 2007, 115(1), 84; d) S.J. Jackson et al. Neurology 2009, 72 (Suppl. 3), A377 (P308.047); e) V.E. Miron et al. Ann. Neurol. 2008, 63(1), 61-71; f) Coetho RP et al. J. Pharmacol. Exp. Ther. 2007, 323(2), 626-635; g) C. Barske et al. Neurology 2008, 70 (11 suppl 1)
4. V. Brinkmann, Br. J. Pharmacol. 2009, 158(5), 1173-1182.
5. C.A. Foster et al. J. Pharmacol. Exp. Ther. 2007, 323(2), 469-475

POSTER COMMUNICATIONS – PC.208

IN VITRO AND IN VIVO EFFECTIVENESS OF NEUROPROTECTIVE L-GLUTAMIC ACID DERIVATIVES FOR THE POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE¹

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Solid phase synthesis was employed to obtain a series of L-glutamic acid derivatives,^{1,2} which exhibit attractive *in vitro* and *in vivo* biological activities for the potential treatment of Alzheimer's disease. At submicromolar concentrations, they efficiently inhibit acetylcholinesterase (AChE), displace propidium from the peripheral anionic site of the AChE, and protect neurons against damage caused by oxidative stress, okadaic acid, calcium overload, and beta-amyloid peptide (Abeta). They show no toxicity and could be able to penetrate into the central nervous system, according to an *in vitro* blood-brain barrier assay.³ A selected glutamic acid derivative shows neuroprotection against oxygen-glucose deprivation in rat hippocampal slices. In animal models, this L-glutamic acid derivative shows neuroprotection in mice subjected to a focal cerebral ischemia by photothrombosis or to an intrahippocampal injection of kainate. Consequently, it is expected that these multifunctional compounds could be able to improve cognitive impairment, diminish the oxidative damage caused by mitochondrial free radicals, and delay the degenerative process related to the excessive deposition of Abeta in patients suffering Alzheimer's disease.

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REFERENCES

- Conde, S.; Rodríguez-Franco, M. I.; Arce, M. P.; González-Muñoz, G. C.; Villarroya, M.; López M. G.; García, A. G. Novel dicarboxylic amino acid derivatives and the use thereof in the treatment of neurodegenerative diseases. PCT/ES2008/070221; WO 2009/074706 A1.
- Arce, M. P.; Rodríguez-Franco, M. I.; González-Muñoz, G. C.; Pérez, C.; López, B.; Villarroya, M.; G. López M.; García, A. G.; Conde, S. Neuroprotective and cholinergic properties of multifunctional glutamic acid derivatives for the treatment of Alzheimer's disease. *J. Med. Chem.*, **2009**, 52, 7249–7257.
- Fernández-Bachiller, M. I.; Pérez, C.; Campillo, N. E.; Páez, J. A.; González-Muñoz, G. C.; Usán, P.; García-Palomero, E.; López, M. G.;

Villarroya, M.; García, A. G.; Martínez, A.; Rodríguez-Franco, M. I. Tacrine – Melatonin Hybrids as Multifunctional Agents for Alzheimer's Disease, with Cholinergic, Antioxidant, and Neuroprotective Properties. *ChemMedChem* **2009**, 4, 828-841.

POSTER COMMUNICATIONS – PC.209

SYNTHESIS OF NOVEL ANTIMITOTIC ANALOGUES OF COMBRETASTATIN

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Combretastatins are natural products with antimitotic activity. They act by binding to tubulin at the colchicine site. One of the combretastatins problems is their low aqueous solubility. To improve this solubility we have synthesized and evaluated analogues with a pyridine moiety¹ replacing the trimethoxyphenyl ring.

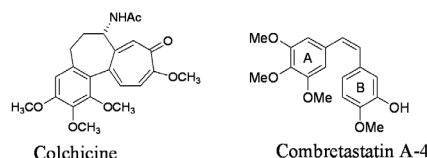


Figure 1. Structure of colchicine and combretastatin A-4.

New pyridine combretastatins have been synthesized by means of Wittig reaction between the corresponding phosphonium salt and 2,6-dimethoxypyridine-4-carbaldehyde, which was synthesized from citrazinic acid in four steps. In order to test the capability of the methoxylated pyridine ring to substitute for the A or B rings of combretastatin, we have combined it with either a 2-naphthyl², or a p-methoxyphenyl, or a 3,4,5-trimethoxyphenyl ring. Dihydrocombretastatins have also been obtained by hydrogenation of the bridge double bonds.

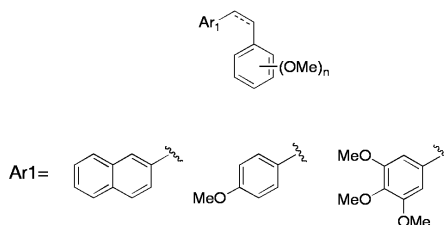


Figure 2. Structure of the synthesized analogues

These compounds have been assayed for antimitotic activity but none of them has shown higher potency in tubulin polymerization inhibition than the 3,4,5-trimethoxyphenyl combretastatin analogues, which indicates that it seems to be necessary for the activity of these compounds.

ACKNOWLEDGMENTS

Financial support came from Spanish MCINN/SAF 2008-04242 and Junta de Castilla y León SA067A09.

REFERENCES

1. Lozano, M. Aramburu, L. Degree Dissertations. University of Salamanca, 2007, 2009.
2. Maya, A. B.; Pérez-Melero, C.; Mateo, C.; Alonso, D; Fernández, J. L.; Gajate, C.; Mollinedo, F; Peláez, R; Caballero, E.; Medarde, M. Further Naphthylcombretastatins. An Investigation on the Role of the Naphthalene Moiety. *J. Med. Chem.* **2005**, 48, 556-568.

POSTER COMMUNICATIONS – PC.210

SYNTHESIS, BIOLOGICAL ACTIVITY, AND DOCKING STUDIES OF NOVEL ACETAMIDINES AS SELECTIVE MODULATORS OF NITRIC OXIDE SYNTHASE

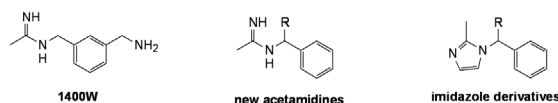
Rosa Amoroso, Alessandra Ammazalorso, Barbara De Filippis, Marialuigia Fantacuzzi, Letizia Giampietro, Fabio Lannutti, Cristina Maccallini, Simona Masella, Antonia Patruno, Nazzareno Re

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Nitric oxide (NO) is an important mediator involved in the regulation of many physiological and pathological processes. [1] The formation of NO is catalyzed by the enzyme nitric oxide synthase (NOS) via the NADPH- and O₂-dependent oxidation of L-arginine. Three distinct isoforms of NOS have been identified: the constitutive endothelial (eNOS) and neuronal (nNOS), predominantly expressed in the vascular endothelium and in the nervous system, respectively, and the inducible (iNOS), which generates high levels of NO that modulates inflammation through multiple pathways and plays an important role in the regulation of immune reactions. When highly regulated, the production of NO is beneficial to the host organism. It is, however, believed that a number of disease states arise from excessive or inappropriate production of NO, including cerebral ischemia, septic shock, and arthritis. It has been proposed that overexpression of iNOS contributes to the progression of inflammatory diseases such as rheumatoid arthritis and osteoarthritis. [2] Moreover, there is evidence that also NO overproduction by nNOS plays a key role in these pathologies, and recently published studies reported promising results from chronic inhibition of nNOS as an adjunct therapeutic strategy for motor nerve repair. [3] Selective inhibition of iNOS and nNOS would be a useful therapy for such diseases. [4]

Recently, we have synthesized and evaluated a series of acetamidines structurally related to N-(3-(aminomethyl)benzyl)acetamide (1400W); among them, compounds characterized by removal of the 3-aminomethyl group and addition of a methyl or an ethyl group on the benzylic carbon connected to

the acetamidine nitrogen, showed good inhibitory potencies and high selectivities toward iNOS. [5] Based on the obtained results, other acetamidines and imidazole derivatives were synthesized and biologically evaluated, and a docking study was also performed to shed light on the effects of the structural modifications on the interaction with the NOS.



REFERENCES

1. R. Bruckdorfer. *Molecular Aspects of Medicine*, **2005**, 26, 3-31
2. P. Ghafourifar, E. Cadenas. *Trends in Pharmacological Sciences*, **2005**, 26, 190-195.
3. L. Zhou, D.-Y. Zhu, *Nitric Oxide - Biology and Chemistry*, **2009**, 20, 223-230.
4. R. Domenico, *Current Pharmaceutical Design*, **2004**, 10, 1667-1676.
5. C. Maccallini, A. Patruno, N. Besker, J. I. Ali, A. Ammazalorso, B. De Filippis, S. Franceschelli, L. Giampietro, M. Pesce, M. Reale, M. L. Tricca, N. Re, M. Felaco, R. Amoroso. *Journal of Medicinal Chemistry*, **2009**, 52, 1481-1485.

POSTER COMMUNICATIONS – PC.211

SYNTHESIS AND EVALUATION OF NEW 1-BENZOYLPIPERIDINES

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Phenstatins are cytotoxic compounds that bind tubulin at the colchicine site. Although they are more polar than combretastatins, their water solubility can still be improved. As trimethoxyphenyl ring is considered necessary for keeping the cytotoxic and antimitotic potency, we have replaced the other aromatic ring by modified piperidine rings in order to increase the solubility.

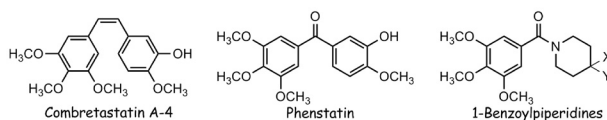


Figure 1. Structure of combretastatins, phenstatins and benzoylpiperidines.

New 1-benzoylpiperidines have been obtained by amide bond formation among the corresponding aromatic acids (3,4,5-trimethoxybenzoic acid, 2,3,4-trimethoxybenzoic acid, 2-(3,4,5-trimethoxyphenyl)acetic acid, 3,4,5-trimethoxycycanic acid) and different piperidines or tetrahydrocarbolines¹.

The ^1H and ^{13}C NMR signals of the nitrogenated methylenes of some piperidines are broadened or missing due to hindered rotation of the amide bond in the NMR time scale.

The activity of these new compounds has been tested by performing the “*in vitro*” tubulin polymerization inhibitory and cytotoxic assays.

In addition, docking studies at the tubulin colchicine site have been carried out to compare the rings disposition of the 1-benzoylpiperidines with respect to the podophyllotoxin pose at the site.

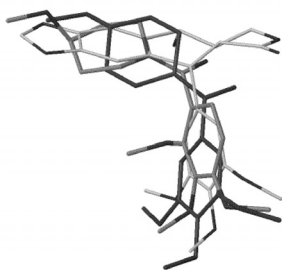


Figure 2. Superposition of podophyllotoxin (green carbons), (4-methoxypiperidin-1-yl)(3,4,5-trimethoxyphenyl) methanone (pink carbons) and (4-methoxypiperidin-1-yl)(2,3,4-trimethoxyphenyl) methanone (grey carbons).

ACKNOWLEDGMENTS

Financial support came from Spanish MCINNSAF 2008-04242 and Junta de Castilla y León SA067A09.

REFERENCES

1. Rodríguez, M., Master's dissertation, University of Salamanca, 2010.

POSTER COMMUNICATIONS – PC.212

A THEORETICAL STUDY OF A FAMILY OF 2-BENZYL-3-METHYLQUINOXALINE 1,4-DI-*N*-OXIDE DERIVATIVES

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The quinoxaline derivatives are a class of compounds that show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, and insecticidal). The oxidation of both nitrogens of this heterocyclic system, needed for obtaining quinoxaline 1,4-di-*N*-oxide derivatives, greatly increases the number of biological properties [1]. In fact, recent studies have demonstrated that quinoxaline 1,4-di-*N*-oxides are endowed with antimycobacterial, antiprotozoal, and anticandida activities as well as mutagenic properties, depending on specific chemical features [1].

In our continuing efforts to find new compounds with therapeutic activities, a series of 2-benzyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives was proposed, synthesized and published [2]. This work recorded the IR and ^1H -NMR spectra of the new species as part of their characterization studies, but no reference to structural parameters was given.

In order to further describe the chemistry of aforementioned compounds, hybrid density functional calculations were performed on them, following a similar methodology as previously described [3]. The conformational space of each one of the ten 2-benzyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives analyzed was scanned using a classical molecular dynamics module of the HyperChem package for the simulation (MM+ Molecular Mechanics Force Field). The 40 lowest-energy molecular conformations of each molecule obtained showed total energies that were mostly grouped, as evidenced by their flat (quinoxaline) structures. These conformations were subjected to further geometry optimization in Gaussian03 program using the density functional theory of B3LYP/6-31G(d,p), and in this way, the most stable conformation resulting from each derivative is selected. The harmonic vibrational frequencies were calculated using the same level of theory. In addition, the isotropic chemical shifts for hydrogen atoms were also calculated, with the isotropic magnetic shielding tensor obtained at the B3LYP/6-311+G(2d,p) level.

In brief, optimized geometries, harmonic vibrational frequencies, and ^1H chemical shifts were reported and compared with experimental data when available. The systematic-theoretical study carried out enabled us to obtain geometric data in quinoxaline 1,4-di-*N*-oxide derivatives that did not pose experimental values, and suggested the preferential conformations that these biological systems would adopt during their interaction with the substrate.

REFERENCES

1. Carta, A.; Corona, P.; Loriga, M., *Curr. Med. Chem.* **2005**, 12(19): 2259-2272.
2. Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A., *Molecules*. **2008**, 13(1): 86-95.
3. Pis Diez, R.; Duchowicz, P.R.; Castañeta, H.; Castro, E.A.; Fernández, F.M.; Albasa, A.G., *J. Mol. Graphics Model.* **2006**, 25: 487-494.

POSTER COMMUNICATIONS – **PC.214**

THE LIMITATIONS OF DATA SETS IN PREDICTION OF DRUG SOLUBILITY

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Drug candidates with poor aqueous solubility run a higher risk of late-stage development failures. The prospect of enabling early screening of combinatorial libraries for such inadequately soluble compounds has prompted many researchers to pursue the development of an *in silico* model for the prediction of solubility, yielding numerous models in the past decade. When challenged with diverse drug structures, however, the predictive capability of the majority of reported models diminishes considerably¹. It has been recognized that the accuracy of experimental data used in model training can be the main limiting factor in achieving good prediction reliability^{2,3}.

For the investigation of the impact of experimental data accuracy on model training, three data sets were used in our study: Data Set 1 (n=319) consisted of solubility values carefully compiled from various literature sources; Data Set 2 (n=319) that was created by substituting solubility values for 28 compounds from Data Set 1 with those determined under standardized experimental conditions; and Data Set 3 (n=375) which was created by expanding Data Set 2 with 56 additional compounds for which uniform experimental data were obtained using the same standardized protocol. All structures were optimized using the AM1 semi-empirical method and then were used for the calculation of a large number of molecular descriptors in CODESSA software. Descriptor selection was performed using the heuristic method which was also applied for establishing one-parameter and multiparameter correlations.

The significance of the selected descriptors was then compared between the three data sets. Constitutional, electrostatic and quantum-chemical (QM) descriptors appeared to be the most significant for drug solubility in each of the data sets. Interpretability of descriptors was found to be favourable: constitutional descriptors accounted for the overall hydrophobicity and aromaticity of the molecule while the electrostatic and QM descriptors were derived from polar surface areas, relative electrostatic charges and H-bond donor/acceptor surface areas, reflecting the presumed solvation mechanism. In one-parameter correlation, the order of significance of the selected descriptors was not largely affected by the changes in experimental data, but there was an overall improvement in individual descriptor correlations found for Data Set 3. When considering the established multiparameter equations for the three data sets, reflecting actual model development more closely, significant differences were observed. The selection of most significant descriptors have been different for three Data sets, and correlation coefficients in multiparameter equations increased with percentage of uniform experimental data used.

Despite significant advances made in developing new modelling techniques, aqueous solubility remains a difficult property to predict. In this work, we have shown that the usage of high-quality, uniformly determined experimental solubility data for drug-like compounds can significantly improve the statistical performance of prospective models. Our findings are in agreement with the growing consensus that improving prediction reliability will require the use of diverse *drug-like* sets with more accurate data.

REFERENCES

1. J. Taskinen and U. Norinder, in *Cprehensive Medicinal Chemistry II* (vol. 5), Elsevier, 2007, p. 627
2. C. A. S. Bergström, *Basic Clin. Pharmacol. Toxicol.* 96 (2005) 156
3. B. Fallner and P. Ertl, *Adv. Drug Delivery Rev.* 59 (2007) 533

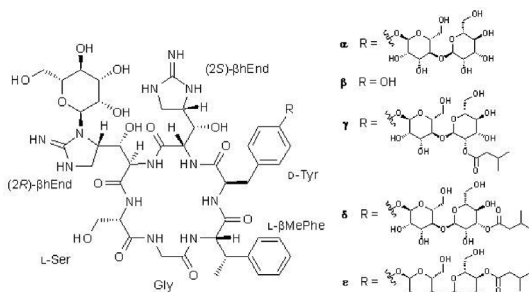
POSTER COMMUNICATIONS – PC.215

TOWARDS THE TOTAL SYNTHESIS OF MANNOPEPTIMYCINS

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Increasing incidence of resistance against therapeutically used antibiotics makes the development of new agents for the treatment of bacterial infections an urgent need. The Manno-peptimycins (MPs), a group of lipoglycopeptides, represent a promising lead-structure for such an undertaking. Some members of this family exhibit an extraordinary activity against various resistant bacterial strains.^[1] Preliminary studies have shown that their mode of action is the inhibition of peptidoglycan biosynthesis by selectively binding to Lipid II and thereby inhibiting its assembly.



Structure of the Manno-peptimycins α - ϵ .

The cyclic hexapeptide core of the MPs contains the unprecedented C2-epimeric amino acids (3S,4S)- β -hydroxy-enduracidines (β hEnd).

Additionally, the (2R)- β hEnd carries an α -linked mannose residue on its C4 nitrogen. The structures of the natural MPs differ in their modification of the tyrosine side-chain. All active compounds (MP γ - ϵ) carry a dimannose residue that is esterified with isovaleric acid.

New and convenient syntheses for both β hEnds were developed^[2] and introduction of the mannosides can be achieved in a high yielding and synthetically useful manner. With those building blocks to hand formation of the hexapeptide and its cyclization can be pursued. This would constitute the first total synthesis of a member of the MP family.

A reliable and convenient chemical synthesis of this promising new lead structure should enable us to gain a deeper insight into the mode of action of the MPs. We plan to vary selected structural elements in order to determine the accompanying changes in antibacterial activity (structure activity relationship). At the same time, investigation of a putative Lipid II/MP complex utilising 2D-nmr techniques should allow the direct observation of potential interactions on a molecular level. By combining these two approaches, Lipid II complexation of MP derivatives may be related to their in vitro activities in MIC assays.

REFERENCES

1. H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen, G. T. Carter, *J. Am. Chem. Soc.* **2002**, 124, 9729–9736
2. C. J. Schwörer, M. Oberthür, *Eur. J. Org. Chem.* **2009**, 35, 6129–6139.

POSTER COMMUNICATIONS – PC.216

HIGHLY POTENT AND SELECTIVE INHIBITORS OF 17 β -HYDROXYSTEROID DEHYDROGENASE TYPE 1 (17 β -HSD1) NOVEL, POTENTIAL THERAPEUTICS FOR BREAST CANCER AND ENDOMETRIOSIS

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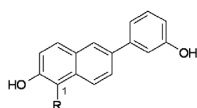
Breast cancer is the most frequent cancer type among women and the most common cause of cancer death. Endometriosis affects approximately 20 % of the female population and can lead to infertility. Both endometriosis and a high percentage of the breast tumours are estrogen-dependent, i.e. growth and proliferation are stimulated by estrogens. Present endocrine therapies - aromatase inhibitors, GnRH-analogues or anti-estrogens - result in a radical and unspecific reduction of estrogenic effects throughout the body leading to severe side-effects like osteoporosis or induction of endometrium carcinomas.

17 β -HSD1 catalyses the reduction of the weak estrogen estrone (E1) to the highly potent estradiol (E2). The enzyme is expressed in different organs like ovaries, breast, endometrium and is pres-

ent in breast cancer tissue as well as in endometriosis. Inhibition of 17 β -HSD1 could selectively reduce the E2-level in specific tissues thus allowing for a novel, targeted approach in the treatment of these diseases resulting in less side-effects compared to established therapies.

As 17 β -HSD2 catalyses the reverse reaction – decreasing the level of active E2 – it should not be affected by inhibitors of the type 1 enzyme. Furthermore, to avoid intrinsic estrogenic effects, 17 β -HSD1 inhibitors should not bind to the estrogen receptors (ERs) α and β .

The combination of a structure- and ligand-based drug design approach led to the development of the previously described steroidomimetics hydroxyphenylnaphthols¹⁻³ as potential inhibitors of the target enzyme. Structural optimisations have been performed by introduction of substituents in the 1-position of the naphthalene core (compound **A**) and led to the identification of new highly potent inhibitors of 17 β -HSD1 with IC₅₀ values in the low nanomolar range displaying good selectivity toward 17 β -HSD2 and ERs α and β .



Compound **A**

REFERENCES

1. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzter, L.; Scherer, C.; Müller Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J Med Chem.* **2008**, *51*, 2158-2169.
2. Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J Med Chem.* **2008**, *51*, 4685-4698.
3. Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. *Mol Cell Endocrinol.* **2009**, *301*, 205-211.

POSTER COMMUNICATIONS – PC.218

THE DISCOVERY OF AZD5672, AN ORALLY AVAILABLE CCR5 ANTAGONIST FOR RHEUMATOID ARTHRITIS

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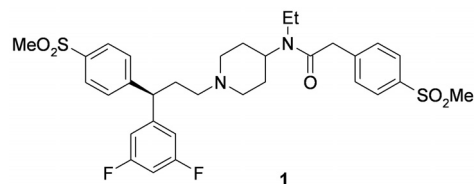
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The chemokine receptor CCR5 has been proposed to be important in the pathogenesis of rheumatoid arthritis (RA).^{1,2} Here we describe the discovery of a series of potent, selective antagonists

of the human CCR5 receptor and its optimisation to the oral clinical candidate AZD5672.

Previous investigations led to the discovery of the pre-clinical candidate **1**. While acceptable in other aspects, compound **1** showed moderate inhibition of the hERG ion channel which is associated with a risk of adverse cardiac events. Therefore subsequent optimisation efforts focused on reducing the activity at the hERG channel while maintaining the excellent potency, selectivity and oral PK properties of compound **1**. Exploration of a related chemical series achieved this goal with the identification of AZD5672. Further profiling of AZD5672 in healthy human volunteers demonstrated it had properties suitable for testing of the clinical hypothesis in RA patients in a Phase IIb clinical trial. The identification of a suitable physical form and improvements to the synthetic route to facilitate kilogram-scale production of AZD5672 will also be described.⁴



REFERENCES

1. Haringman, J.J.; Smeets, T.J.M.; Reinders-Blankert, P.; Tak, P.P. Chemokine and chemokine receptor expression in paired peripheral blood mononuclear cells and synovial tissue of patients with rheumatoid arthritis, osteoarthritis, and reactive arthritis. *Ann. Rheum. Dis.* (2006); *65*: 294-300.
2. Katschke, K.J. Jr.; Rottman, J.B.; Ruth, J.H.; Qin, S.; Wu, L.; LaRosa, G.; Ponath, P.; Park, C.C.; Pope, R.M.; Koch, A.E. Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis. *Arthritis Rheum.* (2001); *44*: 1022-1032.
3. Cumming, John G.; Brown, Simon J.; Cooper, Anne E.; Faull, Alan W.; Flynn, Anthony P.; Grime, Ken; Oldfield, John; Shaw, John S.; Shepherd, Emma; Tucker, Howard; Whittaker, David. Modulators of the human CCR5 receptor. Part 3: SAR of substituted 1-[3-(4-methanesulfonylphenyl)-3-phenylpropyl]-piperidinyl phenylacetamides. *Bioorganic & Medicinal Chemistry Letters* (2006), *16*(13), 3533-3536.
4. Brock, S.; Hose, D.R.; Moseley, J.D.; Parker, A.J.; Patel, I.; Williams, A.J. *Org. Proc. Res. & Dev.* (2008), *12*, 496-502.

POSTER COMMUNICATIONS – PC.219

DESIGN AND OPTIMIZATION OF A
SUBSTITUTED AMINO PROPANAMIDE SERIES
OF RENIN INHIBITORS FOR THE TREATMENT
OF ESSENTIAL HYPERTENSION

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The renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of extracellular fluid and blood pressure¹. Since the cleavage of angiotensinogen by the renin enzyme represents the first and rate limiting step of the RAAS cascade, the maximum benefit that can be gleaned from the modulation of this pathway should come in the form of a direct renin inhibitor². In this regard, research towards the discovery of renin inhibitors with *in vitro* and *in vivo* profiles suitable for clinical development was initiated and this effort culminated in the identification of a novel structural series. In this presentation, the key SAR of this family of substituted amino propanamide renin inhibitors; including the strategies employed to overcome the challenges pertaining to limited oral bioavailability, covalent binding and time-dependent CYP3A4 inhibition, will be discussed³.

REFERENCES

1. Zaman, M. A.; Oparil, S.; Calhoun, D. A. *Nat. Rev. Drug Discovery* **2002**, *1*, 621.
2. (a) Cooper, M. E. *Am. J. Hypertens.* **2004**, *17*, 16S. (b) Norris, K.; Vaughn, C. *Expert Rev. Cardiovasc. Thera.* **2003**, *1*, 51.
3. Chen, A.; Bayly, C.; Benzençon, O.; Richard-Bildstein, S.; Dubé, D.; Dubé, L.; Gagné, S.; Gallant, M.; Gaudreault, M.; Grimm, E.; Houle, R.; Lacombe, P.; Laliberté, S.; Lévesque, J.-F.; Liu, S.; MacDonald, D.; Mackay, B.; Martin, D.; McKay, D.; Powell, D.; Remeň, L.; Soisson, S.; Toulmond, S. *Bioorg. Med. Chem. Lett.* (2010) doi: 10.1016/j.bmcl.2010.02.036.

POSTER COMMUNICATIONS – PC.220

WITHDRAWN

POSTER COMMUNICATIONS – PC.221

MULTI-NUCLEAR NMR AS A METHOD FOR *EX VIVO* DRUG METABOLISM STUDIES

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Metabolic studies of the activation of nucleoside phosphoramidate prodrugs in different biological media (i.e. pig liver carboxylesterase, CEM cell extracts, human serum, mouse serum) and the isolation of subsequent metabolites have been determined by the use of HPLC.^{1,2} Most recently, the use of ³¹P rather than ¹H and ¹³C NMR has been used to evaluate the stability of potential prodrugs in a biological environment. This technique provides a useful tool to monitor the degradation process of organic molecules and the formation of metabolic intermediates.³ We now wish to report the study of metabolic stability by ³¹P NMR carried out in biological media (human serum) on a small library of ProTides with different esters and possible metabolites synthesized in our lab. Enzymatic studies were performed in order to investigate if our ProTides can be metabolised by esterase type enzyme using Carboxypeptidase Y. Moreover chemical stability was evaluated in a buffer at both acidic and basic pH. NMR methodology has proved be a useful, quick and inexpensive tool to gather preliminary information on ProTide metabolism. Moreover the use of multi-nuclear NMR has allowed us to discriminate which drugs would be viable for further progression *in vivo*.

REFERENCES

1. Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. Characterization of the activation pathway of phosphoramidate triester prodrugs of stavudine and zidovudine. *Mol. Pharmacol.* 1999, **56**, 693-704
2. Valette, G.; Pompon, A.; Girardet, J. L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A. G.; Périgaud, C.; Gosselin, G.; Imbach, J. L. Decomposition pathways and *in-vitro* inhibitory effects of isoddA pronucleotides: Toward a rational approach for intracellular delivery of nucleoside 5'-monophosphates. *J. Med. Chem.* 1996, **39**, 1981-1990
3. Bibbo, R. Design and synthesis of novel agents with potential against osteoarthritis, *PhD Thesis* 2008, Cardiff University

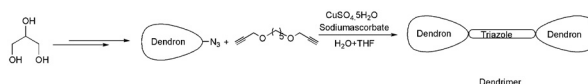
POSTER COMMUNICATIONS – PC.223

SYNTHESIS OF NOVEL GLYCEROL-BASED DRUG DELIVERY VEHICLES AND ENCAPSULATION STUDIES WITH HYDROPHOBIC DRUG MOLECULES

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One of the major challenges faced by pharmaceutical industries is increasing solubility of poorly water soluble drugs¹. Amphiphilic dendrimers have attracted interest over the past few years due to capability of solubilising hydrophobic drug molecules in aqueous medium². Glycerol is an inexpensive³ and biocompatible⁴ molecule which we have utilized for the construction of novel drug delivery vehicles (dendrimers) through "Click chemistry"⁵. These dendrimers possess hydrophilic free hydroxyl groups at the periphery whereas the interior is relatively hydrophobic and were completely characterized by NMR (1D and 2D) and MALDI-TOF spectral analysis. Encapsulation studies were carried out with a model hydrophobic drug molecule. The presentation will discuss details of this work.



REFERENCES

1. E. Atef, A. A. Belmonte, *Eur. J. Pharm. Sci.* 2008, **35**, 257-263.
2. a) S. R. Meyers, F. S. Juhn, A. P. Griset, N. R. Luman, M. W. Grinstaff, *J. Am. Chem. Soc.* 2008, **130**, 14444-14445; b) A. Schmitzer, E. Perez, L. I. Rico, A. Lattes, S. Rosca, *Langmuir* 1999, **15**, 4397-4403; c) J. Sun, M. Ramanathan, D. Dorman, G. R. Newkome, C. N. Moorefield, P. S. Russo, *Langmuir* 2008, **24**, 1858-1862; d) B.K.Cho, A. Jain, J. Nieberle, S. Mahajan, U. Wiesner, S. M. Gruner, S. Tuerk, H. J. Raeder, *Macromolecules* 2004, **37**, 4227-4234; e) D. Joester, M. Losson, R. Pugin, H. Heinzelmann, E. Walter, H. P. Merkle, F. Diederich, *Angew. Chem., Int. Ed.* 2003, **42**, 1486-1490; f) L. Degoricija, M. A. Carnahan, C. S. Johnson, T. Kim, M. W. Grinstaff, *Macromolecules* 2006, **39**, 8952-8958.
3. a) D. T. Johnson, K. A. Taconi, *Environ. Prog.* 2007, **26**, 338-348; b) M. Pagliaro, R. Ciriminna, H. Kimura, M. Rossi, P. C. Della, *Angew. Chem., Int. Ed.* 2007, **46**, 4434-4440.
4. a) H. Frey, R. Haag, *Rev. Mol. Biotechnol.* 2002, **90**, 257-267; b) A. L. Sisson, D. Steinhilber, T. Rossow, P. Welker, K. Licha, R. Haag, *Angew. Chem., Int. Ed.* 2009, **48**, 7540-7545.
5. H. C. Kolb, K. B. Sharpless, *Angew. Chem., Int. Ed.* 2002, **41**, 2596-2599.

POSTER COMMUNICATIONS – PC.224

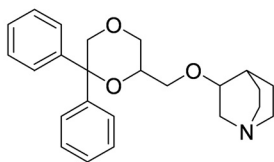
COPRESENCE OF 1,4-DIOXANE AND QUINUCLIDINE NUCLEI IN THE DESIGN OF NOVEL MUSCARINIC ANTAGONISTS

Fabio Del Bello,¹ Aniket Farande,¹ Yogita Farande,¹
 Mario Giannella,¹ Maria Pigni,¹ Alessandro Piergentili,¹
 Wilma Quaglia,¹ Marta Nesi,² Rosanna Matucci²

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The five muscarinic receptors are involved in many physiological functions and, therefore, the potential therapeutical applications of muscarinic agonists and antagonists are numerous. However, the high homology of the orthosteric binding sites of the different subtypes (amino acid sequence identity > 60%) makes particularly difficult the achievement of selective ligands (1). Candidates which are more likely to discriminate among subtypes are obviously antagonists because of their ability to interact with sites in the extracellular loops or with accessory residues of the receptor. In the search for novel muscarinic antagonists we opted for molecules bearing the 1,4-dioxane nucleus, which has proved to be a suitable scaffold for targeting muscarinic receptors (2). Such a scaffold, substituted in position 6 with two phenyl rings, has been linked by an oxymethylene bridge to a quinuclidine nucleus, which is present in numerous muscarinic agents as well. The presence of this nucleus has the advantage of including in the final compound a second stereogenic center, thus achieving a more selective effect, after the separation of the four enantiomers.

The synthesis of the novel compounds, the characterization of their structure, and the preliminary pharmacological results will be discussed.



REFERENCES

1. Eglén, R. M. Muscarinic receptor subtype pharmacology and physiology. *Prog. Med. Chem.* **2005**, 43, 105-136.
2. Piergentili A.; Quaglia, W.; Del Bello, F.; Giannella, M.; Pigni, M.; Barocelli, E.; Bertoni, S.; Matucci, R.; Nesi, M.; Bruni, B.; Di Vaira, M. Properly substituted 1,4-dioxane nucleus favours the selective M₃ muscarinic receptor activation. *Bioorg. Med. Chem.* **2009**, 17, 8174-8185 and references therein.

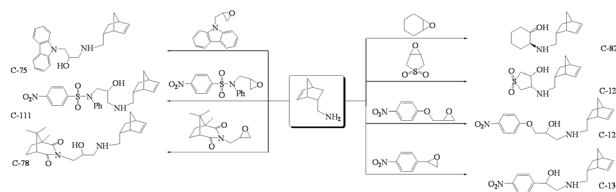
POSTER COMMUNICATIONS – PC.225

SYNTHESIS, ANALGESIC AND ANTICONVULSIVE ACTIVITY OF AMINOALCOHOLS BASED ON BICYCLO[2.2.1]HEPT-5-ENE-ENDO-2-YLMETHYLAMINE

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The present work deals with neurotropic activity of seven novel aminoalcohols C-75, C-111, C-78, C-82, C-123, C-121 and C-13, synthesized from 9-(oxiran-2-ylmethyl)-9H-carbazole, 4-nitro-N-(oxiran-2-ylmethyl)-N-phenylbenzenesulfonamide, glycidylcamphorimide, epoxycyclohexane, 3,4-epoxysulfolane, 4-nitrophenyl glycidyl ether, 2-(4-nitrophenyl)oxirane and stereochemical purity *endo*-aminomethylnorbornene (bicyclo[2.2.1]hept-5-ene-*endo*-2-ylmethylamine) in 2-propanol with yields 60-93% (Figure). Aminoalcohols as the products of regioselective aminolysis of epoxides (according to the Krasusky rule) have been obtained. The structures of individual products have been confirmed by IR spectra, NMR ¹H, ¹³C spectra, using experiments that involve homonuclear and heteronuclear scalar coupling interactions (COSY, NOESY, HMQC, HMBC), and mass spectrometry.



Experiments have been carried out on both genders adult white mice at the doses of 1/10 LD₅₀. Acute toxicity of compounds (LD₅₀) has been measured by Litchfield & Wilcoxon method in Prozorovsky V.B. (1962) modification and varies within 375-1196 mg/kg (they are considerably less toxic than *endo*-aminomethylnorbornene, LD₅₀ 331 mg/kg). The most toxic compound is aminoalcohol C-111 with N-phenylbenzenesulfonamide fragment. Investigation of analgesic activity has been carried out by determination of pain threshold by thermal irritation method (hot plate) at 55°C. Anticonvulsive activity has been studied by corazole spasm test. The activity has been calculated in percentage in relation to control group of animals.

All aminoalcohols by analgesic activity essentially surpass initial amine. For aminoalcohols C-82, C-111 and C-121 the most intense activities are analgesic (+104-127%) and for C-78 – anticonvulsive activity (+213%). For compounds C-111, C-121 and C-123 it is typical

proconvulsive action at a level -14–(-35)%. Compound C-75 show medium analgesic (+39%) and anticonvulsive activity (+18%).

The detection of correlation between chemical structure and biological activity in frame amine row defines an opportunity of the directed synthesis of new active neurotropic agents.

POSTER COMMUNICATIONS – PC.226

SYNTHESIS AND BIOLOGICAL EVALUATION OF SYMMETRIC AND ASYMMETRIC BIVALENT DOPAMINE D2 RECEPTOR LIGANDS

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A growing body of evidence indicates that GPCRs exist as homomers or heteromers. Thus, the role of dimerization/ oligomerization in receptor function is under extensive investigation.^[1] Inventing a functional complementation assay, Han et al.^[2] were able to show, that two protomers of the dopamine D2 receptor are necessary to initiate signalling through one G-protein. Interestingly, agonist binding to a single protomer is sufficient for G-protein activation. Furthermore, the two protomers are able to allosterically influence each other. An enhanced functional response was achieved by agonist binding to one protomer and inverse agonist binding to the second protomer; whereas agonist binding to the second protomer blunted signalling. More generally, the protomer crosstalk depends on the various activation states adopted by each protomer in a GPCR dimer and is believed to influence the G-protein dependent and G-protein independent signalling pathways. Thus, the possibility that homodimers can adopt multiple active states, accounts for the design of symmetric (both pharmacophores are the same) and asymmetric (pharmacophores differ in their activation pattern) bivalent ligands, as potential tools for the selective addressing of different signalling pathways.

Herein we present the design of three types of bivalent dopamine D2 receptor ligands consisting of antagonist-antagonist, agonist-agonist and antagonist-agonist as well as their monomeric counterparts. The recognition elements were connected via copper-catalyzed azide alkyne cycloaddition (CuAAC) to obtain 1,2,3-1H-triazoles containing alkyl chain linkers. The ligands were extensively investigated at the D2R for their binding affinities and intrinsic activities in various functional assays, intending to detect their influence on different signalling pathways.

REFERENCES

1. Milligan G. *Mol. Pharmacol.* **2004**, 66, 1-7.
2. Han Y.; Moreira, I. S.; Urizar, E.; Weinstein, H.; Javitch, J. A. *Nat Chem Biol.* **2009**, 5, 688-95.

POSTER COMMUNICATIONS – PC.227

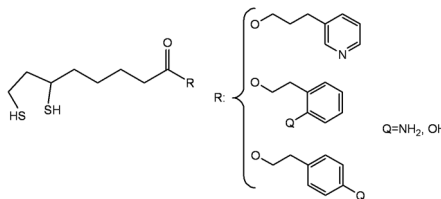
SYNTHESIS AND PHARMACOCHEMICAL STUDY OF NOVEL LIPOIC ACID DERIVATIVES WITH PUTATIVE ACTIVITY AGAINST NEURODEGENERATIVE ALZHEIMER'S TYPE DISEASE

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Senile Dementia Alzheimer's Type (SDAT) is the third cause of morbidity in the western societies. Attempts to find satisfactory drugs for SDAT are a challenge, since there are no efficient drugs for this condition, and the currently applied pharmacotherapy cannot arrest the progress of the disease. However, there are many pathobiochemical changes in the demented brain, which could be used as a basis for designing better agents against SDAT.

Using two or three of these changes, e.g. oxidative stress, neurotrophic agent dysregulation and inflammation, we designed, synthesised and pharmacochémically studied some lipoic acid esters in the hope that they possess beneficial properties. Lipoic acid served as and antioxidant and neurotrophic agent, and the alcohols used were 3-(pyridin-3-yl)propan-1-ol as a neurotrophic structure, 2-(2-aminophenyl)ethanol and 2-(4-aminophenyl)ethanol as potential antioxidants. Synthesis was conducted by conventional or novel methods and structures were identified by modern spectroscopic techniques (IR, ¹H and ¹³C NMR, DEPT NMR) and elemental analysis.



Structures of the synthesised final compounds

They were tested for antioxidant activity assessed as inhibition of lipid peroxidation of hepatic microsomal membranes, induced by the FeSO₄ – ascorbate system, and as interaction with the stable radical DPPH. Lipid peroxidation was determined spectrophotometrically as thiobarbituric acid reactive material. Antiinflammatory action was expressed in vitro as inhibition of lipoxygenase and in vivo as paw edema reduction. Many of the tested compounds demonstrated encouraging results. We believe that multicausal diseases like SDAT can better be treated by polyfunctional compounds^[1, 2], like the above structures incorporating neurotrophic and antioxidant properties.

REFERENCES

1. Doulgeris CM et al., *Bioorg Med Chem Lett.* 16 (2006), 825-829.
2. Tsiakitzis KC et al., *J. Med. Chem.* 52 (2009), 7315-7318.

POSTER COMMUNICATIONS – PC.228

HETEROMERIC DOPAMINE - NEUROTENSIN RECEPTOR INTERACTIONS CONTROL THE BINDING AFFINITIES OF DOPAMINERGICS

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Neurotensin (NT) is a tridecapeptide that is widely distributed in the central nervous system, as well as in the gastrointestinal tract. In many regions of the CNS, NT acts as neurotransmitter and neuromodulator by activating the G-protein coupled neurotensin receptors (NTS1 and NTS2).

Several lines of evidence based on neurochemical, behavioral and anatomical data suggest an interaction of NT with neurotransmitter systems in the CNS, especially the mesolimbic and nigrostriatal dopaminergic pathways. According to these findings, NT is discussed as endogenous antipsychotic agent and might influence the pathophysiology of brain diseases such as schizophrenia, Parkinson's Disease and Alzheimer's Disease. The modulating effect of neurotensin on striatal dopamine D₂ receptors most likely is mediated by activation of NTS1 and, subsequently, causes an attenuation of dopaminergic signalling. [1]

Here, we present a co-expression study of the dopamine receptors (D_{2L} or D₃) and the neurotensin receptors (NTS1 or NTS2). One dopamine receptor and one neurotensin receptor subtype were transiently co-transfected in HEK293 cells, generating four different *in vitro* models for heteromeric GPCR systems. Binding affinities of the dopamine receptor antagonist spiperone and the agonist 7-OH-DPAT, as indicative parameters of the modulating effect of NT on these heteromers, were investigated. When measuring the affinities of the radioligands [³H] spiperone and [³H] 7-OH-DPAT to the dopamine receptors in the absence and presence of NT, a clearly reduced binding of the dopamine agonist could be observed with NT. These results confirm the modulating effect of neurotensin on the dopamine receptors in our *in vitro* systems. Heteromeric systems of GPCRs as models to investigate the molecular interactions of receptor heteromers are presented and exploited for the evaluation of novel dopamine receptor agonists.

REFERENCES

1. E. B. Binder, B. Kinead, M. J. Owens, C. B. Nemeroff Pharmacol. Rev. **2001**, 53, 453-486.

POSTER COMMUNICATIONS – PC.229

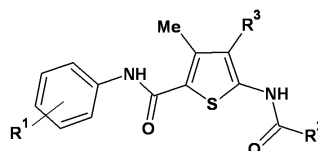
SYNTHESIS OF THIOPHENE CARBOXAMIDE-TYPE TRPV1 ANTAGONISTS

N. Felföldi, Zs. Hadady, É. Szentirmay, A. Visegrády, Zs. Bekes, S. Kolok, M. Vastag, Á. Kis-Varga, J. Nagy, Cs. Horváth, L. Molnár, G. M. Keserű

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Being potent antagonists of TRPV1 drug-like thiophene carboxamides were identified as feasible starting points for further synthetic modifications; regions of the thiophene molecule were changed in a systematic way by simple, few-step syntheses.

It was aimed to improve the potency as well as the metabolic stability of thiophenes by modifications at three positions of the molecule (as it is designated in the figure below). The SAR for the series obtained from these structural modifications suggested that 4-substituted-phenylcarbamoyl moiety and also the R³ group have a determinant role in TRPV1 activity; the presence of the R²-amido moiety believed responsible for the intensive non-CYP metabolism observed in human liver microsomes. As a result, a new class of potent and metabolically stable TRPV1 antagonists were obtained.



POSTER COMMUNICATIONS – PC.230

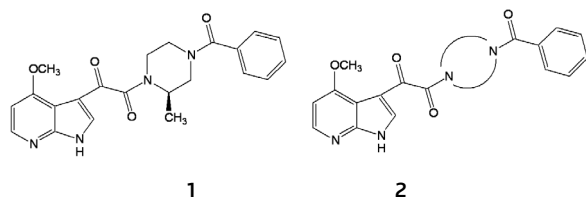
SYNTHESIS, COMPUTATIONAL STUDIES AND HIV-1 INHIBITORY ACTIVITY OF NEW ANTIVIRAL COMPOUNDS

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The AIDS disease is caused by the Human Immunodeficiency Virus (HIV), a retrovirus with RNA genome.^[1] The UNAIDS Report 2009 estimated that 33.4 million people worldwide were living with HIV at the end of 2008.^[2] Since the AIDS pandemic remains

one of the leading global public health threats, the discovery and development of new antiretroviral drugs with reduced toxicity, enhanced potency, different mechanisms of action, and reduced prevalence of adverse drug-drug interactions remain a very high priority. In particular, a promising area of investigation is the identification of agents that inhibit viral attachment and entry into host cells^[3] in order to block HIV infection at the early stages. The azaindole derivative BMS-37806 **1**, discovered at Bristol-Myers Squibb,^[4] has been shown to interfere with the HIV-1 entry process, inhibiting the interaction between the viral gp120 envelope glycoprotein and its cellular CD4 receptors.^[5-7] In this context we have synthesized analogs of compound **1** introducing different changes in the chemical structure instead of the methylpiperazine group. The new compounds of general formula **2** were synthesized and their biological activity evaluated. Finally, in an attempt to correlate the geometrical features of these compounds to their HIV-1 inhibitory activity, a modeling study was carried out to ascertain their conformational preferences. The chemistry, the computational and NMR studies, and biological data will be discussed in the poster.



REFERENCES

1. R. C. Gallo, L. Montagnier, *New Engl. J. Med.* **2003**, 349, 2283–2285.
2. WHO/UNAIDS, AIDS Epidemic Update December 2008.
3. a) V. Briz, E. Poveda, V. Soriano, *J. Antimicrob. Chemother.* **2006**, 57, 619–627; b) S. Rusconi, et al. *Curr. Drug Targets Infect. Disord.* **2004**, 4, 339–355; c) F. Shaheen, et al., *Curr. Opin. Infect. Dis.* **2004**, 17, 7–16; d) I. Markovic, *Curr. Pharm. Des.* **2006**, 12, 1105–1119.
4. T. Wang, et al. *J. Med. Chem.* **2003**, 46, 4236–4239.
5. P. F. Lin, et al. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 11013–11018.
6. R. S. Veazey et al. *Nature* **2005**, 438, 99–102.
7. D. Colombo, S. Villa, L. Solano, L. Legnani, F. Marinone Albini, L. Toma. *Eur. J. Org. Chem.*, **2009**, 3178–3183.

POSTER COMMUNICATIONS – **PC.231**

TRICYCLIC PYRIDAZINONE DERIVATIVES: DEVELOPMENT ON NEW STAT3 INHIBITORS

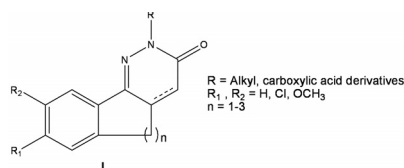
Arianna Gelain¹, Daniela Masciocchi¹, Stefania Villa¹, Alessandro Pedretti¹, Fiorella Meneghetti¹, Daniela Barlocco¹, Byoung-Mog Kwon², Shin Dae-Seop², Laura Legnani³ and Lucio Toma³

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Signal transducer and activator of transcription 3 (Stat3) is a latent cytoplasmic protein that transmits the signals from the cell surface to the nucleus¹ and participates directly in gene regulation by binding to response elements in gene promoters effecting transactivation. Stat3 plays a crucial role in cell growth and survival but the abnormal regulation of its functions is closely linked to tumorigenesis²; in fact, its constitutive activation by aberrant upstream tyrosine kinase activities is evident in a broad spectrum of human solid and blood tumours. Moreover the inhibition of Stat3 induces apoptosis in tumour cells selectively³⁻⁴, which validates Stat3 as a promising cancer drug target. During our ongoing researches on non peptidic small molecules that could directly interact with Stat3, we focused our attention on the tricyclic pyridazinone skeleton **1**. In particular, we explored the effects of substituents both at N-2 and at different positions of the aromatic ring as well as the size of the central ring.



Synthesis, molecular modeling studies, X-ray analysis and biological data will be presented.

REFERENCES

1. Costantino, L.; Barlocco, D. *Curr. Med. Chem.*, **2008**, 15 (9), 834-843.
2. Aggarwal, B.B.; Sethi, G.; Ahn, K.S.; Sandur, S. K.; Pandey, M.K.; Kunnumakkara, A.B.; Sung, B.; Ichikawa, H. *Ann. N. Y. A. S.*, **2006**, 1091, 151-169.
3. Bowman, T.; Broome, M. A.; Sinibaldi, D.; Wharton, W.; Pledger, W. J.; Sedivy, J. M.; Irby, R.; Yeatman, T.; Courtneidge, S. A.; and Jove, R. *PNAS*, **2001**, 98 (13), 7319-7324
4. Turkson, J.; Ryan, D.; Kim, J. S.; Zhang, Y.; Chen, Z.; Haura, E.; Laudano, A.; Sebti, S.; Hamilton, A. D. and Jove, R. *J. Biol. Chem.*, **2001**, 276 (48), 45443-45455

POSTER COMMUNICATIONS – **PC.232**

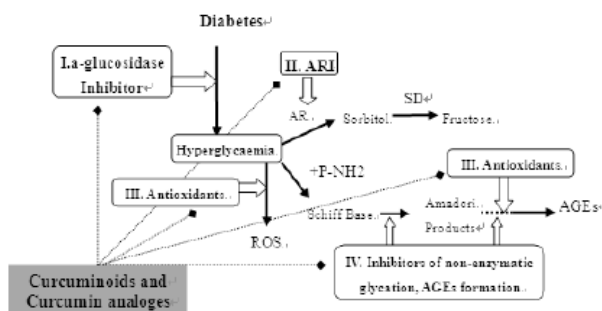
INTERVENTION ON MULTIPLE TARGETS OF DIABETIC COMPLICATIONS BY CURCUMINOIDS AND CURCUMIN ANALOGUES

Zhiyun DU¹, XueTao XU¹, Changzhi DONG², Kun ZHANG¹, Jie ZHENG¹, Huaqian WANG¹

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Diabetes mellitus is a major and growing public health problem throughout the world. The increased flux of glucose results in long-term complications which include neuropathy, nephropathy, retinopathy, cardiovascular disease and cataract formation. It is reported that multiple mechanisms have been implicated in the development of diabetic complications, and three of them appear to play a prominent role (Scheme 1): (i) increased flux of glucose through the polyol pathway; (ii) increased advanced glycation end-product (AGE) formation and (iii) increased oxidative stress [1]. Although a considerable number of therapeutic agents against diabetic complications are available, none is optimal and can achieve a satisfactory effect on its own. Thus, combinations of complementary agents have been suggested and play an increasing role in the treatment of the disease [2]. In the same way, it is more effective if one compound can exhibit more activities on the multiple mechanisms of diabetic complications.

Curcumin, a natural product isolated from Rhizome of the perennial herb *Cucuma Longa L* (turmeric), is a yellow pigment and a known antioxidant in food system. It has already been reported to possess diverse biological activities such as anti-inflammatory, antitumor, antihepatotoxic, anti-HIV. We previously studied the inhibitory activities of natural curcuminoids and synthesized curcumin analogues against aldose reductase (AR) and α -glucosidase [3,4], and found that the ortho-dihydroxy curcumin analogues possessed much more potential bioactivities for both the targets than that of curcumin. Recently, it was reported that curcumin showed potential effects on diabetic animal model as an aldose reductase inhibitor (ARI) [5]. This prompted us to synthesize a new series of novel curcumin analogues with ortho-dihydroxy moiety and evaluated their inhibitory activities against α -glucosidase, aldose reductase, advanced glycation end products (AGEs), and antioxidation ability. The results showed that some curcumin analogues exerted highly potential effects against all above mentioned targets. It suggests that curcumin analogues can be leading compounds to develop an effective drug intervening multiple targets in preventing diabetic complications.



Scheme 1. Possible pharmacological preventions of diabetic complications.

REFERENCES

1. Mark, S.M. The American Journal of Medicine, 123(3), S3-S11 (2010)
2. Kyselova, Z., Stefek, M., Bauer, V. Journal of Diabetes and Its Complications, 18, 129-140 (2004).
3. DU, Z.Y., LIU R.R., SHAO W.Y., et al. Eur. J. Med. Chem. 42(2), 213-218 (2006).

4. DU, Z.Y., BAO, Y.D., LIU, Z., et al. Archiv der Pharmazie, 339(3), 123-128 (2006).
5. Muthenna, P., Suryanarayana, P., Gunda, S.K., et al., FEBS Letters, 583, 3637-3642 (2009).

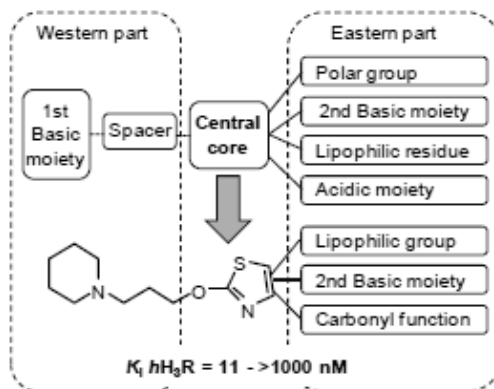
POSTER COMMUNICATIONS – PC.233

THIAZOLE DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS

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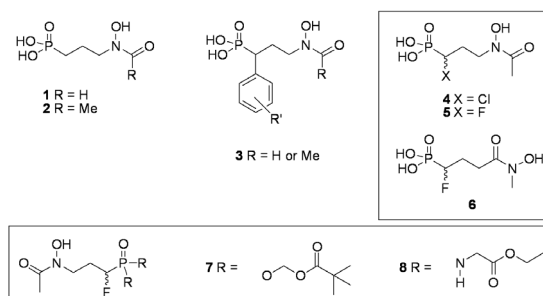
Biogenic amines, such as histamine, play important roles as chemical mediators in human physiology. Histamine mediates its numerous central and peripheral effects *via* four known G protein-coupled receptor subtypes (hH_1R - hH_4R). Human histamine H_3 receptors (H_3R) are acting as autoreceptors on synthesis and liberation of histamine as well as heteroreceptors on modulating the release of several other neurotransmitters (e.g. acetylcholine, dopamine, glutamate, noradrenalin, serotonin, GABA). Due to a distinct expression pattern in the central nervous system (CNS) and resulting involvement in several neuronal functions, e.g. vigilance, attention and learning, the hH_3R are attractive targets for the treatment of CNS disorders like schizophrenia, epilepsy, depression, Alzheimer's disease and sleep disorders.¹



Antagonist H_3R ligands follow a general pharmacophore blueprint, containing a basic moiety, mostly a tertiary amine, linked by a spacer to a central core substituted by a variety of structural elements providing different physicochemical properties.² Based on the well established 1-(3-phenoxypropyl)amine pharmacophore, offering an aromatic ring system in the core region, we investigated the acceptance of polar core elements. Therefore the phenyl ring was replaced by polar thiazole groups.

The thiazole derivatives could be obtained by Hantzsch-thiazole synthesis.^{3,4} A diversity of structural motifs is used as substituents to optimize the *in vitro* receptor binding properties, which ranging from low nanomolar binding strength to completely loss of affinity.

We successfully introduced polar thiazole groups as new central core element in the *h*H₃R antagonist pharmacophore and could establish a new structural class of *h*H₃R antagonists/inverse agonists.



REFERENCES

1. Tiligada, E. et al., *Expert Opin. Investig. Drugs*. 2009, 18, 1519-31.
2. Sander, K. et al., *Bioorg. Med. Chem. Lett.* 2010, 20, 1581-4.
3. Hantzsch, A. et al., *Chem. Ber.* 1888, 21, 938-41.
4. Wiley, R.H. et al., *Organic Reactions*, 1951, 6, 367-409.

POSTER COMMUNICATIONS – PC.234

SYNTHESIS AND EVALUATION OF α -HALOGENATED ANALOGUES OF FR900098 AND THEIR PRODRUGS AS ANTIMALARIALS

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Fosmidomycin (**1**) and its acetyl congener FR900098 (**2**) inhibit DOXP reductoisomerase, a vital enzyme in the non-mevalonate pathway for isoprenoid biosynthesis, and are good leads for further development of new antimalarials. Previously, it has been shown that introduction of an aromatic group in α -position of the phosphonate increases the *in vitro* cellular activity of these analogues.^{1,2}

Assuming that this increase in potency was due to the electron withdrawing effect of the aromatic ring causing a decrease in pKa of the phosphonate, we anticipated that introduction of a halogen atom in α -position of the phosphonate could accomplish the same effect without the steric implications that come with the aromatic rings. Therefore, we synthesized two halogenated retrohydroxamates **4** and **5** as well as the fluorinated hydroxamate **6** via halogenation of a suitable phosphonate precursor. Analogues **4-6** showed submicromolar activity *in vitro* (*P. falciparum* K1 and GHA) as well as good *in vivo* activity in a *P. berghei* mouse model.

To address the high polarity of these phosphonates, resulting in poor bioavailability and cell penetration, we also synthesized and evaluated the bis(pivaloyloxymethyl) **7** and bis(ethoxycarbonylmethylamino) **8** prodrugs of α -fluoro-analogue **5**.

REFERENCES

1. Haemers, T.; Wiesner, J.; Van Poecke, S.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Van Calenberg, S. *BIOORG. MED. CHEM. LETT.* **2006**, 16, (7), 1888-1891.
2. Devreux, V.; Wiesner, J.; Jomaa, H.; Rozenski, J.; Van der Eycken, J.; Van Calenberg, S. *J. ORG. CHEM.* **2007**, 72, (10), 3783-3789.

POSTER COMMUNICATIONS – PC.235

AUTOMATION OF THE CHARMM GENERAL FORCE FIELD FOR COMPUTER-AIDED DRUG DESIGN

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The CHARMM Force Field [1] is widely used in computer-aided drug design for the study of biological macromolecules. The high demand for simulations of these biological systems in interaction with drug candidates motivated development of the CHARMM General Force Field (CGenFF), an organic force field aimed at simulating drug-like molecules in a biological environment represented by the CHARMM biomolecular force field. [2] To facilitate the use of CGenFF, an algorithm was developed that automatically assigns atom types, bonded parameters and charges to an arbitrary molecule. The atom typer is rule-based and programmable, making it easy to update the atom typing scheme as the force field grows. Assignment of bonded parameters is based on substituting atom types in the definition of the desired parameter. A penalty is associated with every substitution and the existing parameter with the lowest total penalty is chosen as an approximation for the desired parameter; the "penalty score" is returned to the user as a measure for the accuracy of the approximation. Charges are assigned using an extended bond-charge increment scheme that should be able to

capture short- and medium-range inductive and mesomeric effects. [3] The resulting program's functionality will be accessible on the web, which makes it possible to apply CGenFF on arbitrary molecules in a matter of seconds. [4] Although the program should ideally accept any molecule, the quality of the resulting parameters (as indicated by their "penalty scores") will vary depending on whether a similar compound has been parametrized before. Therefore, an effort has been started to build a computational engine with the capability of automatically validating and/or optimizing parameters for drug-like model compounds of the user's interest in the framework of CGenFF, thus providing high-quality force field parameters for computer-aided drug discovery. [4]

REFERENCES

1. A. D. MacKerell et al., *J. Phys. Chem. B* **1998**, *102*, 3586-3616
2. K. Vanommeslaeghe et al., *J. Comput. Chem.* **2010**, *31*, 671-690
3. K. Vanommeslaeghe et al., *in preparation*
4. NSF Award #0823198; <https://www.paramchem.org/>

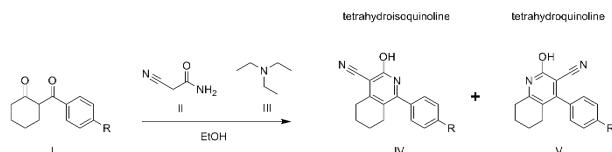
POSTER COMMUNICATIONS – PC.236

ON THE REACTION OF PARA-SUBSTITUTED 2-BENZOYL CYCLOHEXANONES WITH CYANOACETAMIDE

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In the synthesis of a more complex heterocyclic system, we explored 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) as an important intermediate. The reaction of substituted 2-benzoyl cyclohexanone (I) with cyanoacetamide (II) and triethylamine (III) in ethanol at room temperature has been reported to lead to the formation of 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) (1). However, depending on the nature of the *para* substituent on the phenyl ring, formation of regioisomeric 2-hydroxy-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile can compete (V).



No molecular determinants for the regiochemistry have been described. Therefore, we undertook efforts to gain more insights in the mechanism of this reaction. We investigated the effect of different *para*-substituents and their influence on the observed ratio between formed tetrahydroisoquinoline and tetrahydro-

quinoline products. For the characterization and quantification of precursors and products we used an array of techniques, including GC-MS and 2D NMR. We postulate that the electron-donating or -withdrawing properties of the *para* substituents directly determine the ratio of product formation.

REFERENCES

1. Rosowsky, A.; Papathanasopoulos, N. Pyrimido[4,5-*c*]isoquinolines. Synthesis and biological evaluation of some 6-alkyl-, 6-aralkyl-, and 6-aryl-1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinolines as potential folate antagonists. *J. Med. Chem.*, **1974**, *84*, 1140-1143

POSTER COMMUNICATIONS – PC.237

MULTIFUNCTIONAL NEW COMPOUNDS FOR THE TREATMENT OF DEGENERATIVE CONDITIONS WHICH IMPLICATE INFLAMMATION

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used agents for the treatment of inflammation. However, they possess major and even life-threatening side effects, such as damage to the gastric mucosa, prolongation of bleeding time and renal failure.

A number of approaches have been pursued to minimize these adverse effects of NSAIDs. It is well known that reactive oxygen species are implicated in inflammation. Furthermore, since cyclooxygenase (COX) catalysis involves radical intermediates, a radical scavenger would be considered to interfere with the COX reaction. Thus, antioxidant properties seem to be advantageous for anti-inflammatory agents. Derivatives bearing the 3,5-di-*tert*-butyl-4-hydroxybenzylidene moiety with antioxidant activity have been found to be dual inhibitors of COX-2 and 5-lipoxygenase (5-LOX) as well as to suppress interleukin-1 production. In addition, we have found that derivatives of 2,6-di-*tert*-butyl phenol which are amines or amides of thiomorpholine, morpholine and proline residues reduce inflammation and offer protection against free radical attack by direct inhibition of lipid peroxidation as well as by inhibiting LOX, which is considered to play a significant contributory role in the generation of cellular oxidative stress. Considering the above studies, we designed some new derivatives of 2,6-di-*tert*-butyl phenol with known NSAIDs.

Neurodegenerative disorders of Alzheimer's type are one of the top causes of morbidity. There is a series of pathobiochemical changes in the demented brain, which could be used as a starting point for rational design of molecules for the particular condition, such as oxidative stress, inflammation, reduction of nerve growth factor, increased apoE₄ and hypercholesterolemia. Thus, we describe the synthesis of derivatives of NSAIDs esterified with 3-(3-hydroxypropyl)pyridine expected to offer neurotrophic

and/or neuroprotective activity Furthermore, we have found that this moiety enhances anti-inflammatory action.

These compounds were tested in vitro and in vivo for the evaluation of their antioxidant and anti-inflammatory activities. They were found to inhibit in vitro rat hepatic microsomal membrane lipid peroxidation, to exert anti-inflammatory activity, assessed as paw edema reduction and inflammation enzyme inhibition, and to reduce total cholesterol, LDL-cholesterol and triglyceride levels in the plasma of dyslipidemic rats. Thus, compounds combining anti-inflammatory and antioxidant structural features may be proven useful for the treatment of conditions which implicate inflammation.

POSTER COMMUNICATIONS – PC.238

DISCOVERY OF INHIBITORS OF *SCHISTOSOMA MANSONI* NAD⁺ CATABOLIZING ENZYME

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Schistosoma mansoni flatworm is the agent of the human intestinal schistosomiasis disease. *Schistosoma mansoni* NAD⁺ catabolizing enzyme (*SmNACE*) is a distant homolog of mammalian CD38 enzymes. [1] It efficiently converts NAD⁺ into Adenosine diphosphoribose (ADPR), but lacks the ADPR cyclase activity. [2] It is localized to the outer tegument of the adult parasite hosted in human blood vessels, and most probably scavenges the host extracellular/circulating free NAD⁺, therefore impacting NAD⁺-dependent pathways of the human immune system.

To discover *SmNACE* inhibitors, we carried out a virtual screening of commercially available compounds libraries (ca. 2 millions of molecules). Our approach combined docking using a homology model of *SmNACE* and scoring using machine learning and interaction fingerprinting. [3] It has benefited from the preliminary analysis of CD38 apo- and holo- structures, which revealed the rigidity of the catalytic site as well as the key determinants for binding of NAD⁺-like ligands. We selected and experimentally tested 103 compounds, and found four enzyme inhibitors at low micromolar concentrations. Among hits, taxifolin, a natural product of the favonoid family, has an inhibitory constant of 10 μ M.

A parallel experimental screening of an "in-house" chemical library of 6160 molecules [4] yielded only two confirmed hits, cyanidin and delphinidin, with respective inhibitory constants of 1.2 μ M and 3.2 μ M. Cyanidin and delphinidin are both anthocyanidins, which are chemically similar to flavonoids. Docking poses suggest that anthocyanidins and flavonoids can mimic the nicotinamide ribosyl part of NAD⁺.

REFERENCES

1. Goodrich, S. P.; Muller-Steffner, H.; Osman, A.; Moutin, M. J.; Kusser, K.; Roberts, A.; Woodland, D. L.; Randall, T. D.; Kellenberger, E.; LoVerde, P. T.; Schuber, F.; Lund, F. E., *Biochemistry* 2005, 44, (33), 11082-97.
2. Kuhn, I.; Kellenberger, E.; Rognan, D.; Lund, F. E.; Muller-Steffner, H.; Schuber, F., *Biochemistry* 2006, 45, (39), 11867-11878.
3. Marcou, G.; Rognan, D., 2007, 47, (1), 195-207.
4. <http://chimiotheque-nationale.enscm.fr/>

POSTER COMMUNICATIONS – PC.239

DESIGN AND SYNTHESIS OF AZAISOFLAVONES AND THEIR INHIBITORY ACTIVITIES OF NO PRODUCTION IN ACTIVATED MICROGLIA

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Nitric oxide (NO) produced by nitric oxide synthase (NOS) is involved in diverse biological functions including regulation of vascular relaxation, long-term potentiation in neurons and the cytotoxic action of macrophages. The family of NOS is classified as inducible form (iNOS) and two constitutive forms such as neuronal enzyme (nNOS) and endothelial enzyme (eNOS). The overproduction of NO by iNOS is responsible for the severe hypotension, cell cytotoxicity, tissue damage, the pathogenesis of septic shock, various inflammatory processes and cancer. Thus selective inhibition of iNOS may offer possible treatments for such diseases. Many strategies to control NO levels have been applied and mainly directed towards regulation of iNOS by the control of expression level and/or enzymatic activity of iNOS. Several classes of iNOS inhibitors, such as amino acid analogues, amino heterocycles, amidines, guanidines, isoquinolinamines and isothiourea, were reported. Despite the identification of various iNOS inhibitors, there is still strong need for development of selective and low toxic molecules.

Isoflavones are polyphenolic compounds that are widespread in legume plants. Isoflavones exhibit a variety of biological properties and may play a role in the prevention of cancers, coronary heart diseases, menopausal symptoms and osteoporosis. In addition, some isoflavones have been reported to exhibit anti-inflammatory and neuroprotective functions by inhibition of microglia activation. In spite of abundant reports of isoflavones, structural requirements and mechanisms for their anti-inflammatory effects have not been fully understood. Therefore, there is a strong demand for the various chemical tools. Although many synthetic variations of isoflavones have been reported, relatively few quinolone analogs, so called aza-isoflavones which were replaced oxygen of isoflavone skeletons with nitrogen, have been described.

Herein we report design and synthesis of new aza-isoflavones. The prepared compounds were evaluated their inhibitory activities of NO production and iNOS expression in BV-2 microglia cell lines. Several compounds showed significant inhibitory activities of NO production. Among these compounds, *N*-ethyl trihydroxy azaisoflavone was the most potent with IC_{50} 7.83 μ M for inhibition of NO production. This compound inhibited expression of iNOS in LPS-induced BV2 cells. This result suggests that the *N*-ethyl trihydroxy azaisoflavone inhibited the production of NO by suppressing the expression of iNOS.

^a As a first approach, one compound was considered an inhibitor if it inactivated TcTIM by more than 50 % at 100 μ M concentration.

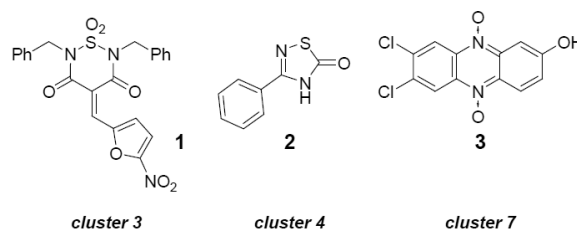


Figure 1

POSTER COMMUNICATIONS – PC.240

T. CRUZI TRIOSEPHOSPHATE ISOMERASE INHIBITORS DERIVED FROM A SCREEN HIT STUDY

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Chagas' disease (CD) is the third largest neglected disease in Latin America, affecting at least 15 million people.¹ The current drugs exhibit unacceptable side effects, do not eliminate the pathogen in the chronic stage, and ineffective due to parasite resistance. Therefore, development of new drugs against CD is urgent. *Trypanosoma cruzi* triosephosphate isomerase (TcTIM) is a ubiquitous glycolytic enzyme that catalyzes the interconversion between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The enzyme exhibits high catalytic rates only in its dimeric form, but each inactive monomer has all three catalytic residues. Recently, we have identified small molecules capable of inhibiting TcTIM by perturbing the interactions between its two subunits, which also have antiproliferative activity against *T. cruzi*.²

Along this line, we have designed a high-throughput assay to identify TcTIM inhibitors studying a 250 compound library that includes other known *T. cruzi* antiproliferative leads from our laboratory.³ The compounds belong to the following twelve clusters: 1) benzofuroxanes; 2) furanes- and thiophenes; 3) 1,2,6-thiadiazines; 4) 1,2-thiazoles; 5) nitroalkenes; 6) furoxanes; 7) 1,4-dioxidoquinoxalines and 5,9-dioxido-phenazines; 8) flavonoids; 9) *N*-oxidebenzimidazoles; 10) indazoles; 11) 1,2,4-triazines; 12) miscellaneous derivatives. The initial results, gathered according to the corresponding cluster, are summarised in the Table. From the dose-response study, compounds **1-3** (Figure 1), belonging to clusters 3, 4, and 7, were identified as new hits for TcTIM inhibition.

cluster	1	2	3	4	5	6	7	8	9	10	11	12	Total
Number of evaluated compound	50	59	19	9	4	25	11	6	18	12	10	28	251
Number of TcTIM inhibitors ^a	13	18	10	7	3	10	5	1	1	0	0	5	73

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REFERENCES

1. J. Rodrigues Coura, S.L. Castro, *Mem. Inst. Oswaldo Cruz*, 2002, **97**, 3.
2. a) V. Olivares-Illana, R. Pérez-Montfort, F. López-Calahorra, M. Costas, A. Rodríguez-Romero, et al., *Biochemistry*, 2006, **45**, 2556; b) V. Olivares-Illana, A. Rodríguez-Romero, I. Becker, M. Berzunza, J. García, R. Pérez-Montfort, N. Cabrera, F. López-Calahorra, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, *PLoS Neg. Trop. Dis.*, 2007, **1**, e01.
3. H. Cerecetto, M. González, *Mini Rev. Med. Chem.*, 2008, **8**, 1355.

POSTER COMMUNICATIONS – PC.241

DESIGN AND SYNTHESIS OF PENTACYCLIC TRITERPENE LIBRARIES WITH MULTIPLE TARGETING ANTITUMOR ACTIVITY

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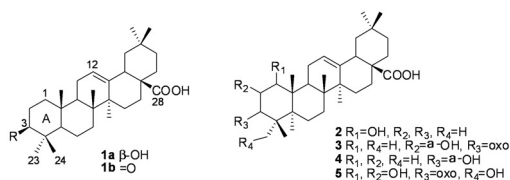
Multitargeting agents are believed to be crucial in the treatment of complex, multiple gene related diseases, such as cancer, neurodegenerative diseases, etc. To discover multitargeting agents, molecules with scaffolds based on privileged structure are of interests to be explored.

Pentacyclic triterpenoids have been known to exert their antitumor actions through various mechanisms, but usually exhibiting moderate activity [1]. We have found that 3-oxo OA (**1b**) exhibited antitumor and angiogenesis inhibitory activity [2]. So we chose to work on this type of privileged structure, in order to search for multitargeting antitumor leads, through the design and parallel synthesis of diverse libraries of oleanic acid (**1a**), with an emphasis on its A ring modified analogues.

The derivatization at A ring of **1** was first realized by introduction of hydroxyl groups in liquid phase, leading to the 1-OH (**2**), 2 α -OH (**3**), 3 α -OH (**4**), 23-OH (**5**) analogs, enabling us to introduce

diverse substituents to various positions on triterpene scaffold. For the library design, we selected a series of carboxylic acids from MDL® Available Chemical Directory, and grouped them by using many structural parameters, such as hydrophobic, electrostatic and stereoscopic factors, as descriptors, to ensure the selected synthetic building blocks from each group are diverse in the chemical space. Those parameters were also used for theoretical prediction of the reactivity order of those building blocks in the esterification reactions. Then we explored the solid phase³ parallel synthesis to prepare a 60 membered compound library of the **1** and **4** analogues. The inhibition of tumor cells proliferation, endothelial cells migration, and angiogenesis for those analogues were tested, and some of them demonstrated better activity than **1** in one or more assays.

Our study demonstrated the usefulness of triterpene scaffolds bearing diverse substituents in the search for multitargeting leads in cancer chemotherapy. Further investigations are warranted, and undergoing in our labs.



REFERENCES

- Wang, S.-R.; Fang, W.-S. *Curr. Top. Med. Chem.* 2009, 9: 1581-1596.
- Huang, D.; Ding, Y.; Li, Y.; Zhang, W.; Fang, W.; Chen, X. *Cancer Lett.* 2008, 233: 289-96.3) Pathak, A.; Singh, S. K.; Biabani, M. A. F.; Kulshreshtha, D. K.; Puri, S. K.; Srivastava, S.; Kundu, B. *Comb Chem High Throughput Screening* 2002, 5: 241-248.

POSTER COMMUNICATIONS – PC.243

HITTING THE RIGHT SPOTS: INTEGRATIVE STRUCTURE-BASED VIRTUAL SCREENING AGAINST INTENDED AND UNDESIED PROTEIN DRUG TARGETS

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To be effective and safe, drug molecules must discriminate successfully the intended protein target from alternative proteins present in the human body. We have developed an integrative structure-based molecular design strategy in which not only intended target-selective hot spots but also unfavorable anti-target-specific interactions are simultaneously considered for the construction of protein-ligand interaction fingerprint (IFP) models. Our virtual screening method enables the identification of target-selective ligands among large chemical databases while steering ligand design away from undesired anti-targets.

Applying this approach to sequentially and structurally unrelated targets and anti/targets, such as G protein-coupled receptors (GPCRs) and cytochrome P450 enzymes (CYPs), is especially challenging. The molecular backgrounds of ligand cross-reactivity between these pharmaceutically relevant systems are not well understood, and general strategies to design selective ligands are lacking. By combining our *in silico* models with experimental site-directed mutagenesis and structure-activity relationship studies we aim to identify the molecular determinants of ligand selectivity and promiscuity.

POSTER COMMUNICATIONS – PC.244

GUANIDINE CONTAINING BENZOFUROXAN AND BENZIMIDAZOLE 1,3-DIOXIDE SCAFFOLDS AS CRUZIPAIN-T. CRUZI INHIBITORS

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Chagas' disease (CD) is the third largest neglected disease in Latin America, affecting at least 15 million people with more than 25 million at risk of infection.¹ Current drugs exhibit unacceptable side effects, do not eliminate the parasite in the chronic stage, and display resistance. For that, development of new drugs against CD is urgent. One of our recent focuses has been the development of multitarget-directed ligands as anti-*T. cruzi* agents, using free-radical releasing moieties linked to cruzipain (CP)-inhibitor- pharmacophores (**1-3**, Fig. 1).²

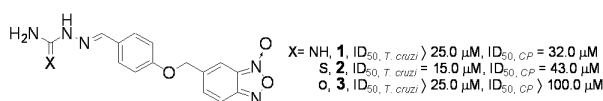


Figure 1

We selected parent compound **1** and benzofuroxan and benzimidazole 1,3-dioxide as core scaffolds linked to guanidine-pharmacophore aiming to produce new anti-*T. cruzi* agents with CP-inhibition capability. The designed compounds (**4-11**) were synthesized following the strategy shown in Fig. 2.

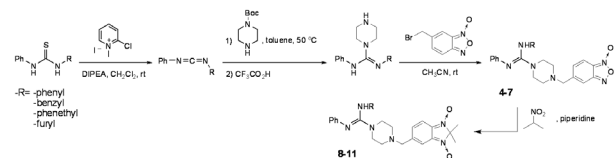


Figure 2

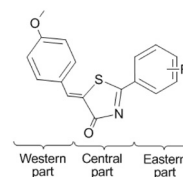
Their anti-proliferative activity was determined *in vitro* against *T. cruzi*, Tulahuen 2 strain, and the unspecific cytotoxicity was evaluated using human blood red (hrb) cells. The inhibition of CP was assayed *in vitro* and ligand-enzyme interactions were studied in terms of docking and NMR analysis. The new guanidine derivatives displayed good anti-*T. cruzi* activity, with IC_{50} values lower than Nifurtimox and **1**, and selectivity indexes ($IC_{50,hrb}/IC_{50,T.cruzi}$) higher than 11. The CP-inhibition was modest, increasing at pH 5.0 and the TI values, from the NMR experiments, and docking analysis clearly indicated that the main interactions with the enzyme are established by the piperazine and the aromatic moieties of the ligands.

ACKNOWLEDGMENTS

FCE, PEDECIBA, ANII, Cooperación CSIC (Spain)-Udelar (Uruguay), RIDIMEDCHAG-CYTED

REFERENCES

1. J. Rodrigues Coura, S.L. Castro, *Mem. Inst. Oswaldo Cruz*, 2002, **97**, 3.
2. a) W. Porcal, P. Hernández, M. Boiani, G. Aguirre, L. Boiani, A. Chidichimo, J.J. Cazzulo, N.E. Campillo, J.A. Paez, A. Castro, R. L. Krauth-Siegel, C. Davies, M.A. Basombrio, M. González, H. Cerecetto, *J. Med. Chem.*, 2007, **50**, 6004; b) W. Porcal, P. Hernandez, L. Boiani, M. Boiani, A. Ferreira, A. Chidichimo, J.J. Cazzulo, C. Olea-Azar, M. González, H. Cerecetto, *Bioorg. Med. Chem.*, 2008, **16**, 6995.



Taking the so-called “western part” and the “central core ring” moiety of the compounds constant we investigated the possibility for variations on the substitution pattern of the phenyl moiety as “eastern part” with different electron-releasing and -withdrawing moieties as well as with small and larger substituents of different lipophilic properties. The results of the activity assays using a cell-free 5-LO assay and on whole cells (PMNL cells)² demonstrated the possibility of large variation maintaining inhibitory potency, respectively.

On the one hand the study shows the possibility for large structural variation maintaining affinity, but also clearly demonstrates the difficulties in significantly increasing their antagonist potencies at 5-LO.

Supported by LOEWE LiFF and LOEWE OSF funding grants.

REFERENCES

1. Rådmark et al., *TrendsBiochemSci* **2007**, 32, 332-41.
2. B. Hofmann et al., *ChemMedChem* **2008**, 3, 1535-38.
3. E.M. Zayed et al., *Pharmazie* **1985**, 3, 194-7.

POSTER COMMUNICATIONS – PC.246

THIAZOLONE DERIVATIVES AS INHIBITORS OF 5-LIPOXYGENASE

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The enzyme 5-lipoxygenase (5-LO) plays an important role in the metabolism of arachidonic acid (AA). It catalyzes the reaction of AA into the leukotrienes (LT). Inhibition of 5-LO is a promising option for the treatment of inflammatory diseases, such as asthma or rheumatic arthritis¹. Actually, two drugs admitted for therapy targeting the LT pathway are on the market only: the antagonist of CysLT₁ receptor Montelukast (Singulair®), and the 5-LO inhibitor Zileuton (Zyflo®). So far Zileuton, a direct 5LO inhibitor of the iron-ligand type, is only admitted in the USA. Both drugs are marketed for the therapeutic indication of asthma bronchiale. Due of further medical need for alternative treatment options, the development of new direct inhibitors at 5-LO is of special interest.

Based on very recently disclosed lead structures² we designed and synthesized novel derivatives of thiazolone-containing compounds as direct inhibitors of 5-LO. The disubstituted thiazolone compounds were comfortably prepared by a three component one-pot-synthesis procedure using *p*-methoxybenzaldehyde, thioglycolic acid and differently substituted benzonitriles³.

POSTER COMMUNICATIONS – PC.247

PYRIMIDO[4,5-*d*]AZEPINES AS POTENT AND SELECTIVE 5-HT_{2C} RECEPTOR AGONISTS: THE DISCOVERY OF PF-3246799

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Pfizer Global Research & Development, Sandwich, Kent, CT13 9NJ, UK.

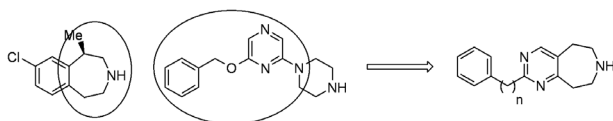
The neurotransmitter serotonin (5-HT) mediates its effects through at least 14 different receptor subtypes that have been classified into seven major families, 5-HT₁₋₇. The 5-HT₂ family has three members 2A, 2B and 2C and unlike 5-HT_{2A} and 5-HT_{2B} receptors, the expression of 5-HT_{2C} receptors appears to be restricted to the central nervous system (CNS). 5-HT_{2C} receptor agonists have become attractive drug targets that have potential use in the treatment of a number of conditions including obesity, schizophrenia, sexual dysfunction and urinary incontinence. For these indications, selectivity over 5-HT_{2A} and 5-HT_{2B} agonism is a key objective as 5-HT_{2A} agonists can potentially be hallucinogenic and have cardiovascular (CV) effects, while 5-

HT_{2B} agonism has been associated with heart valvulopathy and pulmonary hypertension.

The search for potent and selective 5-HT_{2C} agonists has identified lorcaserin (APD-356; Arena) which has been filed with the FDA for the treatment of obesity; vabicaserin (SCA-136; Wyeth/Pfizer), and several other small molecule 5-HT_{2C} agonists that are reported to be in early clinical development.

Pfizer have disclosed several new templates as 5-HT_{2C} receptor agonists and some of these compounds have been progressed to clinical trials. As part of our efforts to identify potential 5-HT_{2C} agonist drug candidates, we adopted a strategy of exploring multiple chemical templates in order to increase our chances of having compounds survive to become advanced clinical candidates. This presentation discloses novel pyrimido[4,5-d]azepines as potent and selective 5-HT_{2C} receptor agonists,¹ and includes:

- The identification of the lead series by "template hopping".
- Discussion of changes in structural features and physicochemical properties that effect CNS permeability.
- The exciting preclinical profile of PF-3246799, including efficacy data in animal models, pharmacokinetic and safety data and a comparison with the profile of lorcaserin.



REFERENCES

1. Pyrimido[4,5-d]azepine derivatives as 5HT_{2C} receptor agonists. M. D. Andrews, J. Blagg, P. Brennan, P. V. Fish, L. R. Roberts, R. I. Storer, G. A. Whitlock. **2008**, WO 117169.

POSTER COMMUNICATIONS – PC.248

DISCOVERY OF D-ASPARTATE LIGASE INHIBITORS BY VIRTUAL SCREENING

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D-Aspartate ligase is an enzyme involved in the peptidoglycan assembly pathway of Gram-positive bacteria, such as multiresistant enterococci and appears as an attractive target for the design of new narrow-spectrum antibacterial agents active against multiresistant *E. Faecium*. Aspartate ligase is a member

of the ATP-grasp protein superfamily, which includes a diverse set of enzymes catalyzing the ATP-dependent formation of carbon-nitrogen bond referred to as carboxylate-amine ligases. The role of aspartate ligase in peptidoglycan synthesis is to ligate the β-carboxylate of D-Asp to the ε-amino group of L-Lys in the nucleotide precursor UDP-*N*-acetylmuramyl-pentapeptide.

Virtual screening of ZINC database guided by 3D shape and electrostatics surface similarity searching using the ROCS and EON software programs was performed based on the structure of β-D-Asp-phosphate and dipeptide L-Lys-β-D-Asp. All known promiscuous inhibitors and compounds containing metal ions or reactive groups were excluded and a total of 50 compounds from virtual screen of 240000 compounds were selected for biological evaluation. By this approach we were able to identify the first inhibitors of D-aspartate ligase, which are a good starting point for further studies on our target enzyme.

REFERENCES

1. Staudenbauer WL, Strominger JL: Activation of D-Aspartic acid for incorporation into peptidoglycan, *J. Biol. Chem.*, **1972**, 247, 5095-5102
2. Staudenbauer WL, Willoughby E, Strominger JL: Further studies of the D-Aspartic acid-activating enzyme of *Streptococcus faecalis* and its attachment to the membrane, *J. Biol. Chem.*, **1972**, 247, 5289-5296
3. Bellais S, Arthur M, Dubost L, Hogunnet J, Gutmann L, Heijenoort J, Legrand R, Brouard J, Rice L, Mainardi J: Asl_{fm}, the D-aspartate ligase responsible for the addition of D-aspartic acid onto the peptidoglycan precursor of *Enterococcus faecium*, *J. Biol. Chem.*, **2006**, 281, 11586-11594

POSTER COMMUNICATIONS – PC.249

NICOTINIC ACID DERIVATIVES AS PRECURSORS OF THIOPEPTIDE ANTIBIOTICS

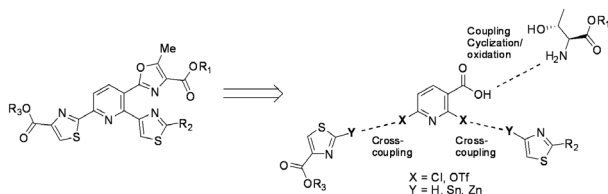
Xavier Just-Baringo¹, *Fernando Albericio^{1,2}* and *Mercedes Álvarez^{1,3}*

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The thiopeptide class of antibiotics¹ has been the centre of much attention during the last years due to the need of new antibacterial agents required to face the antibiotics resistance by strains of bacteria that were not thought to be a threat these days. These natural products, which have been extracted from both soil and marine microorganisms, are highly active inhibitors of protein synthesis in Gram-positive bacteria, and in many cases are effective even against methicillin-resistant *Staphylococcus aureus*, a strain usually resistant to most conventional treatments. Thus, thiopeptides represent a source of new structural diversity and modes of action that offer a unique opportunity to study the protein synthesis and ribosomal machinery in bacteria.



Properly functionalised nicotinic acid derivatives serve as precursors of the 2,3,6-trisubstituted thiopeptide antibiotics of the d series. The adequate halogen substitution pattern on the pyridine and the thiazole cross-coupling partners allow for the synthesis of the desired natural products in a highly convergent manner, thus granting access to analogues that should permit the study of the Structure-Activity Relationships of this family of compounds.

REFERENCES

1. Bagley, M. C.; Dale J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, 105, 685. (b) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, 46, 7930.

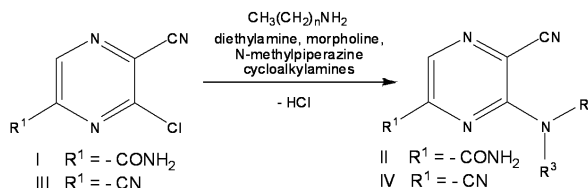
POSTER COMMUNICATIONS – PC.250

SYNTHESIS AND ANTI-INFECTIVE PROPERTIES OF SOME PYRAZINAMIDE ANALOGUES

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The widespread of Mycobacteria strains resistant to current treatment methods is an important epidemiological problem recognized by WHO¹. Pyrazinamide, the first line antituberculous substance, is used as a template model for compounds involved in this research project. Some pyrazinamide analogues, 3-arylaminopyrazine-2,5-dicarbonitriles, have already demonstrated good antimycobacterial and/or antifungal activity in a previous study². 6-chloro-5-cyanopyrazine-2-carboxamide (**I**) treated with various non-aromatic amines yielded **N-substituted 6-amino-5-cyanopyrazine-2-carboxamides (II)**. Similarly, the dehydration product of (**I**), 3-chloropyrazine-2,5-dicarbonitrile (**III**), yielded **N-substituted 3-aminopyrazine-2,5-dicarbonitriles (IV)**. Prepared compounds were chemically characterized and screened for antimycobacterial (*Mycobacterium tuberculosis* Rv H37, *M. tuberculosis* wild stem, *M. kansasii*, *M. avium* 80/72, *M. avium* 152/73) and antifungal activity (*in vitro* testing against *Candida albicans*, *C. tropicalis*, *C. crusei*, *C. glabrata*, *Trichosporon asahii*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and *Absidia corymbifera*).



Starting compound (**III**) possessed better antimycobacterial effect (MIC = 100 µg.ml⁻¹) against non-typical Mycobacteria (*M. avium* 80/72, *M. avium* 152/73) than pyrazinamide (MIC > 200 µg.ml⁻¹). Some other compounds of type (**II**), e.g. 6-heptylamino-5-cyanopyrazine-2-carboxamide (**II**, R¹ = -CONH₂, R² = heptyl, R³ = H) exhibited equal or slightly better activity (MIC ≤ 12.5 µg.ml⁻¹) against *Mycobacterium tuberculosis* Rv H37 than pyrazinamide.

3-heptylamino-5-cyanopyrazine-2,5-dicarbonitrile (**IV**, R¹ = -CN, R² = heptyl, R³ = H) showed a specific activity against *Trichophyton mentagrophytes* (MIC = 3.9 µmol.l⁻¹, standard ketoconazole MIC = 0.98 µmol.l⁻¹), and did not inhibit the growth of other tested fungi species (MIC > 500 µmol.l⁻¹).

This study was supported by the Grant Agency of Charles University (**BCH/120509**). The presentation was supported by ZENTIVA, k.s., Czech Republic.

REFERENCES

1. Anti-tuberculosis drug resistance in the world, Fourth Global Report, WHO/HTM/TB/2008.394, Accessed at: http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf, November 5th, 2008
2. Palek, L.; Dvorak, J.; Svobodova, M.; Buchta, V.; Jampilek, J.; Dolezal, M. *Arch. Pharm.* **2008**, 341, 61

POSTER COMMUNICATIONS – PC.251

A STUDY ON NEW INHIBITORS OF BHMT AND BHMT-2

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Betaine-homocysteine S-methyltransferase (BHMT) and betaine-homocysteine S-methyltransferase 2 (BHMT-2) are enzymes expressed in the liver and kidney of mammals where they are responsible for the conversion of homocysteine into methionine. Both BHMT and BHMT-2 are Zn-metalloenzymes and the Zn-cation was shown to be essential for their activity.^{1,2} While BHMT catalyzes a methyl transfer from betaine to L-Hcy to form dimethylglycine and L-Met, BHMT-2 methylates L-Hcy using S-methyl-L-methionine (SMM), Fig. 1.

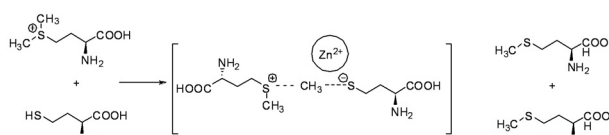


Fig. 1: Reaction catalyzed by BHMT-2

Recently, we synthesized and tested a series of strong BHMT inhibitors mimicking the hypothetical transition state.³ In contrary, BHMT-2 is a much less studied enzyme and up to date, no potent inhibitors are available. A series of rationally designed inhibitors can be very useful tools to study the physiological functions of the BHMT-2 *in vivo* and to understand the structural factors that govern substrate, product or inhibitor binding and/or the catalysis of the enzyme.

Herein, we present a series of first inhibitors of BHMT-2 developed recently in our laboratory and compare their properties to BHMT inhibitors described previously.

REFERENCES

1. Pajares, MA; Perez-Salab, D: Betaine-homocysteine S-methyltransferase: Just a regulator of homocysteine metabolism? *Cell. Mol. Life Sci.* **2006**, 63, 2792-2803.
2. Szegedi, S. S; Castro, C; Koutmos, M; Garrow, TA: Betaine-homocysteine S-methyltransferase-2 is an S-methylmethionine-homocysteine methyltransferase. *J. Biol. Chem.* **2008** 283(14), 8939-8945.
3. Vaněk, V; Buděšínský, M; Kabeleová, P; Šanda, M; Kožíšek, M; Hančlová, I; Mládková, J; Brynda, J; Rosenberg, I; Koutmos, M; Garrow, TA; and Jiráček, J: Structure-Activity Study of New Inhibitors of Human Betaine-Homocysteine S-Methyltransferase. *J. Med. Chem.* **2009**, 52, 3652-3665.

POSTER COMMUNICATIONS – PC.252

STRUCTURAL AND THEORETICAL STUDY OF HIV-1 INTEGRASE INHIBITION: ELUCIDATION OF QUINOLONYL DIKETOACID BINDING MODE

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Human immunodeficiency virus (HIV-1) is the causal agent of acquired immunodeficiency syndrome (AIDS). The mortality and the morbidity of HIV-1 infection led to the design of antiviral agents targeting different steps in the viral replication process. HIV-1 integrase is an especially attractive target for HIV

chemotherapy because the enzyme is essential for viral infectivity by catalyzing the insertion of viral DNA into the host cell genome through a metal ion dependent process^[1].

More recently, some diketoacid derivatives containing the quinolinone scaffold have been reported^[2,3]. Based on this series, we synthesized two quinolone compounds containing a β -diketoacid/ester group as chelating moiety (Figure 1). Both compounds were evaluated for their enzymatic and antiviral activity, acidic compound **2** being more potent than ester compound **1**. X-ray diffraction, UV-visible spectroscopy studies and theoretical calculations were performed in order to identify the most stable conformations of the diketoacid moiety and to study the chelation mode. In order to characterize the binding mode of compound **2**, a model of the enzyme was built by using the very recent crystal structure of PFV integrase^[4]. Based on these docking studies, pharmacomodulations were proposed in order to increase the inhibitory potency of the series of HIV-1 integrase inhibitors.

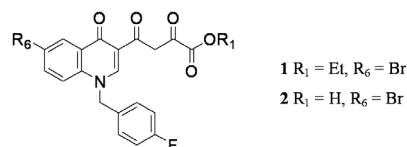


Figure 1

REFERENCES

1. Delelis, O. *et al.* *Retrovirology* 2008, 5,114.
2. Di Santo, R. *et al.* *J. Med. Chem.* 2006, 49, 1939.
3. Di Santo, R. *et al.* *J. Med. Chem.* 2008, 51, 4744.
4. Hare, S. *et al.* *Nature* 2010, 464, 232.

POSTER COMMUNICATIONS – PC.253

SYNTHESIS OF NUCLEOSIDE 5'-PHOSPHONATE ANALOGUES AS POTENTIAL P2Y₂ RECEPTOR LIGANDS

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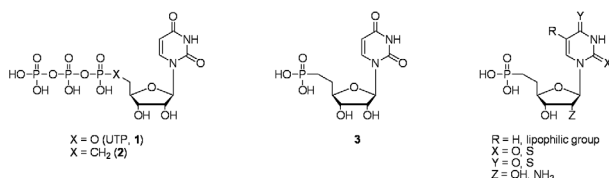
P2Y receptors are a family of eight class A GPCRs that respond to different nucleotides. The human P2Y₂ receptor is activated equipotently by both UTP and ATP. This receptor subtype is distributed in a broad range of tissues¹ and plays important physiological roles in epithelial cells of the lung, gastrointestinal tract and the eye. P2Y₂ receptors are targets for several therapeutic approaches, some of which are currently being explored. Agonists are promising for cystic fibrosis, cancer and dry eye syndrome^{1,2} while P2Y₂ antagonists exhibit anti-inflammatory³ and neuroprotective effects.⁴

The natural ligand UTP (**1**), as well as most reported analogs thereof, are liable to enzymatic degradation at the surface of the airways. This results in a relative short time of action when these molecules are given by means of inhalation. Changing the α -phosphate group for an isosteric phosphonate could raise up the metabolic stability.²

While we initially focused on a diphosphophosphonate mimic of UTP (**2**), it was accidentally discovered that the synthetic precursor **3** was also capable of activating the P2Y₂ receptor, possibly through an allosteric mechanism, while being inactive at the P2Y₄ receptor.²

From the growing series of UTP analogs that have been screened on P2Y receptors, it is known that a 2-thio and a 4-thio modification raise the affinity for the P2Y₂ receptor, while the former also enhances the selectivity vs the P2Y₄ receptor.⁵

Here, we decided to combine these uracil modifications and/or the selectivity-enhancing 2'-amino-2'-deoxy substitution with a 5'-phosphonate group. The synthesis of 5'-modified 5'-phosphonate analogues is another goal.



REFERENCES

1. Kellerman, D.; Evans, R.; Mathew, D.; Shaffer, C. *Adv. Drug Deliv. Rev.* (2002), **54**, 1463-1474.
2. Cosyn, L. et al. *Bioorg. Med. Chem. Lett.* (2009), **19**, 3002-3005.
3. Morse, D. M.; Smullen, J. L.; Davis, C. W. *Am. J. Physiol. Cell. Physiol.* (2001), **280**, 1485.
4. Volonte, C. et al. *Neuropharmacology* (1999), **38**, 1335-1342.
5. Ko, H. et al. *Bioorg. Med. Chem.* (2008), **16**, 6319-6332.

POSTER COMMUNICATIONS – PC.254

HEMODYNAMIC EFFECTS OF LEVOSIMENDAN DURING ISCHEMIA/REPERFUSION IN THE ISOLATED PERFUSED RAT HEART

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Levosimendan exerts a coronary and systemic vasodilator effect through its K_{ATP} channel-opening properties and this action may be of specific interest in the setting of myocardial ischemia. The aim of this experimental study was to investigate whether levosimendan has protective effects on myocardial ischemia-reperfusion injury.

After anesthesia rats heart were removed placed in ice-cold Krebs-Heinseleit and mounted on Langendorff apparatus. Hearts were assigned into three groups: first, after 30 min ischemia in order to induce, ischemic postconditioning 3 times 10 second ischemia separated by 10 second reperfusion protocol was used; second, control; and third, 10^{-7,8,9} mol levosimendan was added to the reperfusion fluid after 30 min ischemia in order to induce chemical postconditioning. In all these groups, 30 min global ischemia was followed by 120 min reperfusion. In all experiments, hemodynamic parameters (LVDP, LVEDP, $\pm dP/dt$, RPP) were evaluated at 30th and the 120th minutes of reperfusion. Left ventricular pressure was measured with a water-filled balloon. After ischemia, the heart tissues were dyed with TTC for infarct size measurement.

LVDP, LVEDP and $\pm dP/dt$ values were similar to the pre-ischemia in ischemic postconditioning and levosimendan (10⁻⁸mol) groups at 30th minute of reperfusion. However at the 120th minute, the values were reduced in the same groups. The infarct size was significantly reduced in levosimendan and ischemic postconditioning groups compared to the control group.

The results showed that, levosimendan and ischemic postconditioning may provide cardioprotection over the myocardial ischemia reperfusion injury.

POSTER COMMUNICATIONS – PC.255

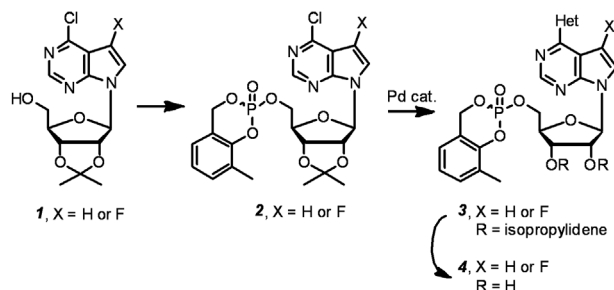
SYNTHESIS AND BIOLOGICAL ACTIVITIES OF CYCLOSAL-PRONUCLEOTIDES DERIVED FROM 6-HETARYL-7-DEAZAPURINE RIBONUCLEOSIDES

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The usage of prodrugs plays a key role in antiviral and antitumor drug development. The *cycloSal* approach is a successful method used for improvement of antiviral activities of nucleosides.¹ Recently, a series of 6-hetaryl-7-deazapurine nucleosides with cytotoxic activity in nanomolar range was synthesized in our group.² The aim of this project was to improve the activity of these derivatives using *cycloSal*-phosphate prodrugs. The 2',3'-isopropylidene 6-chloro-7-deazapurine ribonucleosides **1** were converted to *cycloSal*-pronucleotides **2**. Using Suzuki and Hille cross-coupling reactions of *cycloSal*-pronucleotides **2** with hetarylboronic acids or -stannanes a series of isopropylidene protected 6-hetaryl-7-deazapurine pronucleotides **3** was prepared. These were further deprotected to give the derivatives of free pronucleotides **4**. All the compounds were obtained as 1:1 mixtures of diastereomers as a result of non-stereoselective introduction of the centre of chirality to phosphorus atom. All 12 target compounds **4** were tested for their *in vitro* cytotoxic activity on various cell lines. Mostly, the compounds showed similar or

lower activities compared to the parent nucleosides. Only in the case of three 7-fluoro pronucleotides the cytotoxic activities were improved by one order of magnitude.



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REFERENCES

1. Meier C.: Eur. J. Org. Chem. 2006, 1081.
2. Nauš P., Pohl R., Votruba I., Džubák P., Hajdúch M., Ameral R., Birkus G., Wang T., Ray A.S., Mackman R., Cihlar T., Hocek M.: J. Med. Chem. 53, 460 (2010).

experiments, STD NMR, and by monitoring the $^1\text{H}/^{13}\text{C}$ chemical shift changes of MurD selectively labeled with ^{13}C at the methyl groups of Ile, Val, and Leu upon binding of ligands².

REFERENCES

1. Humljan J., Kotnik M., Contreras-Martel C., Blanot D., Urleb U., Dessen A., Solmajer T., Gobec S. *J. Med. Chem.* **2008**, 51: 7486-7494.
2. Hajduk P. J., Augeri D. J., Mack J., Mendoza R., Yang J., Betz S. F., Fesik S. W. *J. Am. Chem. Soc.* **2000**, 122: 7898-7904.

POSTER COMMUNICATIONS – PC.257

CONFORMATIONAL INSIGHTS INTO A RIGID BODY C-TERMINAL DOMAIN MOVEMENT OF MurD LIGASE

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Bacterial resistance is a worrying phenomenon that urges the need to discover new antibacterial drugs. Since peptidoglycan is a unique component of the cell wall of almost all eubacteria, its biosynthetic pathways represent an attractive targets for new drugs.¹ ATP dependant enzymes of the Mur ligase family have an essential role in peptidoglycan biosynthesis as the stepwise addition of amino acids to the UDP-*N*-acetyl muramic acid to yield UDP-MurNAc pentapeptide is catalyzed by them. MurD (UDP-*N*-acetylmuramoyl-L-alanine: D-glutamate ligase) is the second enzyme in the series of Mur ligases, and catalyzes the addition of D-glutamic acid to UDP-*N*-acetylmuramoyl-L-alanine (UMA).²

MurD has been structurally very well characterized as several crystal structures of apo-enzyme and enzyme co-crystallized with natural substrates have been solved.^{3,5} Structures reveal that a rigid body rotation of the MurD C-terminal domain occurs and its position defines the so called closed and open conformation of the protein.^{4,5} Previous structural studies revealed that, MurD co-crystallized with UMA and 5'-adenylylimido-diphosphate (AMPPNP: A non-hydrolyzable analogue of ATP), was in the closed conformation⁴, on the contrary MurD co-crystallized with UMA or in its apo-form was in the open conformation⁵.

We discovered two new conformations of MurD, which are not in line with previous findings: Closed conformation of apo-MurD and a structure of MurD in an open conformation co-crystallized with UMA and AMPPNP.

POSTER COMMUNICATIONS – PC.256

NOVEL RIGIDIFIED INHIBITORS OF MURD

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MurD is the second enzyme in the series of Mur ligases. It catalyses the addition of D-glutamic acid to the cytoplasmic intermediate UDP-*N*-acetylmuramoyl-L-alanine (UMA). A series of naphthalene *N*-sulfonyl-D-Glu derivatives previously synthesized and the high-resolution crystal structures of MurD from *E.coli* in complex with four small molecule inhibitors revealed details of the binding mode in the active site of MurD¹. An insight into structure-activity relationship presented a solid foundation for optimization of inhibitors of this structural class and was the main reason for the synthesis of novel sulfonamide compounds where a rigid surrogate of D-glutamic acid moiety was incorporated in the structure.

Three compounds displayed significant inhibitory activity, with an IC_{50} values of 38, 46, and 50 μ M, respectively. In addition these compounds were extensively studied with high resolution NMR spectroscopy, in order to determine binding to the enzyme and the location of compounds in protein binding site. This was done by using several different NMR techniques, such as trNOE

New structural information on MurD represents an important contribution to design of novel inhibitors by implementation of computer-aided approach. Furthermore, the loop which is important for the C-terminal domain movement could be used as a potential target of novel allosteric inhibitors, which could prevent the C-terminal domain to rotate and consequently inactivate the enzyme's function.

REFERENCES

1. van Heijenoort J. *Nat. Prod. Rep.* 200; 18(5): 503-19.
2. Barreteau H, Kovač A, Boniface A, Sova M, Gobec S, Blanot D. *FEMS Microbiol. Rev.* 2008; 32(2): 168-207.
3. Bertrand JA, Auger G, Fanchon E, Martin L, Blanot D, van Heijenoort J, Dideberg O. *EMBO J.* 1997; 16(12): 3416-25.
4. Bertrand JA, Auger G, Martin L, Fanchon E, Blanot D, Le Beller D, van Heijenoort J, Dideberg O. *J. Mol. Biol.* 1999; 289(3): 579-90.
5. Bertrand JA, Fanchon E, Martin L, Chantalat L, Auger G, Blanot D, van Heijenoort J, Dideberg O. *J. Mol. Biol.* 2000; 301(5): 1257-66.

POSTER COMMUNICATIONS – PC.259

NEW PHENYLALKYLAMINES AS ISOFORM SELECTIVE HCN-CHANNELS BLOCKERS

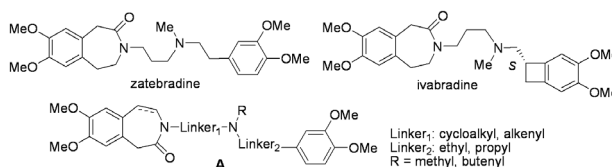
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The "funny current" (I_f) plays a major role in the generation of pacemaker activity and in the regulation of heart rate. [I_f] is carried by the HCN (Hyperpolarization-activated Cyclic Nucleotide gated) channels, a family of membrane proteins formed by four members (HCN1-4) which are differently distributed within tissues such as heart and nervous system. [2] Beside heart, HCN channels regulate rhythmic activity in other excitable cells such as neurons and photoreceptors, they have a role in dendritic integration, long term potentiation, synaptic transmission. [3] The different isoforms vary in their distribution within tissues; therefore, isoform-selective substances could be important pharmacological tools to study the channels in native tissues, and may be useful to design safe drugs. [3]

I_f can be blocked by the so-called «specific bradycardic agents», a group of structurally-diverse drugs among which are zatebradine and ivabradine. Ivabradine is the only specific bradycardic agent available for therapy: in 2005 it has been approved by EMEA for the treatment of stable *angina pectoris*. However, this drug, as well as its congener zatebradine, is not able to discriminate among

HCN channel isoforms. [4] In order to search for isoform-selective I_f blockers, new phenylalkyl amines structurally related to zatebradine have been prepared (general formula **A**). These compounds were tested for their I_f blocking ability on HEK293 cells stably expressing the HCN1, HCN2 and HCN4 channels. It was found that structural manipulations can lead to molecules showing isoform selectivity, the best selectivity profile being toward HCN1 or HCN4. The most interesting compounds were further characterized on heart by electrophysiological studies.



REFERENCES

1. DiFrancesco, D. *Pharmacol. Res.* **2006**, 53, 399-406.
2. Herrmann, S.; Stieber, J.; Ludwig, A. *Pflugers Arch. Eur. J. Physiol.* **2007**, 454, 517-522
3. Wahl-Schott, C.; Biel, M. *Cell Mol. Life Sci.* **2008**,
4. Stieber, J.; Wieland, K.; Stockl, G.; Ludwig, A.; Hofmann, F. *Mol. Pharmacol.* **2006**, 69, 1328-1337

POSTER COMMUNICATIONS – PC.260

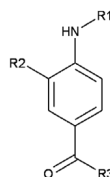
SYNTHESIS OF NOVEL BENZOIC ACID DERIVATIVES AS POTENTIAL ANTICHAGAS AGENTS

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Chagas disease or American Trypanosomiasis is caused by *Trypanosoma cruzi*, and is one of the most important parasitic diseases in Latin America. Currently chemotherapy is only based in nifurtimox or benznidazole, but it has limited efficacy in the chronic stage of the disease and produce toxic side effects. Parasite proteins as trypanothione reductase, trans-sialidase and cruzain cysteine protease among others are key enzymes in the metabolism of *Trypanosoma cruzi* and recently have been proposed as potential drug targets for Chagas disease. Particularly, trans-sialidase from *Trypanosoma cruzi* (TcTS) has emerged as a potential drug target for pharmacological treatment of Chagas disease (1-2). As a part of our ongoing research in the design and synthesis of new antichagas drug candidates, here, we report a series of novel benzoic acid derivatives

(Scheme 1.) that were tested *in vitro* against the trypomastigote form of *Trypanosoma cruzi* (NINOA strain).



Scheme 1. General structure of novel benzoic acid derivatives as potential antichagas agents.

Structure-activity relationship analysis showed that carbonyl or sulfonyl derivatives in position R1 on a 4-amino group decreases compounds activity. Alternatively, only a 4-amino group on aromatic ring enhanced the biological activity. Additionally, a nitro group in position R2 increases the activity compounds. On the other hand, Neres and cols reported benzoic acid derivatives as TcTS (1), thus we included novel substitutions in position R3 to determine their influence in biological activity. The most active compound P-057 showed a 97 percentage of trypomastigotes lysis and both reference drugs, nifurtimox and benznidazole showing a percentage lysis of 46 and 47, respectively, tested at same concentration. In conclusion, these novel benzoic acid derivatives could be used as lead compounds for design and synthesis of new series of promising potential antichagas agents.

REFERENCES

1. Neres J., Bonnet P., Edwards PN., Kotian PL., Buschiazio A., Alzari PM., Bryce RA., Douglas KT. *Bioorganic & Medicinal Chemistry*. 2007, 15(5), 2106-2119.
2. Neres J., Brewster ML., Ratier L., Botti H., Buschiazio A., Edwards PN., Mortenson PN., Charlton MH., Alzari PM., Frasch AC., Bryce RA., Douglas KT. *Bioorganic & Medicinal Chemistry*. 2009, 19(3), 589-596.

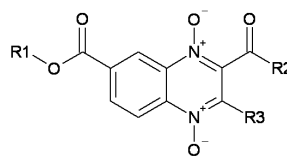
POSTER COMMUNICATIONS – **PC.261**

SYNTHESIS OF NOVEL QUINOXALINE DERIVATIVES AS POTENTIAL ANTITUBERCULOSIS AGENTS

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The importance of heterocyclic compounds has been long time recognized in the field of synthetic organic chemistry. Quinoxalines, including their fused-ring derivatives, showed various pharmacological activities and particularly, their 1,4-di-*N*-oxides derivatives have demonstrated excellent efficacy as antiviral, anticancer, antibacterial, and antiparasitic agents (1-2). As a part of our ongoing research in the design and synthesis of new antituberculosis drug candidates, two new series of quinoxaline 1,4 di-*N*-oxide derivatives with carboxylate substituent in position R1 have been synthesized (Scheme 1.) and tested *in vitro* against *Mycobacterium tuberculosis*. Biological evaluation was carried out within the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program (3). Benzofuroxane 5-substituted and quinoxaline 1,4 di-*N*-oxide derivatives were obtained according to the synthetic methods previously described (4).



Scheme 1. General structure of novel quinoxaline derivatives.

Results of these compounds showed a good antituberculosis activity in the first level screening with IC₉₀ values of ≤10 µg/mL. Structure-activity relationship analysis showed that compounds with electron withdrawing substituent such as trifluoromethyl group in position R3 had better activity than compounds with methyl group substituent. Also, compounds activity is significantly affected when trifluoromethyl substituent on the quinoxaline nucleus is replaced by a rigid structure or electron donating group such as phenyl group and methyl group in position R2 and/or R3, respectively, showed low or not activity. Additionally, when a substitution at *para* position on phenyl ring with a chlorine atom was carried out, resulted in an enhanced activity compounds. Statistical significant differences were not found between the anti-mycobacterial activities in the two series obtained. In conclusion, these quinoxaline derivatives could be used as lead compounds for design and synthesis of new series of new potentially antituberculosis agents.

REFERENCES

1. Gonzalez M, Cerecetto H, Monge A. *Topics in Heterocyclic Chemistry*. 2007, 11, 179-211.
2. Khan SA, Saleem K, Khan Z. *European Journal of Medicinal Chemistry*. 2008, 43, 2257-2261.
3. <http://taacf.org/Process-text.htm#IC90>
4. Jaso A, Zarranz B, Aldana I, Monge A. *European Journal of Medicinal Chemistry*. 2003, 38, 791-800.

POSTER COMMUNICATIONS – PC.262

IDENTIFICATION OF BINDING MODES OF BENZODIAZEPINE BINDING SITE LIGANDS BY A COMBINED DOCKING-PHARMACOPHORE MODELING APPROACH

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Ligand/protein docking uses energetic scoring functions for evaluating and ranking poses. However, if the precise induced fit structure is not known, as it is the case in the benzodiazepine binding site (bz-site) of the GABAA receptor, docking results are often unsatisfying and lead to ambiguous binding mode hypotheses of the ligands. In contrast, ligand-based pharmacophore models are protein and binding mode independent models which represent the mutual alignment of structurally different, biologically active ligands. Thus, the mutual orientation of different ligands which is provided by the pharmacophore model should be recovered in the poses produced by ligand-protein docking as the pharmacophore-supported binding mode (1). We developed a methodology within MOE (Chemical computing Group) using the SVL programming language which automatically screens for this pharmacophore-supported binding mode in the bz-site of the GABAA receptor.

In the first step, a set of rigid ligands is selected for docking from the widely accepted pharmacophore model of the benzodiazepine binding site (2). We prefer ligands which are biologically characterized by mutagenesis or labeling data. Subsequently, the selected ligands are docked and the energetically plausible poses of each ligand are retained and stored within a database. The search for the pharmacophore-supported binding mode starts with selecting an index ligand (in our case diazepam) from the pharmacophore model. At the beginning each diazepam pose represents a candidate binding mode. Each proposed binding mode of diazepam serves as template for superposition of other compounds (reference ligands like β -Carbolines or Imidazopyridines) based on the alignment in the common pharmacophore model. In the next step the docking poses of each reference ligand are compared with their respective orientation in the pharmacophore-based alignment by calculating the RMSD-value of the atoms characterizing the pharmacophoric features. The binding mode which performs best is considered as the pharmacophore-supported binding mode and further validated using experimental data.

The introduced methodology enabled us to identify the binding mode of benzodiazepine ligands within the bz-site of the GABAA receptor.

ACKNOWLEDGMENT

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REFERENCES

1. Zhang W, et al. Development of a comprehensive pharmacophore model for the benzodiazepine receptor. *Drug Des Discov* 1995;12:193-248
2. Wallach I, Lilien R. Predicting Multiple Ligand Binding Modes Using Self-Consistent Pharmacophore Hypotheses. *J Chem Inf Model* 2009;49:2116-28

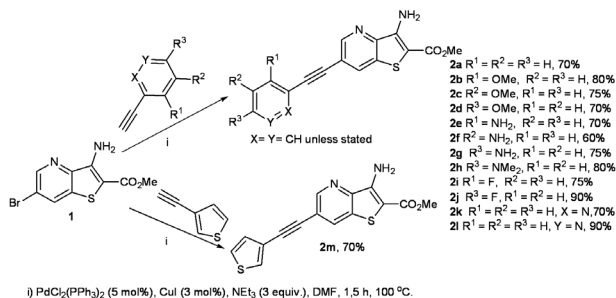
POSTER COMMUNICATIONS – PC.263

SYNTHESIS AND TUMOR CELL GROWTH INHIBITION OF NOVEL METHYL 3-AMINO-6-[(HETERO)ARYLETHYNYL]THIENO [3,2-*b*]PYRIDINE-2-CARBOXYLATES. SARs AND EFFECTS ON THE CELL CYCLE AND APOPTOSIS

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Several novel methyl 3-amino-6-[(hetero)arylethynyl]thieno[3,2-*b*]pyridine-2-carboxylates were prepared by Sonogashira coupling of the methyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate **1**, recently reported by some of us,¹ with (hetero)aryl-acetylenes in high yields (Scheme).



The growth inhibition of all the compounds was evaluated on three human tumour cell lines (MCF-7, NCI-H460, A375-C5) and some SARs were established. Compounds **2d**, **2e** and **2g** are the most promising compounds presenting the lowest GI_{50} values.

Their effects on the normal cell cycle distribution and induction of apoptosis were studied in the NCI-H460 cell line.² All three compounds altered cell cycle distribution and compound **2e** was further shown to induce apoptosis in the same cell line.

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REFERENCES

1. Calhella, R. C.; Queiroz, M.-J. R. P. *Tetrahedron Lett.* 2010, 51, 281-283.
2. Almeida G. M.; Duarte T. L.; Farmer P. B.; Steward W. P.; Jones G.D.D. *Int. J. Cancer* 2008, 122, 1810-1819.

POSTER COMMUNICATIONS – PC.264

PASS BASED VIRTUAL SCREENING OF DRUG-LIKE COMPOUNDS

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The computer program PASS (Prediction of Activity Spectra for Substances) is the product of ideas that originated about forty years ago from the development of a National Registration System for New Chemical Compounds in the former USSR. To select the most promising hits among the newly synthesized compounds submitted for registration, their structural formulae must be analyzed using computer-assisted methods. The current version of PASS 10.1 predicts 4130 kinds of biological activity with average accuracy about 95% based on the analysis of the training set included 266697 drugs, drug-candidates, leads and toxic compounds (in 1993 the appropriate numbers were 114, 9314 and 75%, respectively). Detailed description of PASS approach is presented in the paper [1]. PASS predictions have been obtained for about 250000 molecules from Open NCI database [2] and presented at the NCI web-site (<http://cactus.nci.nih.gov/ncidb2/>). It was shown that, using PASS for virtual screening, it is possible to increase significantly the number of “actives” in the selected sub-set of compounds (85 from 250000 compounds selected as hits, 7 were tested and 4 appeared to be the angiogenesis inhibitors) [2]. In another project medicinal chemists from six countries jointly prepared a virtual library of about 5500 compounds belonging to different chemical classes. 30 hits have been selected for synthesis and experimental testing, and all of them appeared to be anxiolytics, anticonvulsants and cognition enhancers that confirmed the PASS predictions [3,4]. Since 2000 PASS INet service is freely available via Internet (<http://www.ibmc.msk.ru/PASS/>), and over 6000 reg-

istered users from 60 countries obtained PASS predictions for more than 120000 compounds. Results of PASS predictions confirmed by the experiment have been published in more than forty papers (for review see [5]). It was demonstrated that some adverse and toxic effects can be also predicted by PASS [6]. Based on the PASS predictions, multitargeted antihypertensive [7] and anti-inflammatory [8] agents have been identified in virtual libraries of chemical compounds. PASS applicability to the analysis of biological activities of phytoconstituents from medicinal plants has been recently shown [9]. We will present an overview of PASS applications in medicinal chemistry for both virtual screening and design of pharmaceutical agents with the required properties.

ACKNOWLEDGMENT

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REFERENCES

1. Filimonov D.A. and Poroikov V.V. (2008). In: *Chemoinformatics Approaches to Virtual Screening*. Eds. Alexandre Varnek and Alexander Tropsha. Cambridge (UK): RSC, p.182-216.
2. Poroikov V.V. et al. *J. Chem. Inform. Comput. Sci.*, 2003, **43**; 228-236.
3. Geronikaki A. et al. *J. Med. Chem.* 2004, **47**: 2870-2876.
4. Geronikaki A. et al. *Bioorg. Med. Chem.*, 2004, **12**: 6559-6568.
5. Geronikaki A. et al. *SAR & QSAR Environ. Res.*, 2008, **19**: 27-38.
6. Lagunin A. et al. *QSAR and Combinatorial Science*, 2009, **28**: 806-810.
7. Lagunin A.A. et al. *J. Med. Chem.*, 2003, **46**: 3326-3332.
8. Geronikaki A.A. et al. *J. Med. Chem.*, 2008, **51**: 1601-1609.
9. Lagunin A. et al. *Curr. Pharm. Des.*, 2010, in press.

POSTER COMMUNICATIONS – PC.265

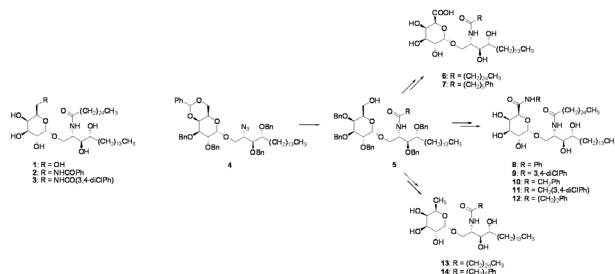
VERSATILE SYNTHESIS OF C-5'-MODIFIED α -GALCER ANALOGUES AS NEW NKT-CELL LIGANDS

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α -Galactosylceramide (**1**), a synthetic glycosphingolipid structurally based on substances extracted from the marine sponge *Agelas mauritanus*, exhibits antitumor activity in mice. Following complexation with the antigen presenting glycoprotein CD1d, α -GalCer is recognized by NKT-cells, which, after activation, release both Th1 and Th2 cytokines. Th1 cytokines are thought to be responsible for the antitumor, antiviral and antibacterial effects of α -GalCer while Th2 cytokines can ameliorate autoimmune diseases. Unfortunately, both responses tend to antagonize each other's biological effect. Hence analogues capable of inducing a Th1/Th2 biased response are highly awaited.

Towards this end, numerous analogues of α -GalCer have been synthesized. Alterations of the galactopyranose ring indicate that the hydroxyls at the 2' and 4' positions of the sugar are critical for biological activity, while modifications of the 3' and 6' positions are more permissive.¹ Modifications at C-6' are possible without loss of antigenic activity,^{2,3} but usually require a laborious synthetic route.⁴ Inspired by the Th1 biased response of certain C-6'-derivatised analogues (**2-3**), designed earlier in our laboratory,³ we decided to further explore modifications at this position. A selective opening of the benzylidene ring of **4** was the key step for a more divergent synthetic approach, in which the C-6'-OH group was modified after the glycosylation. The key intermediate **5** gave access to galacturonic acid derivatives **6-7**, the corresponding uronamides **8-12**, as well as two 6'-deoxygenated analogues (**13-14**), all featuring substituents known to induce Th1 polarisation.



REFERENCES

1. Wu, D. et al. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 3972-3977.
2. Liu, Y. et al. *J. Immunol. Methods* **2006**, 34-39.
3. Trappeniers, M. et al. *J. Am. Chem. Soc.* **2008**, 16468-16469.
4. Stallforth, P.; Adibekian, A.; Seeberger, P.H. *Org. Lett.* **2008**, 1573-1576.

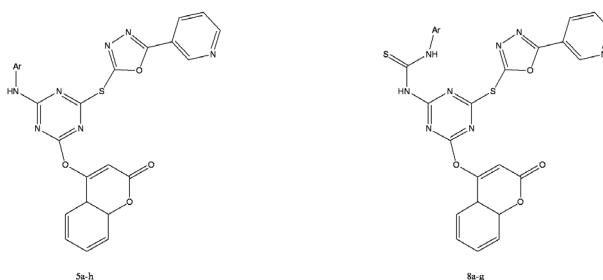
POSTER COMMUNICATIONS – PC.266

A SMALL LIBRARY OF TRI-SUBSTITUTED S-TRIAZINES AS ANTIMICROBIAL AND ANTITUBERCULAR AGENTS

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A small library of tri-substituted *s*-triazines were synthesized by sequential selective substitution at controlled temperature. All the synthesized compounds were evaluated for their *in vitro* antimicrobial and antitubercular activity. Out of total screened compounds seven compounds have shown activity against gram positive bacteria in the range of 3.12-6.25 μ g/mL and seven compounds have shown antitubercular activity against *Mycobacterium tuberculosis* H₃₇Rv, in the range of 1.76-6.25 μ g/mL.



REFERENCES

1. Design, synthesis and antimicrobial evaluation of *s*-triazinyl urea and thiourea derivatives. *Arkivoc*, 2009 (xi), 326-335
2. Synthesis and antimicrobial studies of *s*-triazine based heterocycles. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2010, 25 (1), 121-125

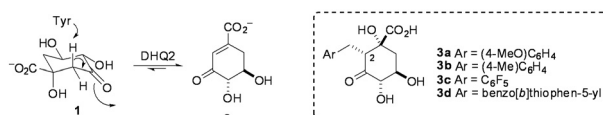
POSTER COMMUNICATIONS – PC.267

STEADY-STATE FLUORESCENCE AND MOLECULAR DYNAMIC STUDIES ON THE INHIBITION OF TYPE II DEHYDROQUINASE BY (2R)-3-BENZYL-3-DEHYDROQUINIC ACIDS

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In recent years, we have focused on the inhibition of type 2 dehydroquinase (DHQ2) from *Mycobacterium tuberculosis* (DHQ2-Mt) and *Helicobacter pylori* (DHQ2-Hp), bacteria in which this enzyme are essential,¹ for the development of new antimicrobial agents against the diseases caused by them.² We have shown recently that the substitution of the equatorial hydrogen of the 3-dehydroquinic acid (**1**), the natural substrate of the DHQ2, by benzyl groups provide potent reversible competitive inhibitors of these enzymes.³ *p*-Methoxybenzyl derivative **3a** and benzothiophene **3d** proved to be the most potent competitive inhibitors against both enzymes, with *K_i* values of 26 nM and 28 nM against *M. tuberculosis* and 170 nM and 160 nM against *H. pylori*, respectively. The structure-activity relationship of these substrate analogues has been studied by steady-state fluorescence techniques and molecular dynamics and it will be presented.



REFERENCES

1. www.essentialgene.org
2. González-Bello, C.; Castedo, L. *Med. Res. Rev.* **2007**, *27*, 177.
3. Hatakeyama, M. *J. Gastroenterol* **2009**, *44*, 239.
4. González-Bello, C. *et al. ChemMedChem* **2009**, *4*, 1980.

POSTER COMMUNICATIONS – PC.268

VIRTUAL SCREENING WORKFLOW FOR GLYCOGEN SYNTHASE KINASE 3 β INHIBITORS

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Glycogen synthase kinase 3 β (GSK-3 β) is a serine/threonine protein kinase participating in important insulin and Wnt pathways. Inhibitors of this kinase show antidiabetic properties, may be used for the treatment of Alzheimer's disease and inflammation, have memory-stabilizing effect and so on. Unique combination of various effects in the same target makes the kinase very attractive for design of new perspective drugs.

We have performed computational search for ATP-competitive GSK-3 β inhibitors in ZINC database with the use of two opposite but complementary methods: structure-based screening (docking with FRED) and ligand-based screening (combination of similarity search with ROCS and electrostatic similarity scoring with EON). ZINC database was filtered according to rules of lead-likeness; additionally, it was required for potential inhibitors to have at least one ring structure with the aim to restrict flexibility of a molecule and make it more synthetically accessible.

Comparative analysis of usability of various scoring functions applied to structure-based computational search for GSK-3 β inhibitors was carried out. The best performance was observed for functions based on the complementarity between the ATP-binding cavity of the kinase and inhibitor molecule (Shapegauss, Chemgauss3, Chemscore). The structure-based screening system was evaluated with the help of ROC analysis to assess overall enrichment of database and BEDROC metrics for early recognition of the hits. After visual inspection of docking results, 2500 reasonable hits were identified, some of them belonging to previously unexplored structure classes.

Ligand-based search was performed using the structure of hymenialdisine as a template. This molecule is a very potent conformationally restricted inhibitor of GSK-3 β possessing rather unusual structure. Consequently, similar molecules may be interesting leads for the design of GSK-3 β inhibitors; similarity search was performed for the same ZINC subset as docking studies. Six thousand hits were identified, and docking was performed to compare them with the hits found during the structure-based search. Despite the fact that hits identified with ligand-based screening were smaller than ones found based on

the structure of the kinase, certain scaffolds appear in both hitlists. We may conclude that the sequential use of ROCS, EON and FRED may be considered as a quick way to identify hits, whereas docking of large databases can be used when chemical diversity is needed to be explored.

POSTER COMMUNICATIONS – PC.269

NOVEL AZULENE-OXINDOLE CLASS KINASE INHIBITORS THAT INDUCE COMPLETE TUMOR REGRESSION IN AML MICE

On Lee, Ching-Huai Ko, Yuan-Jang Tsai, Chih-Peng Liu, Yen-Chun Chen, Hsiang-Wen Tseng, Ying-Chu Shih, Ting-Shou Chen, Chih-Hung Chen, Shu-Jiau Chiou, Ling-Mei Wang, Chrong-Shiong Hwang

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Approximately 30% of AML patients harbor activating internal tandem duplication (ITD) of the FLT3 receptor are associated with poor prognosis. This result suggests FLT3 might be a valid target for the development of AML medication. In the development of potential kinases inhibitors, we focused on utilizing azulene as pharmacophor for constructing novel drug-like compounds to overcome the issues of crowded chemical spaces and intellectual property. Among one of the strategies to bring azulene into drug molecules, we linked azulene to another well-recognized pharmacophor (aza)oxindole to generate a series of novel azulene-oxindoles. These compounds showed potential inhibitory activity toward various kinases such as FLT3-ITD, PDGFR and c-Kit. Among the candidate compounds, ITRI-227 inhibited FLT3-ITD dependent MV4-11 cells growth with IC₅₀ of 307 nM. Western blot analysis confirmed a direct effect of ITRI-227 on phosphorylation of FLT3 in RS4-11 cells upon drug treatment for 1 hr. ITRI-227 inhibited autophosphorylation of FLT3 and phosphorylation of downstream effectors, STAT5 and ERK, in a pattern similar to that seen for cellular proliferation. In *in vivo* PK study, ITRI-227 showed medium volume of distribution (Vss 1.9 L/kg), moderate clearance (1.4 L/hr/kg) and half-life about 1.2 hr after IV dosing. Its oral BA is around 10%. In PO dose escalation studies, ITRI-227 does not reach saturation in Cmax and AUC from 10 to 100 mg/kg. From normalized result, we predicted the drug exposure of ITRI-227 could still push upward to 150 mg/kg dosing level. Following the 50 and 150 mg/kg/bid oral dosing regimen, the therapeutic efficacy of ITRI-227 towards human leukemia MV4-11 subcutaneous xenograft model in female BALB/c nude mice appeared at day 22 and 30 respectively, with complete tumor regression. No specific clinical signs or body weight loss were attributed to the test article during the treatment period. In conclusion, the *in vitro/in vivo* PK/PD/toxicology studies have provided the basis for the clinical developmental potential of ITRI-227 in AML. The bridge studies from discovery to development such as CMC, GLP toxicology, safety and pre-formulation are now in progress.

POSTER COMMUNICATIONS – PC.270

DEVELOPMENT OF NOVEL 1-AZAAZULENE CLASS KINASE INHIBITORS THAT INDUCE COMPLETE TUMOR REGRESSION AND PROLONGS SURVIVAL IN AML MICE

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In continuing development of new drugs, we found 1-azaazulene is a potential pharmacophor with suitable drug-like properties and exhibits potency toward many targets like enzymes, receptors and kinases. In one of our projects, we linked 1-azaazulene to another well-recognized pharmacophor benzimidazole to generate a series of novel azaazulene-benzimidazole compounds. These compounds showed potential inhibitory activity toward a variety of kinases such as FLT3-ITD, RET, TrkA, TrkB and FGFR. Further studies on structure-activity relationship (SAR) provided several compounds meet the criteria for further development. Among these candidate compounds, ITRI-260 inhibited FLT3-ITD dependent MV4-11 cell growth with IC₅₀ of 21 nM and cellular phosphorylation with IC₅₀ of 17 nM. In *in vivo* PK study, ITRI-260 showed favorable oral PK profiles. The maximum concentration of ITRI-260 was 700 nM after a single 100 mg/kg oral dosing in mice, with dose proportional exposure from 10 to 150 mg/kg. ITRI-260 also showed therapeutic efficacy in a human AML orthotopic SCID mouse model following a 100 mg/kg/qd oral dosing regimen. The treatment group showed 100% survival of animals at day 90 after tumor cell inoculation, while the vehicle control group showed 100% mortality. In the other study, ITRI-260 also increased survival in primary tumor cell engraftment model inoculated with cancer cells isolated from sunitinib- and sorafenib-resistant AML patients. Through hERG assay with patch clamp technology, we found higher IC₅₀ of ITRI-260 compared with sunitinib and MLN-518, which indicates that ITRI-260 might arise less cardiotoxic concern. In a 14-day sub-chronic toxicology study in mice, ITRI-260 was generally tolerated at 300 mg/kg/qd. The most prominent adverse effect appeared at 600 mg/kg/qd group is starry sky appearance in spleen, which is moderate and reversible. No specific clinical signs or body weight loss were attributed to the test article. In conclusion, the *in vitro/in vivo* PK/PD/toxicology studies have provided the basis for the clinical developmental potential of ITRI-260 in AML. The bridge studies from discovery to development such as CMC, GLP toxicology, safety and pre-formulation are now in progress.

POSTER COMMUNICATIONS – PC.271

DESIGN AND SYNTHESIS OF GUANOSINE DERIVATIVES FOR THE CHARACTERIZATION OF THE PUTATIVE GUANOSINE RECEPTOR

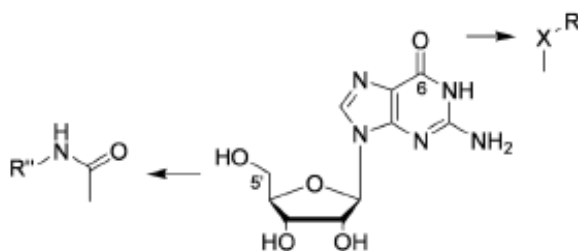
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Guanosine and its nucleotides are released from a variety of cells under physiological and pathological conditions, and in particular astrocytes release fivefold time guanine-based purines than adenine-based counterparts (1). Guanosine has many trophic effects in the CNS, including the stimulation of neurotrophic factor synthesis and release by astrocytes, which protect neurons against excitotoxic death (1).

Rat brain membrane preparations showed specific binding sites for [³H]-guanosine, which are not recognized by other purinergic receptor ligands (2). These findings supported the hypothesis of the existence of a specific G protein-coupled receptor (GPCR) for guanosine.

On this basis, various ligands were designed and synthesized for the further characterization of this elusive binding site. In particular, new guanosine analogues modified at the 6- and 5-positions have been designed and evaluated through an innovative Eu-GTP binding assay. This time-resolved fluorescence technique allows to assess the activation of a receptor through the coupling coupling to a G protein (3).



Biological results confirmed the existence, on rat brain, of a GPCR, which is activated by guanosine and 6-thioguanosine, but not by other reference purinergic receptor ligands. Furthermore, the newly synthesized compounds showed high potencies and resulted, in some cases, more active than guanosine itself.

REFERENCES

1. Ciccarelli, R.; Di Iorio, P.; Giuliani, P.; D'alimonte, I.; Ballerini, P.; Caciagli, F.; Rathbone, M. P. Rat Cultured Astrocytes Release Guanine-Based Purines in Basal Conditions and After Hypoxia/Hypoglycemia. *Glia*, **1999**, *25*, 1, 93-8.
2. Traversa, U.; Bombi, G.; Camaioni, E.; Macchiarulo, A.; Costantino, G.; Palmieri, C.; Caciagli, F.; Pellicciari, R. Rat Brain Guanosine

Binding Site: Biological Studies and Pseudo-Receptor Construction.
Bioorg. Med. Chem., **2003**, *11*, 5417-25.

3. Volpini, R.; Buccioni, M.; Dal Ben, D.; Lambertucci, C.; Lammi, C.; Marucci, G.; Ramadori, A.T.; Klotz, K.-N.; Cristalli, G. Synthesis and Biological Evaluation of 2-Alkynyl-*N*⁶-methyl-5'-*N*-methylcarboxamidoadenosine Derivatives as Potent and Highly Selective Agonists for the Human Adenosine A₃ Receptor. *J. Med. Chem.*, **2009**, *52*, 7897–900.

POSTER COMMUNICATIONS – PC.272

STRUCTURAL INSIGHT INTO MONOACYLGLYCEROL LIPASE, THE ENZYME DEGRADATING 2-ARACHIDONOYLGLYCEROL

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Together with anandamide, 2-arachidonoylglycerol (2-AG) constitutes one of the main representatives of a family of endogenous lipids known as endocannabinoids¹. These act by binding to CB₁ and CB₂ cannabinoid receptors, the molecular targets of the psychoactive compound Δ^9 -THC, both in the periphery and in the central nervous system, where they behave, among others, as retrograde messengers to modulate synaptic transmission. These last years, evidence has accumulated to demonstrate the lead role played by the fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL) in the regulation of anandamide and 2-arachidonoylglycerol (2-AG) levels, respectively. Considering the numerous physiological functions played by endocannabinoid, MAGL is now considered as promising targets for therapeutics. Indeed, it is now well known that inhibitors of this enzyme could reveal useful for the treatment of pain and inflammatory disorders, as well as for cancer therapy.

Therefore, as a first step toward the rational design of such compounds, we report the elucidation of the tridimensional structure of the human MAGL², which was solved by X ray diffraction at the resolution of 2.2 Å.

The overall fold of the enzyme is that of the α/β hydrolase superfamily, with a central core that is similar to that of the other proteins of this family. However, the enzyme distinguishes itself by the particular architecture of its “cap”, a flexible device covering the central core²⁻⁴, and by adopting subtle structural features that adapted the enzyme for the degradation of its natural substrates.

Besides giving us a structural insight into the process that regulates 2-AG signalling, we have now high expectations of developing new MAGL inhibitors on a rational basis. Indeed, there is now an urgent need for the design of valuable inhibitors of 2-AG degradation for both pharmacological and therapeutic purposes, with a special emphasis on improving potency and selectivity versus other serine hydrolases (FAAH and other carboxylesterases). Along this line, the exploitation of a so far unexploited pocket could provide new opportunities for the development of selective drugs that modulate the endocannabinoid signalling.

REFERENCES

1. Di Marzo V. *Nat Rev Drug Discov.* 2008; 7: 438-55.
2. Labar G, Bauvois C, Borel F, Ferrer JL, Wouters J, Lambert DM. *Chembiochem.* 2010; 11: 218-27.
3. Bertrand T, Augé F, Houtmann J, Rak A, Vallée F *et al.* *J Mol Biol.* 2010; 396: 663-73.
4. Schubert C, Grasberger B, Schalk-Hihi C, Maguire D, Lewandowski F *et al.* *WO/2009/132267*

POSTER COMMUNICATIONS – PC.274

HIT-TO-LEAD OPTIMIZATION OF PYRROLO[1,2-A]QUINOXALINES AS NOVEL CANNABINOID TYPE 1 RECEPTOR ANTAGONISTS

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Hit-to-lead optimization of a novel series of N-alkyl-N-[2-oxo-2-(4-aryl-4H-pyrrolo[1,2-a]quinoxaline-5-yl)-ethyl]-carboxylic acid amides are described. High throughput screening (HTS) campaign yielded several hits featuring a pyrrolo[1,2-a]quinoxaline core, as represented by (Figure 1).

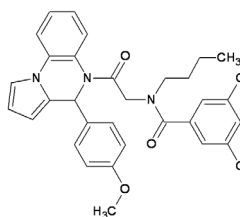


Figure 1

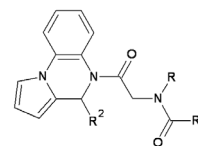
$$K_i = 831 \text{ nM}$$


Figure 2

We aimed at reducing the molecular weight and improving the potency of compound. In order to that, three areas were addressed (Figure 2): (R) the effect of the alkyl side chain, (R1) the effect of acyl groups, and (R2) the effect of groups of the pyrrolo-quinoxaline ring. This optimization process led to iden-

tification of in vitro potent cannabinoid 1 receptor (CB₁R) antagonists representing a new class of compounds in this area.

POSTER COMMUNICATIONS – PC.275

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL SERIES OF MONOACYLGLYCEROL LIPASE INHIBITORS

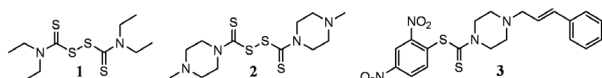
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The monoacylglycerol lipase (MAGL) is an enzyme of 33 kDa belonging to the superfamily of serine hydrolase. It is involved in the hydrolysis of the 2-arachidonoylglycerol (2-AG) in glycerol and arachidonic acid. 2-AG is an endogenous ligand of cannabinoid receptors CB₁ and CB₂ and acts as retrograde lipid messenger regulating the release of other neurotransmitters. 2-AG is involved in many pathophysiological processes, including regulation of appetite, pain control and cell proliferation.¹ Therefore, inhibition of monoacylglycerol lipase appears as a promising approach for the treatment of disease states in which increased levels of 2-AG may be beneficial.

Our group discovered the MAGL inhibitory properties of disulfiram (**1**), a bis(dialkylaminethiocarbonyl)disulfide derivative,² and showed that arylthioamide scaffold represents a useful template for designing new MAGL inhibitors.³ In this connection, we report here results from pharmacomodulations around these structures.

Among disulfiram analogues, bis(4-methyl-1-piperazinylthiocarbonyl)disulfide (**2**) was found to inhibit pure human MAGL with a pI₅₀ of 6.97±0.05, along with a selectivity ratio of 1825 compared to FAAH inhibition, another endocannabinoids hydrolyzing enzyme. MAGL inhibition within this series was highly dependent on the presence of the disulfide group, as similar compounds lacking this functionality were less potent or unable to inhibit MAGL. Additionally, these derivatives constitute irreversible MAGL inhibitors that probably interact with the sulfhydryl moiety of Cys208 and/or Cys242, located in the vicinity of MAGL active site.



Regarding dithiocarbamate derivatives, 2,4-dinitrophenyl-4-cinnamylpiperazine-1-carbodithioate (**3**) inhibits human MAGL with a pI₅₀ of 6.03 ± 0.09, along with a good selectivity compared to FAAH inhibition (40% maximal inhibition at 10⁻³M). MAGL inhi-

bition within this series was also found to be dependent on the presence of the lipophilic group, as similar compounds lacking, for instance, the cinnamylpiperazine moiety were found less potent inhibitors.

These compounds therefore constitute useful pharmacological tools to explore the endocannabinoid signalling.

REFERENCES

1. Lambert D.M et al. J.Med Chem 2005, 48, 5059-87.
2. Labar et al.ChemBioChem 2007, 8, 1293 – 1297.
3. Kapanda et al. Med. Chem. Res.2009, 18: 243-254.

POSTER COMMUNICATIONS – PC.276

INDEXING CAVITIES IN PROTEIN STRUCTURES

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The identification and characterisation of cavities in protein structures is an aspect of key relevance in molecular biology and drug discovery. Usually, multiple cavities are identified in each protein structure and there is currently no means for indexing those cavities in a unique meaningful manner. To this aim, we introduce an algorithm that allows for generating a unique identifier for each protein cavity based on the pharmacophoric features exposed.

The process leading to the generation of a unique protein environment identifier (PEI) for each existing cavity in a protein structure starts by detecting all cavities present in the protein structure. Once detected, the surface exposed is fragmented on the basis of six pharmacophoric features (hydrophobic, aromatic, donor, acceptor, negatively charged and positively charged) according to the nature of the contributing atoms. Each pharmacophoric patch is represented by a centroid. The ensemble of pharmacophoric centroids of a cavity constitute a cavity graph. This cavity graph is ultimately taken to generate a unique haschcode (PEI) using a modified and further extended adaptation of the Morgan algorithm.

The indexing approach introduced here will be used i) to analyse the dynamics of cavities found in multiple structures for a given protein and ii) to locate at which point of a molecular dynamics run cavities with an optimal druggability profile are created.

POSTER COMMUNICATIONS – PC.277

SOME NEW 3H-PYRROLO[3,2-f]QUINOLIN-9-ONE DERIVATIVES SHOW POTENT AND SELECTIVE ANTILEUKEMIC ACTIVITY

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In the context of pharmacomodulating the properties of 7-phenyl-3H-pyrrolo[3,2-f]quinolin-9-ones we focused on making molecules more hydrophilic in comparison with previous analogues^[1] by binding suitable functional groups onto 1, 3 and 7 positions (figure 1). Such compounds should be of value for *in vivo* studies on pharmacokinetics, metabolism and toxicity. The novel substituted angular 3H-pyrrolo[3,2-f]quinolinones were evaluated for their antiproliferative activity. The most active derivatives showed high selectivity against human leukemia cell lines and potently inhibited their growth, with nM IC₅₀ values. The active compounds strongly inhibited tubulin assembly and the colchicine binding to tubulin. Their activities were equal to or greater than that of the reference compound CA-4.

Flow cytometry study showed that Jurkat cells treated with the most active compounds **11** and **12** (nanomolar IC₅₀s), having an ethyl propanoate and a trimethoxybenzyl group in the 3 position respectively, were arrested in the G₂/M phase of cell cycle in a concentration-dependent manner. This effect was associated with apoptosis of cells, mitochondrial depolarization, generation of reactive oxygen species, activation of caspase-3 and cleavage of the enzyme polyADP-ribose cleavage polymerase. Very interesting structure-activity relationships regarding the position and the nature of substitutions for obtaining potent and selective anti-leukaemias drugs will be discussed.

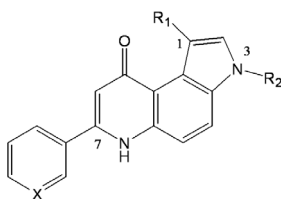


Figure 1. 3H-pyrrolo[3,2-f]quinolin-9(6H)-ones

REFERENCES

1. V. Gasparotto, I. Castagliuolo, M. G. Ferlin *J. Med. Chem.* 2007, 50, 5509-5513.

POSTER COMMUNICATIONS – PC.279

SYNTHESIS OF VLA-4 INTEGRIN LIGANDS AND THEIR GRAFTING TO BLOOD FILTER SURFACES

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The reduction of the number of leukocytes in blood products is essential to avoid harmful reactions "donor-receiver" occurring after blood transfusions. Different methods for leukodepletion exist but the filtration is nowadays the most efficient and practical.

The retention of leukocytes by a specific adhesion effect is considered. The integrins expressed on leukocytes could react with synthetic ligands, which mimic the natural ligands of the integrins and are covalently fixed on the surface of the filters. These strong "ligand-receptor" interactions combined with the physico-chemical effects of the filters should improve their performance.

Different peptidomimetics, related to the active sequence "LDV" (Leu-Asp-Val), recognized by the integrin $\alpha_4\beta_1$, have been studied. The synthesis of a first peptidomimetic ligand will be presented¹ (Figure 1).

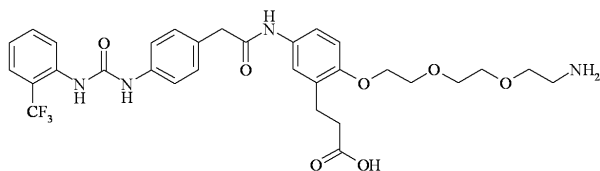


Figure 1

The ligands are grafted on the surface of polypropylene membranes, which are currently used as part of filtration devices of blood products. These oxygen-plasma treated membranes are activated by "wet chemistry" treatments using cyanuric fluoride² as reagent.

Modified materials are characterized by physico-chemical and morphological analyses and then evaluated towards their ability of selectively retaining leukocytes from blood products during filtration experiments.

REFERENCES

1. Momtaz, M.; Rerat, V.; Gharbi, S.; Gérard, E.; Pourcelle, V.; Marchand-Brynaert, J., *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1084-1090.
2. Momtaz, M.; Dewez, J.-L.; Marchand-Brynaert, J., *J. Membr. Sci.*, **2005**, *250*, 29-37.

POSTER COMMUNICATIONS – PC.280

A NEW FRAGMENT BASED LIGAND DESIGN TOOL TO BOOST COMMUNICATION BETWEEN MEDCHEMS AND COMPCHEMS

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From our experience some facts emerge concerning the relationship between MedChems and CompChems today:

- Effective and fast communication between these disciplines is of crucial importance.
- Management is well advised to support both groups by exploiting their own, respective strengths instead of letting MedChems do partial CompChem tasks and vice versa.
- When it comes to computer interaction, MedChems think 2D rather than 3D; CompChems think more 3D.
- Learning curves for computational tools to be used by MedChems must be avoided entirely.

We present a novel piece of software, LeadIT, which has been co-developed with Hoffmann-LaRoche; its user interaction model has been designed from scratch to support MC/CC team building following the principles above. One of the core computation pieces is an interactive *fragment based ligand design* engine which needs 3 mouse clicks to be triggered by MedChems, but has a totally open and configurable chemistry model behind so that the computational specialists can adapt it to corporate needs if requested. Because result delivery is within seconds, the tool has proven to be highly usable in a slideshow-like fashion in teams of Medicinal and Computational chemists at various big pharma world-wide. Results can be refined iteratively and within teams so that time consuming communication loops are avoided.



Basic FBLD and SBDD tasks to be accomplished using LeadIT are:

- a) Growing from seed fragment binders into other parts of protein binding pockets,
- b) Fragment linking of two or more fragment binders, or
- c) Merging multiple overlapping binders into a single potent lead,
- d) Protein-ligand docking using the built-in industry standard FlexX.

For a)-c), based on a vast 3D fragment library, the tool finds fragments which provide an optimal fit with the 'dangling bonds' and which comply with optional filters and pharmacophore features. Synthetic access of results – a major weakness in the early days of de novo design – is taken care of in LeadIT at three levels: during fragment creation, within the query definition, and when creating the results.

Our basic design and computation dogmas will be discussed; examples which map onto experimental data and evolve into novel lead ideas will be presented.

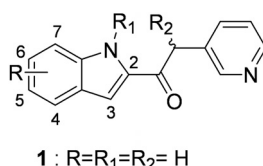
POSTER COMMUNICATIONS – PC.281

DISCOVERY AND OPTIMIZATION OF INDOLE-PYRIDINYL-ETHANONES AS NOVEL INHIBITORS OF INDOLEAMINE-2,3-DIOXYGENASE (IDO), A PROMISING TARGET FOR ANTI-CANCER IMMUNOTHERAPY

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Immunotherapy is a promising novel and validated strategy for cancer therapy. It consists of the therapeutic vaccination of patients to stimulate their (natural) immune system against cancer cells. However, this approach showed a limited efficacy *in vivo* because cancer cells develop enzymatic mechanisms allowing tumours to resist or escape immune rejection. Among the enzymes involved, indoleamine 2,3-dioxygenase (IDO) was identified as a potential actor. IDO catalyses the rapid degradation of tryptophan (Trp) into *N*-formylkynurenine. This results in a local Trp depletion that severely affects T-cells proliferation and is thereby deeply immunosuppressive. Recently, the team of Prof. Van den Eynde demonstrated that many human tumours express IDO in a constitutive manner and that this expression allows cancer cells to escape immune rejection.¹ IDO was thus clearly identified as an attractive target for the development of inhibitors.



The recent elucidation of the three-dimensional structures of IDO,² in complex with phenylimidazole and the cyanide ion (CN⁻), provide important results for the structure-based drug discovery and design of novel IDO inhibitors. In the present work, we applied virtual screening for the discovery of new IDO inhibitors. As a result, five novel scaffolds with inhibitory potencies in the micromolar range were identified. Among these, the most promising candidate (**1**: IC₅₀ = 65 μM) was selected and its inhibitory potency improved by chemical modifications. This led to a 7-fold improvement of the inhibitory potency of the hit selected.

In this communication, the identification of **1**, the synthesis and biological evaluation of analogues as well as a modeling study explaining the SAR's will be presented.³

REFERENCES

1. Uyttenhove, C.; Pilotte, L.; Theate, I.; Stroobant, V.; Colau, D.; Parmentier, N.; Boon, T.; Van den Eynde, B. J. *Nat Med* **2003**, 9, 1269-74.
2. Sugimoto H., Oda S., Otsuki T., Hino T., Yoshida T., Shiro Y. *PNAS* **2006**, 103(8), 2611-6
3. This work is supported in part by the FNRS and Biowin (CANTOL : Convention n° 5678)

POSTER COMMUNICATIONS – **PC.282**

RESTRICTED DOCKING OF THE DHFR INHIBITORS TO THE DHFR RECEPTOR

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"Tuberculostatic drug – DHFR" complexes were modeled with algorithm Infant. The dataset of the drugs includes dihydropyrimidine derivatives, their annealed analogs and the analogs with a podand chain. For the docking procedure, we used the X-ray crystallography data of dihydrofolate reductase with well-known drugs (methotrexate, trimethoprim, 4-bromo-WR99210 [13]) and the data of the molecular orientation obtained with the CiS algorithm. The drug is orientated to the cavity bottom by oxalic radical and methyl group, pyrimidine ring is turned to the protein outlet. Aminoacid residues responsible for binding with drugs were determined using algorithm CoCon [1]. These are: Gln28, Ser49, Thr46, Trp6, Leu50, Arg23, Ile20 and three water molecules (see Fig.).

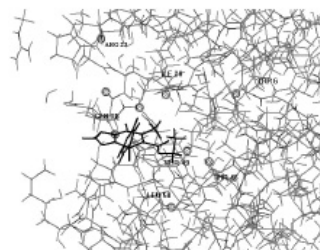


Fig. "Drug – DHFR" complex (atoms of aminoacid residues responsible for binding with the drug are marked)

Low-active molecules have the following shorter contacts than high-active molecules: 1) of Leu50 with the oxyacyclic oxygen, 2) Ile20 with the hydrogen of the aromatic substituent and dihydropyrimidine cycle, 3) Ser49 with the hydrogen of methyl. The following regression model was obtained:

$$\text{pMIC} = 4.47 - 5.6x_1 - 0.015x_2$$

is energy of "bond – angle" stretch-bending interactions (MM3 force field), x_2 is electrostatic energy computed by MERA force field [1]. The cross validation quality is 0.9779 and Fisher's criterion is 43. So, the steric stresses of the complexes lead to decrease of the activity. Moreover, tuberculostatic activity depends on the total energy of the complex. It has been shown that more active drug molecules form more stable complexes.

ACKNOWLEDGMENTS

SKIF–GRID Supercomputer Initiative.

REFERENCES

1. Potemkin V.A., Pogrebnoy A.A., Grishina M.A. Technique for Energy Decomposition in the Study of " Receptor-Ligand" Complexes// J. Chem. Inf. Model., 49: 1389-1406

POSTER COMMUNICATIONS – **PC.284**

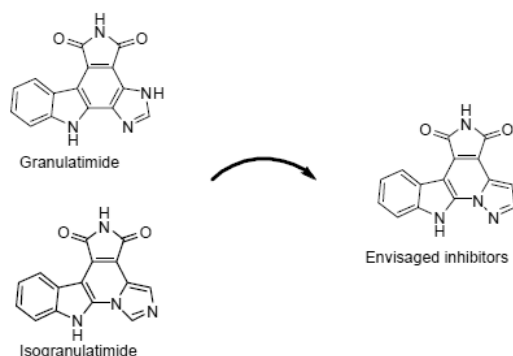
SYNTHESIS AND ANTICANCER EVALUATION OF PYRAZOLIC ANALOGUES OF THE MARINE CHK1 INHIBITORS, GRANULATIMIDE AND ISOGRANULATIMIDE

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Therapeutic intervention targeting the cell cycle has emerged as an important strategy for the development of new anticancer drugs. Chk1 is a major kinase that regulates the G2 checkpoint. As almost 50 to 70 % of tumor cells are defective at G1 due to mutations in tumor suppressor genes, they rely heavily on the G2

checkpoint and thus Chk1 to correct otherwise fatal genetic errors¹. As such, Chk1 inhibitors should potentialise the effect of a genotoxic agent². Granulatimide and isogranulatimide are alkaloids issued from marine source which have been shown to be effective inhibitors of Chk1³. Pyrazolic analogues of these natural compounds have been investigated in order to define new effective and specific inhibitors of Chk1. Both synthetic and biological results will be discussed.



REFERENCES

1. Kaufmann, W. K.; Paules, R. S. *FASEB J.* **1996**, *10*, 238.
2. Weinert, T.; Lydall, D. *Semin. Cancer Biol.* **1993**, *4*, 129.
3. Jiang, X.; Piers, E.; Andersen, R. J.; Roberge, M. *Mol. Cancer Ther.* **2004**, *3*, 1221.

POSTER COMMUNICATIONS – PC.285

CONFORMATIONAL CHANGES INDUCED BY FRAGMENT GROWING: A STRUCTURAL AND THERMODYNAMIC ANALYSIS

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Neuronal nicotinic acetylcholine receptors (nAChRs) are potential therapeutic targets for the treatment of pain, epilepsy and a wide variety of neurodegenerative and psychiatric disorders e.g. Alzheimer's disease, Parkinson's disease, schizophrenia and depression. Several important physiological processes appear to be regulated by a single or a few nAChR subtypes.¹ Subtype selective ligands are needed to aid target validation for the development of safe and selective drugs. However, due to high homology between the binding sites of the different subtypes, the design of selective ligands poses an interesting challenge.

Recently, high-quality X-ray structures of several acetylcholine-binding proteins (AChBPs) co-crystallized with ligands, have become available.^{2,3} These proteins are water soluble homologs of the ligand-binding domain of nAChRs.⁴ Fragment library screening using a variety of orthogonal biochemical and biophysical approaches has resulted in the identification of novel hit fragments. Several of these fragments have been co-crystallized with AChBP. We here describe the growing of one of these fragments into a sub-pocket of the binding site that becomes accessible upon a ligand-induced change in rotameric state of a gate-keeper tyrosine residue. These studies were guided by pharmacological, structural and thermodynamic data and illustrate the detailed insights and molecular understanding that can be obtained by fragment-based design approaches.

REFERENCES

1. Jensen et al. *J. Med. Chem.* **2005**, *48*, 4705-4745
2. Celie et al., *Neuron* **2004**, *41*, 907-14
3. Hansen et al. *EMBO journal* **2005**, *24*, 3635-46
4. Sixma et al. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 311-34

POSTER COMMUNICATIONS – PC.286

CHEMOGENOMIC LIBRARY DESIGN PRODUCTION AND USE

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To date most screening within the pharmaceutical industry has relied on chemical diversity for example High Through-put screening.¹ There has been limited effort to generate screening libraries using the vast amount of published pharmacological data, to pick the mode of action and then ensure the compound is selective by comparison with all known screening data.² The ideal situation is a screening set with potent, selective compounds for every mechanism. Such a collection in combination with a functional disease model would allow phenotypic screening for the identification of new mechanisms of interest.³ This poster will describe our efforts to automatically trawl internal and external databases to identify compounds with these properties. Compounds were selected that had sub 500nM potency, solid availability for multiple screening cycles and potency data for five or more secondary mechanisms. Multi-parameter optimisation was used to select up to five compounds for each target with the best possible combination of potency and selectivity. The resulting Chemogenomics set contains 3155 compounds with coverage of 722 mechanisms split into 11 Gene Family groups as shown in Figure 1, coverage is uneven and is a reflection of areas of traditional interest at Pfizer.

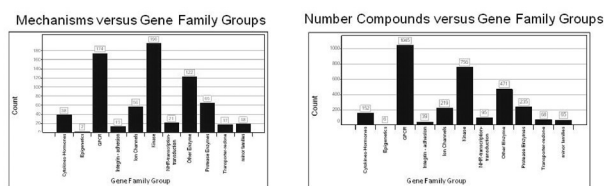


Figure 1

The libraries are available for researchers to use as initial tools in a phenotypic screen where the introduction of selectivity data increases the chance that a particular mechanism has been accurately identified, especially if all five chemogenomic tools for a given target are active. Use of this library within Pfizer has already led to the discovery of a new approach for the conversion of Pancreatic Progenitor Cells to Beta-cells, potentially useful for the treatment of diabetes.

REFERENCES

1. K Bleicher. (2003) Hit and Lead generation: Beyond high-throughput screening. *Nature Rev. Drug Discov.* 2: 369-378.
2. Lee Harland Anna Gaulton. (2009) Drug Target Central. *Expert Opin. Drug Discov.* 4(8):857-872.
3. Charles P.Hart. (2005) Finding the target after screening the phenotype *Drug Discovery Today.* 10: 513-519.

POSTER COMMUNICATIONS – PC.287

NEW SUGAR-DERIVED COMPOUNDS TARGETING SELECTIVELY CD14 AND MD-2 RECEPTORS AS ANTI SEPSIS AND ANTI NEUROPATHIC PAIN AGENTS

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In this poster we present the discovery and the biological characterization of new chemical entities derived from natural sugars targeting selectively the extracellular Toll-like Receptor 4 (TLR4) complex. The main focus of an active collaboration between the Universities of Milano (Italy) and Iowa (USA) is to gain information on the molecular basis of the TLR4 pathway using small molecules capable to interact selectively with the extracellular receptors LBP, CD14, MD-2 and TLR4^[1]. Positively charged monosaccharides with lipid chains have been developed by our group, that inhibit lipopolysaccharide (LPS) and lipid A-induced cytokine production in innate immunity cells^[2-5]. These molecules are also active *in vivo* in contrasting septic shock and other syndromes,

such as neuropathic pain, caused by TLR4 activation^[6]. Biochemical studies indicated that these monosaccharides inhibit the TLR4 pathway by selectively antagonizing the endotoxin binding to the CD14 receptor. The discovery of new lipodisaccharides containing negatively charged sulfate groups, active as mild agonists of TLR4, is also described. These molecules selectively target the MD-2 receptor and are promising leads for the development of non-toxic vaccine adjuvant. All molecules presented, besides being hit or lead compounds for the development of anti-sepsis and anti-inflammatory agents, and immunotherapeutics, are also unique tools for investigating the structural and functional biology of the TLR4 pathway with the chemical genetic approach.

REFERENCES

1. 5-years NIH grant, Award No 2R01 AI059372-06A1 "Regulation of MD-2 Function and Expression".
2. F. Peri, F. Granucci, B. Costa, I. Zanoni, C. Marinzi, F. Nicotra Amino and Hydroxylamino Monosaccharides Inhibit Lipid A Stimulated Activation of Human Dendritic Cells and Macrophages, *Angew. Chem. Int. Ed.* 2007, **46**, 3308-3312.
3. M. Piazza, C. Rossini, S. Della Fiorentina, C. Pozzi, F. Comelli, Bettoni, P. Fusi, B. Costa, F. Peri Glycolipids and benzylammonium lipids as novel anti-sepsis agents: synthesis and biological characterization, *J. Med. Chem.* 2009, **52** (4), 1209-1213.
4. M. Piazza, L. Yu, A. Teghanemt, T. Giannini, J. Weiss, F. Peri, "Evidence of a specific interaction between new synthetic antiseptic agents and CD14", *Biochemistry* 2009, **48**, 12337-12344.
5. F. Peri, F. Nicotra, F. Granucci, B. Costa PCT/EP2007/002279 published the 15/03/2007, LIPID A ANTAGONISTS WITH ANTI-SEPTIC SHOCK, ANTIINFLAMMATORY, ANTI-ISCHEMIA AND ANALGESIC ACTIVITY.
6. I. Bettoni, F. Comelli, C. Rossini, F. Granucci, G. Giagnoni, F. Peri, B. Costa Glial TLR4 Receptor as New Target to Treat Neuropathic Pain: Efficacy of a New Receptor Antagonist in A Model Peripheral Nerve Injury in Mice, *Glia* 2008, **56**, 1312-1319.

POSTER COMMUNICATIONS – PC.288

COMPUTATIONAL STUDIES AND SYNTHETIC APPROACHES FOR THE IDENTIFICATION OF NEW PROTEIN-PROTEIN INTERACTION INHIBITORS (PPI)

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Human Immunodeficiency Virus (HIV) infection is one of the leading causes of death in the world. An essential step in the virus-retroviral life cycle is the integration of the proviral DNA into the cellular genome of the host cell to generate a provirus that remains present as long as the cell survives.

Highly Active Antiretroviral Therapy (HAART) is the standard therapy of infection based on potent cocktails of drugs targeting viral proteins.

Considering that this treatment is associated with severe side effects and emergence of drug-resistant strains, the discovery and development of new anti-HIV agents with novel mechanisms, high potency, less toxicity and broad-spectrum activity against wild-type and mutant HIV-1 strains remain continuously warranted.

In this context the search of small molecules as protein-protein interaction inhibitors (SMPPIIs) represents a new attractive strategy to identify new anti-HIV-1 agents blocking HIV replication, through a different mechanism of action than all other drugs so far used in therapy.

Several cofactors were recently identified as important proteins involved in the HIV-1 life cycle and among them lens epithelium-derived growth factor LEDGF/p75 plays a key role during the integration process.

Taking into account these findings and employing different computational approaches we performed molecular modeling studies with the aim to explore the structural requirements necessary to disrupt the protein-protein interaction.^{1,2}

Our studies resulted in the discovery of small molecules able to prevent PPIs between IN and its cofactor LEDGF/p75 at micro-molar concentration.

The most innovative eco-friendly methods and technologies have been used for the synthesis of the new compounds. In particular the application of microwave-assisted organic synthesis was adopted in the steps requiring heating in order to shorten reaction times and increase the yields.

Research supported by THINC project (HEALTH-F3-2008-201032)

REFERENCES

1. L. De Luca, M.L. Barreca, S. Ferro, F. Christ, N. Iraci, R. Gitto, A.M. Monforte, Z. Debyser, A. Chimirri. *ChemMedChem* **2009**, *4*, 1311-1316
2. Submitted for publication

POSTER COMMUNICATIONS – PC.289

NOVEL PYRAZOLE DERIVATIVES AS INHIBITORS OF CELLULAR LTB₄ BIOSYNTHESIS

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Leukotrienes (LTs) are pro-inflammatory lipid mediators derived from arachidonic acid which play pivotal roles in various biological

and pathological processes [1]. Recent studies suggest a crucial role of 5-LO products in cell proliferation and survival particularly of prostate and pancreatic cancer cells. Additionally, the cardiovascular actions of LTs have also been well recognized for many years, especially in atherosclerosis and in myocardial infarction in which each disease is characterized by an inflammatory component at some stage of the associated vascular pathology. Therefore, the inhibition of LT formation became an attracting pharmacological strategy to intervene with these diseases [2]. In present study, we show that certain derivatives of 1,5-darylpyrazole-3-propanoic acid inhibit formation of LTB₄ biosynthesis in intact human neutrophils when stimulated with ionophore A23187. Our efforts for structural optimization resulted compounds with IC₅₀ values between 1.2 and 4.8 μM. Interestingly, the compounds showed no considerable inhibition of 5-LO product formation in a cell-free system suggesting that the compounds may interfere with a regulatory mechanism of 5-LO in intact cells resulting in inhibition of LT formation rather than directly acting on the 5-LO enzyme. We are currently studying the identification of the exact mode of action underlying inhibition of the cellular LT formation. Based on their high selectivity and efficacy, these novel structures might represent promising candidates for further development as suitable inhibitors of 5-LO product synthesis (This work is partly supported by a TUBÝTAK Research Grant 108S210).

REFERENCES

1. Werz, O. 5-lipoxygenase: cellular biology and molecular pharmacology. *Curr. Drug Targets Inflamm. Allergy*, *1*(1), 23-44 (2002).
2. Werz, O., Steinhilber, D. Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacol. Ther.* *112*(3), 701-18 (2006).

POSTER COMMUNICATIONS – PC.290

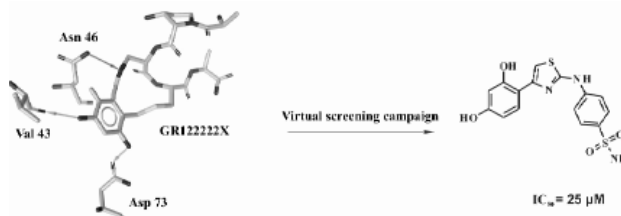
DISCOVERY OF 2-AMINO-4-(2,4-DIHYDROXYPHENYL)THIAZOLES AS NOVEL INHIBITORS OF DNA GYRASE B BY STRUCTURE-BASED VIRTUAL SCREENING APPROACH

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The emergence of bacterial resistance to most of the clinically used antibiotics is driving an urgent need for the development of novel and effective antibacterial agents.¹ The main challenge remains the discovery of highly potent antibacterials with broad spectrum of efficacy and improved safety profile.^{2,3} One of the well established targets of the antibacterial agents is the DNA gyrase, a unique bacterial type II topoisomerase originating from

the gyrase, HSP90, histidine kinase, MutL (GHKL) enzyme super-family that catalyzes the introduction of negative supercoils into the DNA using the concurrent ATP hydrolysis.



Cyclothalidines⁴ are a class of bacterial DNA gyrase B (GyrB) sub-unit inhibitors, targeting its ATP-binding site. Starting from the available structural information on cyclothalidine GR122222X,⁵ an *in silico* virtual screening campaign was designed combining molecular docking calculations with three-dimensional structure-based pharmacophore information. A novel class of 2-amino-4-(2,4-dihydroxyphenyl)thiazole based inhibitors with low micromolar antigyrase activity was discovered.⁶

REFERENCES

1. Brown, E.D.; Wright, G.D. *Chem. Rev.* 2005, 105, 759.
2. Silver, L.L. *Biochem. Pharm.* 2006, 71, 996.
3. Perdihi, A.; Kovač, A.; Wolber, G.; Blanot, D.; Gobec, S.; Solmajer, T. *Bioorg. Med. Chem. Lett.* 2009, 19, 2668.
4. Anghern, P.; Buchmann, S.; Funk, C.; Goetschi, E.; Gmuender, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbbers, T.; Masciadri, R.; Nielsen, J.; Reindl, P.; Ricklin, F.; Schmitt-Hoffmann, A.; Theil, F-P. *J. Med. Chem.* 2004, 47, 1487.
5. Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* 1996, 15, 1412.
6. Brvar, M.; Perdihi, A.; Oblak, M.; Peterlin Mašič, L.; Solmajer, T. *Bioorg. Med. Chem. Lett.* 2010, 20, 958.

POSTER COMMUNICATIONS – PC.291

OPTIMIZATION OF THE FIRST LEAD COMPOUND TARGETING THE *TRYPANOSOMA BRUCEI* METACASPASE 2

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Metacaspases (MCAs) are important cysteine proteases in *Trypanosoma brucei* and are considered as a new attractive drug target for antiparasitic chemotherapeutics. A first series of inhibitors has been prepared and were found to possess modest antiparasitic activity and excellent selectivity when compared to mammalian caspases.¹ Further optimization efforts include P₁', P₁ and P₂ modifications of the lead compound UAMC-00599 in order to obtain more potent and selective inhibitors (Fig. 1). Different warheads at the P₁' position will be investigated. Then, various functional groups will be introduced in the P₁ side chain to lower basicity and thus to enhance membrane permeability. Furthermore, hydrophobic uncharged moieties at the P₂ position will be extensively explored to enhance the selectivity of these compounds.

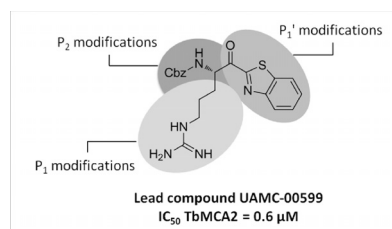


Figure 1: P₁', P₁ and P₂ modifications of the lead compound UAMC-00599 targeting TbMCA2.

REFERENCES

1. Berg, M., Van der Veken, P., Joossens J., et al. *Bioorg. Med. Chem. Lett.* **2010**, 20, 2001-2006.

POSTER COMMUNICATIONS – PC.292

FIRST SULFONIC ACID ANALOGUE OF IDRAPARINUX, THE ANTICOAGULANT PENTASACCHARIDE

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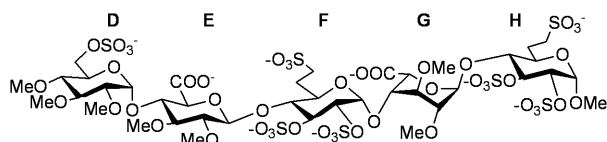
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Heparin, a highly sulfated linear polysaccharide of animal origin is widely used as an anticoagulant drug. Heparin binds to the protease inhibitor antithrombin III (AT-III) through a unique pentasaccharide sequence; upon binding, a conformational change occurs in AT-III, allowing inhibition of coagulation factors Xa and thrombin. A synthetic pentasaccharide analogue of the antithrombin-binding domain of heparin [1] possessing higher anti-Xa activity and longer half-life than heparin was developed by Sanofi-Synthelabo and Organon, and it was marketed as a new antithrombotic drug under the name Arixtra. The lead compound of the second-generation, "non-glycosamino" glycan Xa-inhibitors is Idraparinux, a fully O-sulfated, O-methylated pen-

tasaccharide, which is not only much easier to synthesize, but also displays higher activity and a longer duration of action than analogues synthesized before. [2]

The aim of our Research Group is the preparation of sulfonic acid analogues of Idraparinux in which the sulfate esters are replaced systematically with isosteric sulfonic acid moieties. These negatively charged sulfonic acid analogues can be resistant towards esterases, accordingly the desired biological effect in the organism can be sustained for a longer period of time. Last year we reported the synthesis of three sulfonic acid analogues of the GH fragments, and three analogues of the EF fragments. [3]

Here, we present the synthesis of the first sulfonic acid analogue of the anticoagulant Idraparinux containing methylsulfonic acid moieties at the F and H units. Preliminary results of anti-Xa assay of the pentasaccharide also will be discussed.



Structure of the 6,6''-disulfonic acid analogue of Idraparinux

REFERENCES

1. M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinäy, J. C. Jacquinet, G. Torri *Carbohydr. Res.* **1986**, 147, 221-236.
2. M. Petitou, C. A. A. van Boeckel *Angewandte Chemie Int. Ed.* **2004**, 43, 3118-3133.
3. M. Herczeg, L. Lázár, A. Borbás, A. Lipták, S. Antus: *Org. Lett.* **2009**, 11, 2619-2622

POSTER COMMUNICATIONS – PC.293

EVALUATION OF NUCLEOSIDE HYDROLASE INHIBITORS FOR THE TREATMENT OF AFRICAN TRYPANOSOMIASIS

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In the search for potential chemotherapeutic agents against trypanosomiasis, a library of 52 *N*-arylmethyl-substituted iminoribitol derivatives was designed and synthesized as potent and selective inhibitors of *Trypanosoma vivax* inosine-adenosine-guanosine nucleoside hydrolase (TviAG-NH). However, when tested against bloodstream form *Trypanosoma brucei brucei*, only one inhibitor, *N*-(9-deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-D-ribitol (UAMC-00363 Fig. 1), displayed a significant activity ($IC_{50} = 0.49 \pm 0.31 \mu M$). A validation in an *in vivo* model of African trypanosomiasis showed promising results for this compound.¹

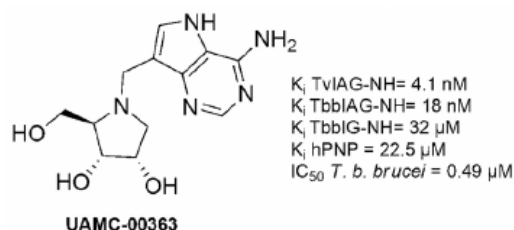


Fig. 1. Structure of UAMC-00363. Inhibition constants (K_i) are provided against IAG-NH from *T. vivax* (TviAG-NH) and *T. b. brucei* (TbbiAG-NH), IG-NH from *T. b. brucei* (TbbiG-NH) and human purine nucleoside phosphorylase (hPNP). IC_{50} value (μM) is provided for *T. b. brucei*.

Several experiments were performed to investigate why only UAMC-00363 showed antiparasitic activity.¹ First, the compound library was screened against *T. b. brucei* IAG-NH and IG-NH to confirm the inhibitory effect of the compounds previously demonstrated on *T. vivax* IAG-NH. Second, to verify the uptake of these compounds by *T. b. brucei*, their affinity for the nucleoside P1 and nucleoside/nucleobase P2 transporter of *T. b. brucei* was tested. Only UAMC-00363 displayed a significant affinity towards the P2 transporter. It was also shown that UAMC-00363 is concentrated into the cell via at least one additional transporter, since it displayed no resistance against P2 knockout mutants of *T. b. brucei*. Consequently, no cross-resistance with the diamidine or the melaminophenyl arsenical classes of trypanocides is expected. Third, three enzymes of the purine salvage pathway of procyclic *T. b. brucei* (IAG-NH, IG-NH and MTAP) were investigated using RNAi knockdown. All these studies showed that it is probably not sufficient to target only the nucleoside hydrolase activity to block the purine salvage pathway of *T. b. brucei* and therefore it is possible that UAMC-00363 acts on an additional target.

REFERENCES

1. Berg, M., Kohl, L., Van der Veken, P. et al. *Antimicrob. Agents Chemother.*, **2010**, doi: 10.1128/AAC.01787-09

POSTER COMMUNICATIONS – PC.294

INVESTIGATIONS ON NEW DIARYLPYRRAZOLE DERIVATIVES FOR THE SYNTHESIS OF POTENT ANTIPLATELET AND ANTI-INFLAMMATORY COMPOUNDS WITH COX-2/TXA₂ INHIBITION

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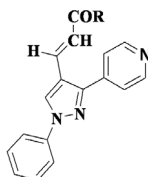
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It is well known that inhibition of the enzyme cyclooxygenase (COX) is the principal mechanism for the efficacy of NSAIDs. Although COX-2 inhibitors are better tolerated than classical NSAIDs with improved gastric safety profile, their prolonged use causes cardiovascular side effects which limit their clinical use [1].

Thromboxane A₂ (TXA₂) is a potent platelet activator and constrictor of vascular and bronchial smooth muscles, while prostacyclin (PGI₂), potent endogenous inhibitor of platelet function, possesses a strong vasodilator effect. Furthermore, PGI₂ is currently presented as the physiologic antagonist of TXA₂. So, balance between TXA₂ and PGI₂ is crucial for the cardiovascular system [2].

The recent doubts about cardiovascular toxicity of COX-2 inhibitors prompted us to synthesize dual inhibitor of COX-2 and thromboxane synthase enzymes. We have aimed to have safer COX-2 inhibitor by suppressing formation of TXA₂ by COX-1 at platelets to protect balance between TXA₂ and PGI₂.

In our previous study, we synthesized diarylpyrrazole compounds possessing 3-methyl-2(3H)-benzoxazolinone moieties and evaluated their antiinflammatory and antiplatelet activities. To overcome solubility problems and achieve better biological results, we have designated and synthesized novel compounds which bear a central pyrrazole ring whose 1,3,4-positions are substituted with phenyl, pyridyl and substituted cinnamic acid residues, respectively. The preparation of 1,5-diarylpyrrazole derivatives to screen their COX-2 and thromboxan synthase inhibitory activities is currently under investigation in our laboratory.



REFERENCES

1. Menozzi G, Merello L, Fossa P, Mosti L, Piana A, Mattioli F, Il Farmaco, 58, 795-808, 2003.
2. De Leval X, Hanson J, David JL, Masereel B, Pirotte B, Dogne JM., Curr. Med. Chem. 11, 1243-52, 2004.

POSTER COMMUNICATIONS – PC.295

MANNANOSE-BASED DC-SIGN INHIBITORS; THE DISCOVERY OF ALTERNATIVE HYDROPHOBIC BINDING POCKETS

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DC-SIGN is a C-type lectin on dendritic cells that binds invading pathogens and mediates an adaptive immune response from T cells.¹ Some pathogens (as HIV-1) bind to DC-SIGN and use it as entering point to DCs. Accordingly, inhibition of pathogen interaction with DC-SIGN specific inhibitors is considered as a plausible concept for new anti-infectives. DC-SIGN specifically binds branched mannose and fucose-containing ligands on the pathogen surface with high affinity and their oligosaccharides with modest affinity.² We have recently reported a pseudo-1,2-mannobioside with moderate antiviral activity in the Ebola infection model (IC₅₀ = 0.62 mM), while its azide derivative, inhibited DC-SIGN adhesion with IC₅₀ = 1.1 mM (measured by surface-plasmon resonance).³ To improve binding affinities of monovalent glycoconjugates, we have designed glycoconjugates based on pseudo-1,2-mannobioside that could bind into hydrophobic binding pockets on DC-SIGN CRD unoccupied by native ligands. These binding pockets were identified by careful examination of crystal structure of DC-SIGN CRD in complex with tetramannoside Man₄ (PDB code: 1SL4, Figure 1). We have synthesized promising candidates and determined their affinities to DC-SIGN by an in vitro assay that measures inhibition of DC-SIGN-mediated immature dendritic cell adhesion. Additionally, we have performed docking studies to rationalize the results and to suggest further optimization. The assay data demonstrate that our efforts to design and synthesize mannose-based DC-SIGN inhibitors resulted in compounds, which inhibit DC-SIGN-mediated adhesion in low micromolar range.

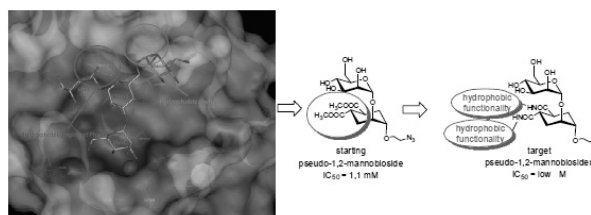


Figure 1.

REFERENCES

1. U Švajger, M Anderluh, M Jeras, N Obermajer. Cell. Signal., 2010, accepted for publication.

2. Y Guo, H Feinberg, E Conroy et al. *Nat. Struct. Mol. Biol.*, **2004**, 11(7), 591-8.
3. JJ Reina, S Sattin, D Invernizzi et al. *ChemMedChem*, **2007**, 2 (7), 1030-1036.

POSTER COMMUNICATIONS – PC.296

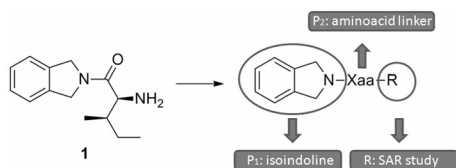
STRUCTURE-ACTIVITY RELATIONSHIP STUDY ON ISOINDOLINE INHIBITORS OF DIPEPTIDYL PEPTIDASE 8 AND 9

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Dipeptidyl peptidase 8 (DPP8) and DPP9 are members of the prolyl peptidase family, which also includes DPPIV (EC 3.4.14.5), fibroblast activation protein (FAP), prolyl oligopeptidase, and others. Compared with well-studied DPPIV, the functions of cytoplasmic enzymes DPP8 and DPP9 are yet to be elucidated. Nevertheless DPP8 and DPP9 have garnered much attention following research results from Merck suggesting that the use of the DPP8/9 inhibitor *allo*-Ile-isoindoline **1** is associated with severe toxicity in animal models.¹ Contrasting with these findings, two recent studies suggest that DPP8/9 inhibition alone cannot account for these toxic symptoms.² Another study claims the observation that DPP8 and DPP9 are up regulated in experimentally induced asthma and that these peptidases specifically respond to the inflammatory stimulus.³ In addition DPP9 appears to be a rate-limiting enzyme for degradation of proline-containing peptides.⁴ These intriguing data call for further investigation.

In order to verify whether the reported toxicity observations are related to DPP8/9 inhibition, or solely due to compound mediated off-target effects and to further study the clinical relevance of potential enzyme involvement in pathologies, there is an urgent need for new, preferably structurally distinct inhibitors of these enzymes. The goal of the present project was to conceive the structure-activity relationship on a chemically diverse set of inhibitors containing an isoindoline moiety. The inhibitors' affinity toward the different DPPs will be discussed, including their potential to discriminate between DPP8 and DPP9.



REFERENCES

1. Lankas, G. R., et al. Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes – Potential importance of selectivity over dipeptidyl peptidase 8 and 9. *Diabetes* **2005**, 54, 2988-2944
2. Roseblum J. S., et al. The case against GI toxicity from acute DPP8/9 inhibition. *Diabetes*, **2007**, 56, A138, Poster presented at the American Diabetes Association congress 2007; Burkey B. F., et al Adverse effects of dipeptidyl peptidases 8 and 9 inhibition in rodents revisited. *Diabetes, obesity and metabolism* **2008**, 10, 1057-1061
3. Schade J., et al. Regulation of expression and function of dipeptidyl peptidase 4 (DP4), DP8/9, and DP10 in allergic responses of the lung in rats. *J. Histochem. Cytochem.*, **2007**, 56, 147-155
4. Geiss-Friedlander R. et al The cytoplasmic peptidase DPP9 is rate-limiting for degradation of proline-containing peptides **2009**, 284, 27211-27219

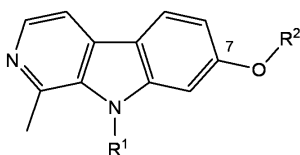
POSTER COMMUNICATIONS – PC.297

INHIBITION STUDY OF MONOAMINE OXIDASES BY β -CARBOLINE DERIVATIVES

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Harmine is a reversible inhibitor of human monoamine oxidase A (MAO-A) (1) and the crystal structure of human MAO-A in complex with harmine has been recently published. (2) This crystal structure shows that close to the methoxy group of the harmine moiety, a lipophilic pocket is left vacant within the binding site of hMAO-A. Our objective was to optimize the β -carboline series against MAO-A in order to explore this pocket. Therefore, a series of β -carboline derivatives has been synthesized. The compounds were evaluated for their human monoamine oxidase A and B inhibitory potency and their K_i values were calculated. The results show that O-alkylated compounds with lipophilic groups like cyclohexyl, phenyl and aliphatic chains increase the inhibition of MAO-A compared to harmine. Compound with the trifluorobutyloxy group, was the most active of this series, with a K_i against MAO-A of 3.6 nM. Molecular docking studies show that the trifluorobutyloxy chain occupies the hydrophobic pocket vacant with harmine. The O-alkylated compounds are less active on MAO-B than on MAO-A. However, several compounds show a better inhibition on MAO-B compared to harmine.



R¹ = H, benzyl

R² = Alkyl, Allyl, (CH₂)₃CF₃, (CH₂)₂OCH₃, (CH₂)₂OH, CH₂-c-Hx, CO-Ar, (CH₂)_n-Ar (n = 1,2), CH₂-pyridyl, (CH₂)_n-N(CH₃)₂ (n = 2,3), (CH₂)₂-morpholine

Keywords: β -carboline; MAO inhibition; Docking; X-ray.

REFERENCES

1. Kim, H.; Sablin, S. O.; Ramsay, R. R. *Arch Biochem Biophys* 1997, 337, 137-42.
2. Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. *Proc Natl Acad Sci U S A* 2008, 105, 5739-44.

POSTER COMMUNICATIONS – PC.298

ENOL MIMICS WITH A FLEXIBLE SIDE CHAIN AS INHIBITORS OF MYCOBACTERIUM TUBERCULOSIS DEHYDROQUINASE

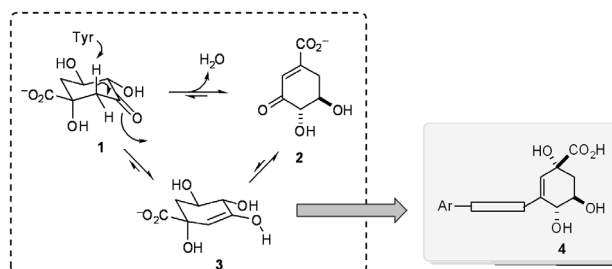
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For years, antibiotics have been fundamental in combating infectious diseases caused by bacteria. Despite the enormous therapeutic arsenal available today, we face a serious problem because the infections caused by pathogenic bacteria have become resistant to the actual antibiotic therapies. For example, tuberculosis, pneumonia or gonorrhea are increasingly more difficult to treat. This phenomenon is especially significant in important diseases such as tuberculosis, where the commonly used therapy has become less efficient. It is therefore necessary to develop updated therapies targeting infectious diseases, in particular, drugs with new mechanisms of action to combat the growth of antibiotic-resistant bacteria.

One of the strategies for the development of new antibiotics is to design compounds capable of interfering with essential processes for bacterial survival. This possibility has been studied in our research group in the last few years.¹ It consists in the design and the synthesis of compounds whose mode of action is based on the selective and the effective inhibition of an essential enzyme, dehydroquinase. Based on the enzymatic mechanism, we have designed and synthesized several analogues of the reaction intermediate **3**, compounds **4**, that have a flexible arm which blocks the action of two key residues in catalysis. Our recent progress made on the design and the synthesis of *M. tuberculosis*

sis type II dehydroquinase inhibitors will be presented in this communication.



REFERENCES

1. González-Bello, C.; Castedo, L. *Med. Res. Rev.* **2007**, 27, 177

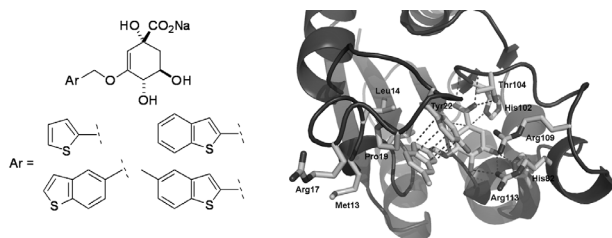
POSTER COMMUNICATIONS – PC.299

STEADY-STATE FLUORESCENCE AND MOLECULAR DYNAMIC STUDIES ON THE INHIBITION OF TYPE II DEHYDROQUINASE BY (2R)-3-BENZYL-3-DEHYDROQUINIC ACIDS

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The ulcer-causing *Helicobacter pylori* bacterium with strains resistant to clarithromycin is becoming more and more prevalent.^{1,2} Specifically, it has been estimated that the widespread of *H. pylori* resistance to clarithromycin is <5% for adults in northern Europe, but as high as 20% in southern Europe. It is present in approximately 13% of patients in USA, but in selected regions such as Alaska has been reported to have rates as high as 30%.² In addition, *H. pylori* infected persons are at increased risk for mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma.³ Therefore, the development of new agents that are able to overcome existing resistance mechanisms or that have novel mechanisms of action is much needed. In the last few years, our research group has been studying the possible development of new antibiotics whose action is based on the selective and effective inhibition of an essential route in bacteria, the shikimic acid pathway. Herein, we present the synthesis of novel inhibitors with high affinity for *Helicobacter pylori* type II dehydroquinase, the third enzyme of this route, and efficient inhibition characteristics. The structure of *Helicobacter pylori* type II dehydroquinase in complex with the most potent inhibitor shows the aromatic functional group interacts with the catalytic Tyr22 by π -stacking, expelling the Arg17 side-chain, which is essential for catalysis, from the active site. The structure therefore explains the favorable properties of the inhibitor and will aid in design of improved antibiotics.⁴



REFERENCES

1. Duck, W. M. et al. *Emerg. Infect. Diseases* **2004**, *10*, 1088.
2. Megraud, F. *Gut* **2004**, *53*, 1374.
3. Hatakeyama, M. *J. Gastroenterol* **2009**, *44*, 239.
4. González-Bello, C. et al. *J. Med. Chem.* **2010**, *53*, 191.

POSTER COMMUNICATIONS – PC.300

SYNTHESIS AND BIOLOGICAL EVALUATION OF OPTIMIZED THIAZOLIDIN-4-ONE-BASED MURD LIGASE INHIBITORS

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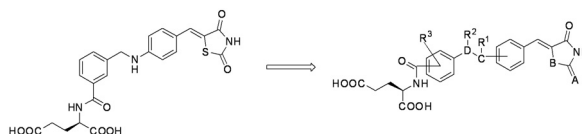
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The search for novel antibacterial agents with new mechanisms of action is one of the key strategies emerging for combating drug-resistant bacteria.¹ Recently, there has been an increased interest in exploiting the enzymes involved in the early cytoplasmic steps of peptidoglycan biosynthesis for antibacterial drug discovery. Among them are the ATP-dependent Mur ligases (MurC to MurF) that catalyze a series of reactions leading to UDP-MurNAc-pentapeptide by sequentially adding L-Ala (MurC), D-Glu (MurD), L-Lys or *meso*-diaminopimelic acid (MurE) and D-Ala-D-Ala dipeptide (MurF) to the starting MurC substrate UDP-MurNAc.²

We synthesized and biologically evaluated a series of 5-benzylidenerhodanine- and 5-benzylidenethiazolidine-2,4-dione-based inhibitors of MurD ligase from *E. coli*.³ The most potent compounds inhibited the enzyme with IC₅₀ values in the low micromolar range. The binding mode of this structural type of MurD inhibitors was established by X-ray crystallography and protein NMR. The crystal structures of MurD in complex with thiazolidine-4-one inhibitors provide useful information for structure-based design of novel inhibitors with improved inhibitory activity against MurD. D-Glutamic acid moiety of the inhibitor binds into the D-Glu binding pocket of MurD active site, where it forms several hydrogen bonds with the protein. Rhodanine or thiazolidine-2,4-dione ring occupies the uracil-binding pocket of natural MurD substrate UDP-MurNAc-L-Ala and interacts with the side chain hydroxyl group of Thr36. Other hydrogen bond

interactions observed between inhibitors and the active site residues are mediated through crystal water molecules.

To further explore the structure-activity relationship of thiazolidine-4-one-based compounds for MurD inhibition, rhodanine and thiazolidine-2,4-dione rings were replaced by pseudothiohydantoin, hydantoin and succinimide, which can also form observed hydrogen bonds with Thr36 in the uracil-binding pocket. Furthermore, the middle part of the known inhibitors was also a subject of extensive structural modification in order to investigate additional interactions in the MurD active site.



REFERENCES

1. Chopra, I.; Schofield, C.; Everett, M.; O'Neill, A.; Miller, K.; Wilcox, M.; Frère, J.-M.; Dawson, M.; Czaplewski, L.; Urleb, U.; Courvalin, P. *Lancet Infect. Dis.* **2008**, *8*, 133-139.
2. Barreateau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* **2008**, *32*, 168-207.
3. Tomašič, T.; Zidar, N.; Rupnik, V.; Kovač, A.; Blanot, D.; Gobec, S.; Kikelj, D.; Mašič, L.P.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 153-157.

POSTER COMMUNICATIONS – PC.301

THE SYNTHESIS, MOLECULAR MODELLING AND PHARMACOLOGICAL TESTING OF NOVEL ANALOGUES OF OLANZAPINE

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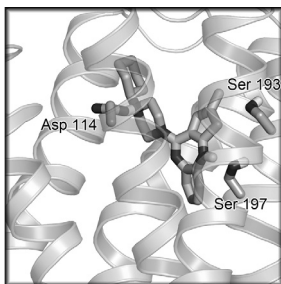
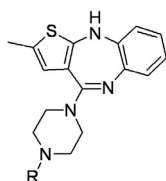
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Atypical antipsychotic drugs offer several notable benefits over typical antipsychotics, including greater improvement in the treatment of negative symptoms, cognitive function, prevention of the deterioration of the quality of life, and fewer extrapyramidal symptoms (EPS). Olanzapine is one such drug; however concerns about EPS have been replaced by concerns about other side effects such as weight gain, hyperlipidemia, type II diabetes mellitus and neutropenia. Olanzapine was introduced to the market in 1996 by Eli Lilly, and exhibits affinity towards a multitude of receptors, predominantly D₂ and 5-HT_{2A} as well as H₁ and M₁ receptors. This multi-acting receptor profile is believed to be unique to olanzapine's therapeutic mechanism of action, but also contributes to an array of undesirable effects.

The presented work examines potential new drug candidates as novel analogues of olanzapine by the incorporation of varied

aliphatic groups to the distal nitrogen, including structures with variations in chain length and branching (acyclic), carbocyclic, heterocyclic (isosteric replacement), saturation and ionization.

The presented work also incorporates rational drug design with the assistance of molecular modelling techniques using the recently published β_2 -adrenoceptor as a template for homology models of the dopamine D_2 and serotonin 5-HT_{2A} receptors to analyse *in silico* interactions between the ligands and the receptor. Furthermore, preliminary *in vitro* analyses were undertaken to assess the affinities of these novel analogues towards D_2 and 5-HT_{2A} receptors, thus aiding validation of molecular modelling, and shedding light on potentially improved antipsychotic therapies for the future.



POSTER COMMUNICATIONS – PC.302

EFFICIENT ACCESS TO ORTHOGONALLY PROTECTED DISULFIDE BRIDGE MIMETICS

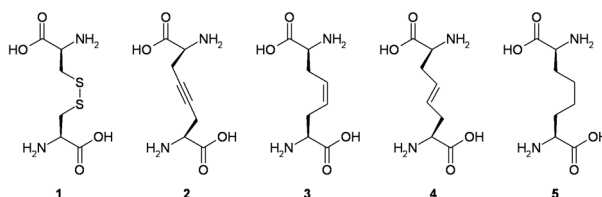
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Many naturally occurring and engineered biologically active peptides are cyclized via disulfide bridges. However, the disulfide bond is not as strong as a carbon-carbon bond, it can also be easily reduced *in vivo* to the open chain peptide. Interest in peptidomimetics and *in vivo* stabilization of peptides has led us to examine disulfide bridge analogues where the disulfide bridge is replaced by different carbon chain linkers. Our primary interest is the bicyclic peptide C-4 that stimulates the activity of human tissue kallikrein 3 (KLK3, also known as prostate specific antigen, PSA).¹ The KLK3 stimulating peptide has the potential to provide a new non-invasive therapy for prostate cancer.² The peptide consists of 13 amino acids, with one terminal disulfide bridge between cysteines 1 and 13 and one internal between cysteines 5 and 10.

We have successfully developed efficient, high-yielding, enantiopure synthetic procedures of four disulfide bridge mimetics **2–5**, possessing an alkyne, a *Z*-alkene, a *E*-alkene and an fully saturated linker instead of the disulfide bridge. These linkers have different geometries and should be able to mimic any conformation of the disulfide bridge **1**. The mimetics are protected

by completely orthogonal protecting groups (Fmoc and Boc as *N*-terminal protecting groups and *tert*-butyl and methyl esters as *C*-terminal protecting groups). We have utilised alkyl copper-zinc/haloalkyne chemistry (previously developed by Knochel) to synthesise a disulfide bridge mimetic containing an alkyne moiety (**2**). Selective and full reduction of the triple-bond using Pd/BaSO₄ and Pd/C respectively, enabled access to the *Z*-alkene and the fully saturated mimetics (**3** and **5**). For the enantiopure synthesis of the corresponding mimetic possessing an *E*-alkene (**4**), we successfully employed a Grubbs cross-metathesis reaction.



REFERENCES

- Wu P., Leinonen J., Koivunen E., Lankinen H., Stenman U.-H. *Eur. J. Biochem.* **2000**, 267, 6212-6220.
- Koistinen H., Närvalä A., Pakkala M., Hekim C., Mattsson J.M., Zhu L., Laakkonen P., Stenman U.-H. *Biol. Chem.* **2008**, 389, 633-642.

POSTER COMMUNICATIONS – PC.303

S-NITROSYLATION AND ATTENUATION OF CALCIUM FLUX BY PENTACYCLOUNDECANE DERIVATIVES

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Glutamate-related excitotoxic neuronal cell injury and death has been reported to contribute to almost every major neurodegenerative disorder.^{1,2} Excitotoxicity can occur by over activation of NMDA-sensitive glutamate receptors and the subsequent excessive calcium influx into the associated cells.^{3,4} There are numerous insults that can lead to excessive glutamate release and stimulation of glutamate receptors, with subsequent excitotoxicity and cell death. All of them share the same pathway in that large amounts of glutamate is released from the injured cells which then reach the thousands of nearby cells, causing them to depolarise, swell, lyse and die by necrosis. In chronic diseases, it is proposed that modest amounts of glutamate over an extended period are to blame for the excitotoxic damage.^{3,5,6} To attenuate the effects of excitotoxicity, three mechanisms of action are proposed for this study. The first is S-nitrosylation of crucial cysteine residues in the NMDA receptor channel, the second is the

blockage of excessively stimulated voltage gated Ca^{2+} channels and the third is through NMDA receptor channel antagonism by the polycyclic amines.

A novel series of polycyclic amines, containing nitrogen monoxide donating moieties, were synthesised and tested for calcium channel and N-methyl-D-aspartate (NMDA) receptor modulating activity. These included unsaturated nitro compounds and nitro esters, or nitrates. The nitrates were obtained via the reaction of hydroxyl functionalities with thionylchloride nitrate. All of the compounds synthesised exhibited significant ($p < 0.01$) S-nitrosylation capacity. The calcium channel activity of the polycyclic amines was evaluated using a KCl mediated fluorescent calcium flux assay. All the compounds exhibited better calcium channel antagonism than the lead structure, NGP1-01, with 8-aminoethyl-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-2-(4-nitrobenzoate) exhibiting calcium channel blockade comparable to that of nimodipine at 10 μM and 1 μM . All the compounds exhibited NMDA/glycine mediated N-methyl-D-aspartate receptor (NMDAR) calcium influx inhibition at 100 μM , with 8-aminoethyl-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-2-[4-(2-nitroethenyl)benzoate] showing the most significant NMDAR inhibitory activity.

No clear correlation was observed between the S-nitrosylation capabilities of the compounds and their calcium channel activity or NMDAR channel antagonism. All the compounds synthesised exhibited promising calcium channel and NMDAR channel inhibitory activity and thus show promise as potential agents against neurodegeneration.

REFERENCES

1. R.P. Simon, J.H. Swan, and B.S. Meldrum, *Science*. 1984, 244, 798-800.
2. D.W. Choi, *Neurosci Lett*. 1985, 58, 293-297.
3. S.A. Lipton, *Nat. Rev. Drug Discovery*. 2006, 5, 160-170.
4. M. Arundine and M. Tymianski, *Cell. Mol. Life Sci*. 2004, 61, 657-668.
5. P. Lipton, 1999. *Physiol. Rev*. 1999, 79, 1431-1568.
6. J.A. Kemp and R.M. McKernan, *Nature Neurosci*. 2002, 5, 1039-1042.

POSTER COMMUNICATIONS – PC.304

NOVEL CCR9 ANTAGONISTS SYNTHESIS, OPTIMIZATION AND BIOLOGICAL ACTIVITY

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Crohn's disease is an autoimmune disease affecting mainly the small intestine in which large numbers of antigen-experienced T

cells accumulate within the intestinal lamina propria. The chemokine receptor CCR9 is expressed by most T cells trafficking to the small intestine. Blockade of the interaction of CCR9 on gut-homing T cells with its only ligand TECK, also expressed in the small intestine, is believed to specifically inhibit the migration of pro-inflammatory T cells into the small intestine and will result in a therapeutic benefit. As shown for other chemokine receptor programs high *in vitro* potency is a necessary prerequisite however successful drug candidates will have also to fulfil other parameters like adequate physico-chemical and ADME properties.¹⁾

Here we report on the synthesis and biological profiling of a novel type of potent CCR9 antagonists. The first generation of compounds is featuring an aromatic *tert*.butyl group which contributes to very high logP values and secondly is prone to metabolic attack. Therefore, our derivation program focussed on replacing this *tert*.butyl group by metabolically more stable groups with similar space filling. We investigated two compound series and identified a SF5-substituted derivative which is combining improved stability and adjusted logP values with high potency in a CCR9-dependent Ca^{2+} mobilization assay.

In summary, we show here that in our case the *tert*.butyl group can be successfully replaced by a SF5 group² leading to a candidate with favourable overall properties. Furthermore, this protocol of replacing a *tert*.butyl by a SF5 group might also be useful in other Medchem programs where an aromatic *tert*.butyl group is creating an issue of high lipophilicity and metabolic instability.

REFERENCES

1. Amanda El Proudfoot; *Expert Opin. Investig. Drugs* 19(3), 345-355 [2010]
2. John T. Welch; *Bioorg. Med. Chem.* 15(21), 6659-6666 [2007]

POSTER COMMUNICATIONS – PC.305

BIOLOGICAL EVALUATION OF THE ANTITUMOUR ACTIVITY OF IMIDOSELENOCARBAMATES DPR235 AND DPR380

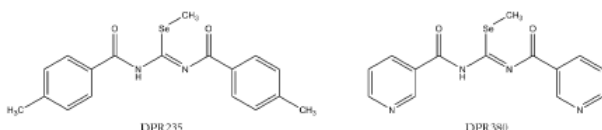
Iranzu Lamberto^{1*}, Iosu Jiménez¹, Elena Ibáñez¹, Daniel Plano¹, Ignacio Encío³, María Font², Juan Antonio Palop¹, Carmen Sanmartín¹

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Cancer is becoming an increasingly significant disease worldwide¹. It's well known that the trace element selenium (Se) appears to have cancer preventive properties based on a con-

verging body of evidence from epidemiologic, clinical and experimental studies^{2,3}. Though mechanisms of action for seleno-compounds as anticancer agents are not fully understood, it has been reported that the dose and chemical form of selenium are determinant factors on its antitumour activity⁴.

Among the growing list of seleno-compounds with desirable anticancer activity, we previously reported the synthesis of various imidoselenocarbamates with significant *in vitro* antiproliferative activity against human prostate cancer cells PC-3⁵. To further characterize the antitumour activity of these compounds, here we extend the evaluation of the antiproliferative action of two of them, DPR235 and DPR380, to a panel of four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and two non-malignant cell lines (184B5 and BEAS). We also analyze the ability of DPR235 and DPR380 to induce ROS overproduction and apoptosis, as well as their effect on cell cycle distribution in MCF-7 cells.



REFERENCES

1. Rizky Abdulah, Kaori Miyazaki, Minato Nakazawa, Hiroshi Koyama; Journal of Trace Elements in Medicine and Biology, Volume 19, Issues 2-3, 2 December 2005, Pages 141-150.
2. Gurusamy, K. Biol. Trace Elem. Res, 2007, 118, 191-206.
3. Ceung, E.; Wadhwa, P.; Dorff, T.; Pinski, J. Expert Rev. Anticancer Ther. 2008, 8, 43-50.
4. Rikiishi, H. J. Bioenerg. Biomembr. 2007, 39, 91-98.
5. Plano, D.; Sanmartin, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. Bioorg. Med. Chem. Lett., 2007, 17, 6853-6859.

POSTER COMMUNICATIONS – **PC.306**

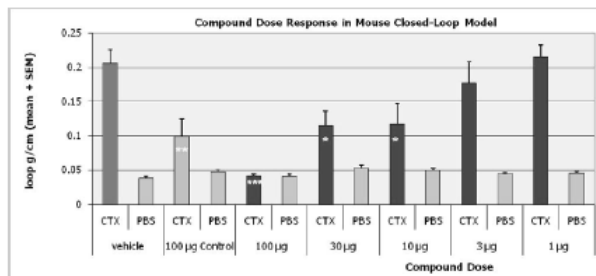
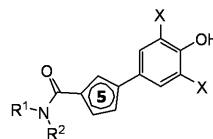
INHIBITORS OF THE CFTR CHLORIDE ION CHANNEL AS POTENTIAL TREATMENT FOR ACUTE SECRETORY DIARRHEA: DEVELOPMENT OF 5-MEMBERED HETEROCYCLES SUITABLE FOR PRE-CLINICAL EVALUATION

Kevin Doyle¹, Graham Jones¹, Michael Russell¹, Joanne Peach¹, Amanda Van de Poël¹, Carrie-Anne Molyneaux¹, Jackie Macritchie¹, Angus MacLeod¹, Joanne Shearer¹, David Cronk¹, Gary Clark¹, Alan Beresford¹, Eugenio de Hostos², Tue Nguyen², David Brown²

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Acute secretory diarrhea is responsible for the deaths of up to 2 million children a year in the developing world. The current standard of care is a glucose/salt solution, known as oral rehydration therapy (ORT), which is a palliative treatment for the disease. However, the labor intensive regimen alongside the lack of symptomatic relief leads to a high level of noncompliance. The identification of an adjunct therapy to work alongside ORT and improve time to symptomatic relief is paramount.

Acute secretory diarrhea is caused by an intestinal infection with pathogens that include *vibrio cholera* resulting in the output of large volumes of watery stools. The major mechanism for this infection is the over-activation of the chloride ion channel, CFTR (cystic fibrosis transmembrane conductance regulator), present in the epithelial cells within the intestinal wall. We demonstrate that inhibition of this ion channel with 5-membered heterocyclic compounds gives blockage of chloride transport in a variety of *in vitro* systems. In addition, we show that a selection of these compounds is efficacious in two animal models for diarrheal disease. The identification and optimization of a series of 5-membered heterocyclic inhibitors suitable for pre-clinical evaluation will be discussed.



POSTER COMMUNICATIONS – **PC.307**

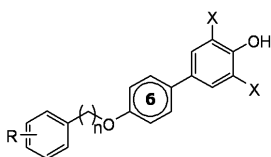
INHIBITORS OF THE CFTR CHLORIDE ION CHANNEL AS POTENTIAL TREATMENT FOR ACUTE SECRETORY DIARRHEA: DEVELOPMENT OF 6-MEMBERED HETEROCYCLES

Kevin Doyle¹, Michael Russell¹, Joanne Peach¹, Stephen Penrose¹, Amanda Van de Poël¹, Graham Jones¹, Jackie Macritchie¹, Angus MacLeod¹, Sebastian Brückner¹, Joanne Shearer¹, David Cronk¹, Gary Clark¹, Alan Beresford¹, Eugenio de Hostos², Tue Nguyen², David Brown²

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POSTER COMMUNICATIONS – PC.308

N-SUBSTITUTED DERIVATIVES OF DEXTROMETHORPHAN ARE INHIBITORS OF $\alpha 3\beta 4$ NICOTINIC ACETYLCHOLINE RECEPTOR

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Dextromethorphan (DM) is widely used ingredient of antitussive OTC medicaments. Due to its complex pharmacology scientists suggested several other potential therapeutic uses (e.g., in Huntington's and Parkinson's diseases or cocaine dependence). Our previous studies indicated DM as potent inhibitor of neuronal nicotinic acetylcholine receptor (nAChR). Molecular modeling studies allowed us to identify a binding site within the nAChR ion channel and to design a series of N-substituted DM derivatives with optimized interactions with $\alpha 3\beta 4$ subtype of nAChR. Some of these molecules were synthesized and characterized structurally [Fig.1].

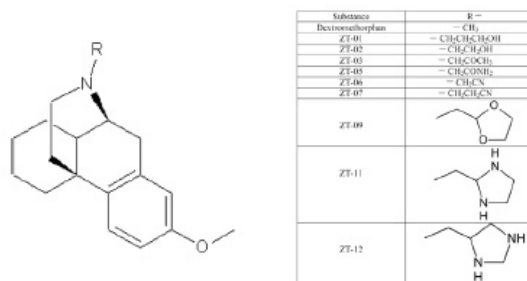


Fig. 1 The structure of dextromethorphan and selected synthesized derivatives.

Currently new derivatives are tested for functional activity against $\alpha 3\beta 4$ nAChR in electrophysiological assay. *In vitro* experiments are performed on HEK-293 cells stably transfected with rat $\alpha 3$ -nAChR and $\beta 4$ -nAChR genes. DM and its new derivatives could play a pivotal role in developing new more efficient therapy for nicotine addiction. The latest scientific evidence demonstrates that $\alpha 3\beta 4$ nAChRs are densely expressed in habenular - interpeduncular (Hb – IPN) system regulating the dopaminergic pathway responsible for addiction development. In next step of the project the most promising compounds will be investigated in animal tests for blocking of nicotine dependence.

POSTER COMMUNICATIONS – PC.309

NOVEL ORGANOSELENIUM DERIVATIVES: A NEW CLASS OF POTENT ANTILEISHMANIAL AGENTS

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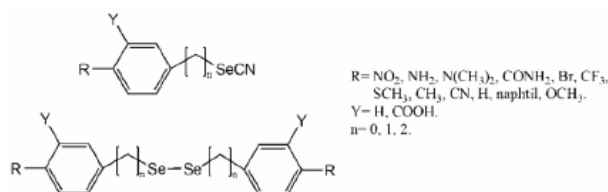
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The leishmaniasis are a group of tropical diseases. World Health Organization (WHO) estimates that the disease results in 2 million new cases a year and that there are 12 million people currently infected worldwide (1).

Different studies recognised the trace element selenium as a new defense strategy against *Leishmania* infection (2), and also the importance of possible potential interference of selenium derivatives with the redox system of the parasites (3).

Due to the urgent need for innovative drugs and previous work of our research group in organoselenium compounds (4-5), we carried out the synthesis and biological evaluation of selenocyanate and diselenide derivatives, according with these general structures:



All the synthesized compounds were subjected to *in vitro* screening against *L. infantum* promastigote and the most active were tested in amastigote model. In order to establish the selectivity index (SI) their cytotoxic effect was carried out against Jurkat and THP-1 cell lines. Nine derivatives exhibited IC_{50} values better than miltefosine and six of them better than edelfosine than were used as standard drugs. Among all, some compounds combine high potency and low cytotoxicity with selectivity index better than reference drugs.

REFERENCES

- Kedzierski, L.; Zhu, Y.; Handman, E. *Parasitology* **2006**, *133*, S87-S112.
- Culha, G.; Yalin, E.; Sanguen, K. *Asian J. Chem.* **2008**, *20*, 3104-3108.
- Sculaccio, S.A.; Rodríguez, E.M.; Cordeiro, A.T.; Magalhães, A.; Braga, A.L.; Alberto, E.E.; Thiemann, O.H. *Mol. Biochem. Parasitol.* **2008**, *162*, 165-171.
- Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6853-6859.
- Sanmartín, C.; Plano, D.; Domínguez, E.; Font, M.; Calvo, A.; Prior, C.; Encío, I.; Palop, J.A. *Molecules* **2009**, *14*, 3313-38.

POSTER COMMUNICATIONS – PC.310

NOVEL INDOLYL ACRYLIC ACID DERIVATIVES AS POTENT INHIBITORS OF DIACYLGLYCEROL ACYLTRANSFERASE

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Triglyceride (TG) is a major form of stored energy in eukaryotes, and it is synthesized primarily through acyl CoA: diacylglycerol acyltransferase (DGAT) reaction that catalyzes the final and the only committed step in the glycerol phosphate pathway involving in the biosynthesis pathways of TG within the insulin-sensitive tissues including the adipose tissue, skeletal muscles, liver, and the β -cells of the pancreas. TG synthesis is important in mammals for energy storage in the adipose tissue and muscle, fat absorption in the intestine, and the assembly of lipoprotein in the liver and in the small intestine as well. As TG is also essential for the accumulation of adipose tissue in the body, inhibition

of TG synthesis may reduce the incidence of obesity and its related diseases. Recently, it has been reported that diet-induced obesity caused by high fat diet could be effectively inhibited in DGAT-1 knock-down mice, and abnormal glucose metabolism was improved by increase of the susceptibility of insulin and leptin, the dietary hormones. As inhibition of DAGT has emerged as a therapeutic target for inhibiting TG synthesis, a potent DGAT inhibitors would be useful for the treatment of metabolic diseases. In the search of novel DGAT inhibitors, it was found that novel indolyl acrylic acid derivatives showed potent DGAT inhibitory effect. Herein, the structure-activity relationships of the indolyl acrylic acid derivatives will be presented.

POSTER COMMUNICATIONS – PC.311

PEROXIREDOXIN INHIBITORS TO SELECTIVELY KILL CANCER CELLS THROUGH A ROS-MEDIATED MECHANISM

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Most cancer cells exhibit an increase in reactive oxygen species (ROS) generation, which enable to maintain their malignant phenotypes. Cancer cells, under oxidative stress associated with increased metabolic activity and production of ROS, are highly dependent on antioxidant system for survival and thus more susceptible to oxidative insults by ROS-stressing agents than normal cells. Peroxiredoxin (Prx), the novel H_2O_2 eliminating enzyme, is known to be elevated in a variety of human cancers. Recent studies have suggested that this biochemical property of Prxs in cancer cells can be exploited for therapeutic benefits. In our search for novel anticancer agents to inhibit Prx I, it was found that N-(3-acetylphenyl)-4-(biphenyl-4-ylmethyl)piperidine-1-carboxamide effectively inhibits the reduction of Prx I from cysteine sulfenic acid to cysteine sulfhydryl acid through interruption of electron transfer from reducing system and causes severe ROS accumulation in cancer cells. Further biochemical study suggests that the Prx-mediated H_2O_2 elimination may serve as a novel therapeutic target for cancer intervention. Herein, the structure-activity relationships and biochemical study of the piperidine-1-carboxamide derivatives will be presented.

POSTER COMMUNICATIONS – PC.312

DESIGN AND SYNTHESIS OF QUINOLINE DERIVATIVES AS A NOVEL CLASS OF ACYL COA: CHOLESTROL ACYL TRANSFERASE INHIBITORS

Jail Goo,¹ Sung Yoon Lee,¹ Jung Ho Choi,² Seung Ju Park,¹ Young Kook Kim,^{2*} Yongseok Choi^{1*}¹ College of Life Sciences and Biotechnology, Korea University, Seoul, Korea. ² Korea Research Institute of Bioscience and Biotechnology, Chungbuk, Korea

Acyl-CoA: Cholesterol acyltransferase (ACAT, E.C.2.3.1.26) is the primary enzyme responsible for the intracellular esterification of free cholesterol with fatty acyl-CoA to produce cholesterol esters in various tissues. Accumulation of cholesteryl ester leads to the formation of foam cells and a hallmark of atherosclerosis lesions. Inhibition of ACAT is expected to reduce plasma lipid levels by inhibiting intestinal cholesterol absorption and to prevent progression of atherosclerotic lesions by inhibiting the accumulation of cholesteryl esters in macrophage. Thus, the inhibitor of ACAT activity could be the competitive drug in the prevention and treatments of hypercholesterolemia and atherosclerosis by inhibiting a formation of cholesteryl ester within the cell. Recently, it was reported that shikonin derivatives with naphthoquinone template possessed ACAT inhibitory activity in vitro as well as cell-based assay system. Based on the activity of the naphthoquinone template, a series of novel quinoline derivatives were synthesized. Herein, the structure-activity relationships of the quinoline derivatives will be presented as potential ACAT inhibitors.

POSTER COMMUNICATIONS – PC.314

A NOVEL CHEMOATTRACTANT PROTEIN, KARATOXIN, FROM THE DORSAL SPINES OF THE REDFIN VELVETFISH, *HYPODYTES RUBRIPINNIS*Ohura K^{1*}, Shinohara M¹, Nagasaka K², Nakagawa H³, Edo K³¹ Department of Pharmacology, Osaka Dental University, Osaka, ² Fukae Nagasaka Clinic, Hiroshima, ³ University of Tokushima, Tokushima, Japan.

A chemoattractant protein from the dorsal spines of the redfin velvetfish, *Hypodytes rubripinnis* was isolated by a combination of affinity chromatography. The glycoprotein with a molecular mass of 110 kDa is named Karatoxin.

The redfin velvetfish, *Hypodytes rubripinnis* is a small congiopodid fish, that belongs to the Scorpaenidae group known as scor-

pionfish. However, *H. rubripinnis* is dangerous to man. More recently, we have observed that Karatoxin, the glycoprotein purified from the dorsal venom have cytotoxic activity on murine P388 leukemia cells (1).

In our search for bioactive compounds we have been investigating for chemoattractant activity from the dorsal venom of *H. rubripinnis*. Here we report biological activities of Karatoxin as a novel chemoattractant lectin from the dorsal venom of *H. rubripinnis*.

Hypodytes rubripinnis were collected by the local fishermen from the coast of Hiroshima Prefecture, Japan. The collected fish were transported alive or frozen to our laboratory. The dorsal spines (total 14) from *H. rubripinnis* were cut from their base.

Mitogenic activity by the murine splenocytes was determined by the cell culture assay using the MTT tetrazolium salt.

Polymorphonuclear leukocytes (neutrophils) and macrophages were induced by intraperitoneal injection of 1 % glycogen solution into male guinea-pigs, and collected by centrifugation in PBS. Chemotaxis were measured by membrane filter method using 48-well chemotaxis chamber.

We had reported that the dorsal venom protein from *H. rubripinnis* showed weak agglutination with rabbit erythrocytes and degranulation of the rat mesenteric mast cells. The agglutination caused by the venom was inhibited with the treatment of D-mannose. The venom also induced mitogenic activity on the murine splenocytes in the dose range of 10 to 100 µg/ml. These results suggested that the dorsal venom protein contain lectin components.

In this study, we report some biological activities of Karatoxin (110 kDa), a dorsal mannose-containing glycoprotein from *H. rubripinnis*. Karatoxin exhibited not only cytotoxic activity on the murine P388 leukemia cells, but also mitogenic activity on the murine splenocytes. In addition, Karatoxin had chemotactic activity of guinea-pig neutrophils and macrophages in a dose of low ranges (0.625 µg/ml - 5.0 µg/ml). Thus, Karatoxin appears to be a unique lectin.

Therefore, we have suspected that Karatoxin might affect inflammatory and immunomodulatory processes. Karatoxin appears to be a D-mannose-binding lectin. Our data suggest that *H. rubripinnis* venom may be a resource for pharmacologically active substances, such as lead compounds and research tools.

REFERENCES

1. Nagasaka K, Nakagawa H, Satoh F, Hosotani T, Yokoigawa K, et al. A novel cytotoxic protein, Karatoxin, from the dorsal spines of the redfin velvetfish, *Hypodytes rubripinnis*. Toxin Rev. 2009;28:260-265.

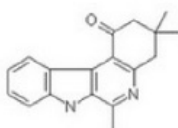
POSTER COMMUNICATIONS – PC.315

NEW HIGH-EFFECTIVE NEUROPROTECTOR – CARBACETAM

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As a result of systematic searches of the substances with influence on CNS our laboratory together with Donetsk Medical Institute worked out new full original high-efficient neuroprotector Carbacetam relating to the class of β -carbolines



Carbacetam is endogenous modulator of GABA_A- benzodiazepine receptor complex (diazepin binding inhibitor). This is powerful nootropic agent with very low tranquilizer activity, anti-amnesic action is 100 times and antihypoxic action 29-35 times as higher by piracetam, has any hypnotic and muscle relaxation activity and also any irritating, allergic, gonado- and embriotoxic or mutagenic action. It has IV toxicity class with LD₅₀ for mouse 346 and 1250mg/kg for rats. The drug was successfully passed following trials: full pre clinic and I and II clinical stages and was patented as stress protector, anti-amnesic, antihypoxic, antianxiotic and antishocking agent.

Friendly technology of this API was considerably improved by exclusion of dangerous materials: perchloric acid and diethyl ether from synthesis scheme.

POSTER COMMUNICATIONS – PC.316

ANTIOXIDANT PROPERTIES OF 4-QUINOLONES COMPARED TO STRUCTURALLY RELATED FLAVONES

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Oxidative stress, the result of overproduction of free radicals such as the hydroxyl radical, is a common occurrence in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. It is suggested that oxidative stress exists before the onset of these neurodegenerative diseases, leading to, or exacerbating deterioration.¹

Flavones are naturally occurring plant compounds with hydrogen donating substituents responsible for well established antioxidant activity.² Hydroxy-substituted flavones were selected as lead compounds, compared to similarly substituted 2-phenylquinolin-4(1H)-ones which were synthesised using the Conrad-Limpach method.³ The compounds, 2-phenylquinolin-4(1H)-one, 6-hydroxy-2-phenylquinolin-4(1H)-one, 7-hydroxy-2-phenylquinolin-4(1H)-one and 8-hydroxy-2-phenylquinolin-4(1H)-one were characterised by NMR, IR and MS. Quinoline, 4-hydroxyquinoline and 8-hydroxyquinoline were also included as reference compounds.

Antioxidant activity was evaluated using different *in vitro* assays to evaluate the potential value of the synthesised 2-phenylquinolin-4(1H)-ones compared to the existing structurally related flavones. The oxygen radical absorbance capacity⁴ was performed to establish the ability of the synthesised compounds to scavenge peroxy radicals. The 2-phenylquinolin-4(1H)-ones demonstrated better activity than the related flavones with 7-hydroxy-2-phenylquinolin-4(1H)-one observed as the best scavenger in the group.

The ferric reduction/antioxidant power assay⁵ assesses the theoretical inhibition of the Haber-Weiss reaction, leading to reduced hydroxyl radical production. In this assay the 2-phenylquinolin-4(1H)-ones also demonstrated better activity than the related flavones with 8-hydroxy-2-phenylquinolin-4(1H)-one having activity comparable to Trolox, a water soluble derivative of vitamin E.

Results from the nitroblue tetrazolium assay⁶ which evaluates the ability of a compound to scavenge superoxide anions, indicated moderate activity for the 2-phenylquinolin-4(1H)-ones, with 7-hydroxy-2-phenylquinolin-4(1H)-one demonstrating the best activity.

Results from the lipid peroxidation assay⁶ showed 6-hydroxy-2-phenylquinolin-4(1H)-one to be the best scavenger of hydroxyl radicals in the 2-phenylquinolin-4(1H)-ones series.

The results from the different assays indicated that the 2-phenylquinolin-4(1H)-ones acted as chain breaking antioxidants, with a postulated hydrogen donor mechanism of action and inhibiting radical mediated damage better than the related flavones, showing promise as possible neuroprotective agents

REFERENCES

1. W.R. Markesbery, T.M. Montine and M.A. Lovell, Pathogenesis of neurodegenerative diseases. 2001, 21-52
2. J. Robak and J. Gryglewski, Biochemical Pharmacology. 1988, 37, 837-841
3. R. Somanathan and K.M. Smith. Journal of Heterocyclic Chemistry. 1981, 18, 1077-1079
4. B. Ou, M. Hampsch-Woodill, R.L. Prior. Journal of Agriculture and Food Chemistry, 2001. 49, 4619-4626
5. I.F.F. Benzie and J.J. Strain. Analytical Biochemistry, 1996. 239, 70-76
6. P. Ottino and J.R. Duncan. Free Radical Biology and Medicine. 1997, 22, 1145-1151

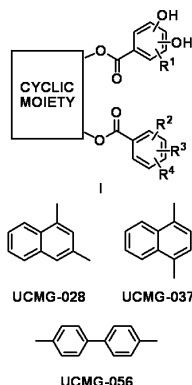
POSTER COMMUNICATIONS – PC.317

NOVEL SYNTHETIC INHIBITORS OF FATTY ACID SYNTHASE (FASN) WITH *IN VITRO* AND *IN VIVO* ANTICANCER ACTIVITY

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Fatty acid synthase (FASN) is highly expressed in breast carcinoma and other human cancers.¹ However, its therapeutic exploitation has been limited by the lack of pharmacological inhibitors that are potent, specific and stable enough.



Targeting FASN inhibition, in the present work we have synthesized a new series of compounds of general formula **I**, that were evaluated for their cytotoxic capacity in a panel of human breast cancer cell lines (SK-Br3, MCF-7, and MDA-MB-231) and in two types of non-malignant cells (fibroblasts and adipocytes), as well as for their effect in FASN activity.^{2,3}

We have identified three potent FASN inhibitors: UCMG-028, UCMG-037 and UCMG-056 (69-90% inhibition of FASN activity) that are highly cytotoxic against SK-Br3 (IC₅₀ < 30 μM) cells. Importantly, these compounds do not stimulate carnitine palmitoyltransferase-1 (CPT-1) activity *in vitro* and they do not decrease body weight *in vivo*, in contrast to previously reported FASN inhibitors such as C75. In addition, UCMG-028 blocked the growth of BT474 breast cancer xenografts.

Taken together, our results show that synthesized compounds **I** represent a novel class of synthetic FASN inhibitors endowed

with *in vitro* and *in vivo* anticancer activity, that do not exhibit adverse effects on body weight *in vivo*. Therefore, they hold promise for further target directed anticancer drug studies either alone or co-administered with other antitumoral drugs. We are currently exploring their full potential with the synthesis of a larger structural field to carry out an in-depth structure-activity relationship (SAR) study, as well as assessing their *in vivo* anticancer efficacy in breast cancer animal models. These results will enable the definitive validation of FASN as a therapeutically useful option for cancer treatment.

This work was supported by grants from Instituto de Salud Carlos III (FIS PI04/1417, FIS PI082031, and RD06-0020-0028; ISCIII-RETIC RD06; and FIS PI082031), the Susan G. Komen Breast Cancer Foundation (PDF-0504073), the Ministerio de Ciencia e Innovación (MICINN, CIT-090000-2008-10 and SAF-2007-67008-CO2-01), Comunidad Autónoma de Madrid (S-SAL-249-2006), and the Spanish Society of Medical Oncology (SEOM08), Ramón y Cajal (SO-G), and FPU (CT) programs from MICINN.

REFERENCES

1. Kuhajda, F. P. *Cancer Res.* **2006**, 66, 5977.
2. López-Rodríguez, M. L.; Benhamú, B.; Turrado, C.; Ortega-Gutiérrez, S.; Puig, T.; Brunet, J.; Colomer, R. Patent WO/2009/000864, **2008**.
3. Puig, T.; Turrado, C.; Benhamú, B.; Aguilar, H.; Relat, J.; Ortega-Gutiérrez, S.; Casals, G.; Marrero, P. F.; Urruticoechea, A.; Haro, D.; López-Rodríguez, M. L.; Colomer, R., *Clin. Cancer Res.* **2009**, 15, 7608.

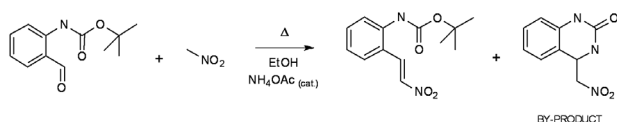
POSTER COMMUNICATIONS – PC.318

MICROWAVE-ASSISTED ONE-POT SYNTHESIS OF SUBSTITUTED 3,4-DIHYDRO-1H-QUINAZOLIN-2-ONES

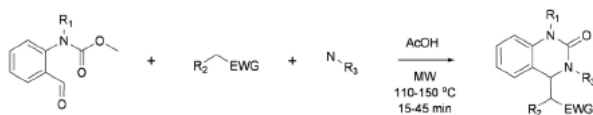
Krzysztof Więckowski, Luke Odell

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Quinazoline- and quinazolinone-derivatives constitute an important group of pharmacologically-active compounds. As inhibitors of enzymes and receptor ligands they have been investigated for decades and found application in treatment of various prevalent diseases (e.g. doxazosin, methaqualone, erlotinib) [1-4]. This study presents a new, facile and environmentally-friendly method for the synthesis of functionalized 3,4-dihydro-1H-quinazolin-2-ones. The procedure described herein was developed in the course of an optimisation of side process accompanying the Henry reaction between *N*-alkoxycarbonyl-2-aminobenzaldehydes and nitromethane.



The method worked out in the optimization allows total conversion of the benzaldehyde to the product (LCMS) with good isolated yields and short reaction time. Further studies revealed a broad scope of the reaction. Tolerance of different types of amines and nucleophiles makes this reaction a facile way to obtain various 1-, 3- and 4-substituted 3,4-dihydro-1H-quinazolin-2-ones.



Considering the simplicity of the method and the functional group compatibility (e.g. EWG, carbonyl) this transformation has significant potential, especially in medicinal chemistry applications - as a source of easily-accessible building blocks or final structures.

REFERENCES

1. Smits R. A., Adami M., Istyastono E. P., Zuiderveld O. P., van Dam C. M., de Kanter F. J., Jongejan A., Coruzzi G., Leurs R., de Esch I. J.; *J Med Chem.* 2010 Mar 25;53(6):2390-400.
2. Garofalo A., Goossens L., Lemoine A., Farce A., Arlot Y., Depreux P. J.; *Enzyme Inhib. Med. Chem.* 2010, 25 (2), 158-71.
3. Alagarsamy V., Raja Solomon V., Dhanabal K.; *Bioorg. Med. Chem.*, 2007, 15 (1), 235-241
4. Rzasa R. M., Kaller M. R., Liu G., Magal E., Nguyen T. T., Osslund T. D., Powers D., (...), Norman M.H.; *Bioorg. Med. Chem.* 2007, 15 (20), 6574-6595.

POSTER COMMUNICATIONS – PC.319

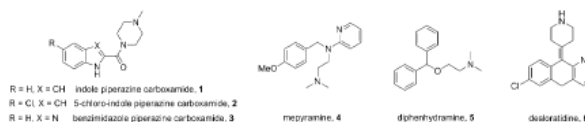
MOLECULAR MODELLING AND SYNTHESIS OF DUAL HISTAMINE H₁/H₄R-ANTAGONISTS

Wagner Eva, Gobleder Susanne, Straßer Andrea

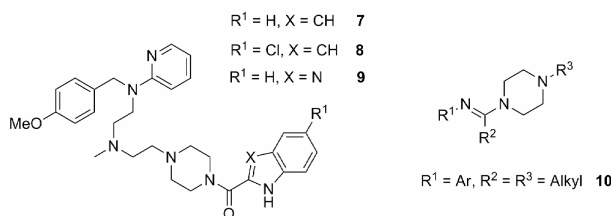
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Indole and benzimidazole piperazine carboxamides **1**, **2**, **3** are known to be potent histamine H₄-receptor antagonists [1]. The histamine H₄-receptor is discussed to be involved in inflammation and regulation of the immune system [2]. Histamine H₁-receptor antagonists like mepyramine **4**, diphenhydramine **5** and desloratadine **6** are used for treatment of allergic diseases. New studies show that combined application of **2** and **4** in the acute murine asthma model exhibits synergistic inhibitory effects on eosinophil accumulation in the bronchoalveolar lavage fluid [3]. Thus, the design of dual H₁/H₄R-antagonists may result in a new class of drugs for therapy of type-I allergic diseases, like rhinitis and con-

junctivitis. Therefore, we pursued two different strategies: 1) Development of ligands with one H₁- and one H₄-pharmacophore, coupled by an alkyl spacer; 2) Development of ligands with one common H₁/H₄-pharmacophore.



Based on the molecular modelling results, the most promising compounds were synthesized. Compounds **7**, **8** and **9** represent examples with regard to strategy 1, whereas **10** represents a general structure for strategy 2.



All compounds will be analyzed in GTPase and binding assays at hH₁R and hH₄R with regard to potency, efficacy and affinity.

REFERENCES

1. Venable et al. *J. Med. Chem.* **2005**, 48, 8289-8298.
2. Thurmond et al. *Nature Reviews Drug Discovery*, **2008**, 7, 41-53.
3. Deml et al. *Mol. Pharmacol.* **2009**, 76, 1019-1030.

POSTER COMMUNICATIONS – PC.320

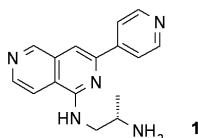
2,6-NAPHTHYRIDINES AS POTENT AND SELECTIVE INHIBITORS OF THE NOVEL PROTEIN KINASE C ISOTYPES

Maurice J. van Eis, Jean-Pierre Evenou, Philipp Floersheim, Christoph Gaul, Sandra W. Cowan-Jacob, Gabriele Rummel, Walter Schuler, Andre Strauss, Anette von Matt, Eric Vangrevelinghe, Juergen Wagner and Nicolas Soldermann

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Multiple members of the protein kinase C (PKC) family play a central role in the signalling cascades of the immune system, the key players being the classical PKC isotypes α/β and the novel PKC isotype θ .¹ In particular for PKC θ a crucial role in T cell receptor (TCR) – mediated T-cell activation and proliferation has been established.² The different PKC isotypes and in particular PKC θ , are therefore considered to be attractive therapeutic targets for the treatment of autoimmune diseases and the prevention of allograft rejection.³

Here we describe the discovery of a novel series of protein kinase C inhibitors with drug-like properties based on the 2,6-naphthyridine template. Example compounds potently inhibit the novel PKC isotypes δ , ϵ , η , θ and display 10-100 fold selectivity over the classical PKC isotypes α/β . Prototype compound **1** was found to be strongly immunosuppressive *in vitro* and *in vivo*. *In vitro*, anti-CD3 / anti-CD28-induced lymphocyte proliferation could be effectively blocked in 10 % rat whole blood. In mice, **1** dose-dependently inhibited *Staphylococcus aureus* enterotoxin B-triggered increase in IL-2 serum levels after oral dosing.



REFERENCES

1. Roffey, J.; Rosse, C.; Linch, M.; Hibbert, A.; McDonald, N. C.; Parker, P. J. *Curr. Opin. Cell Biol.* **2009**, *21*, 268.
2. (a) Kingeter, L. M.; Schaefer, B. C. *J. Immunol.* **2008**, *181*, 6244. (b) Sun, Z.; Arendt, C. W.; Ellmeier, W.; Schaeffer, E. M.; Sunshine, M. J.; Ghandi, L.; Annes, J.; Petrzilka D.; Kupfer, A.; Schwartzberg, P. L.; Littman, D. R. *Nature*, **2000**, *404*, 402.
3. (a) Baier, G.; Wagner, J. *Curr. Opin. Cell Biol.* **2009**, *21*, 262. (b) Lee, M. R.; Duan, W.; Tan, S. *Expert Opin. Ther. Targets*, **2008**, *12*, 535.

POSTER COMMUNICATIONS – PC.321

EVALUATION OF PAMPA TECHNIQUE FOR THE EARLY PREDICTION OF PASSIVE PERMEATION OF NANOCARRIERS THROUGH THE BLOOD-BRAIN BARRIER

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Blood-brain barrier (BBB) is a complex organization separating the brain from the systemic circulation and regulating cerebral homeostasis. It represents the main route followed by chemical entities to reach the central nervous system (CNS). This barrier is located at the endothelial cells of cerebral micro vessels which are sealed by tight-junctions, preventing paracellular diffusion of most compounds. Transcellular route is therefore the predominant pathway to reach the CNS either by passive diffusion or active transport. Actually, efflux pumps such as P-glycoproteins (P-gp) or multi-drug resistance associated proteins (MRP), expressed at the BBB, prevent xenobiotics to enter in cerebral parenchyma. The development of strategies to enable the permeation of low-permeant drugs and/or substrates of these efflux transporters is therefore of prime importance. In this field, nanocarriers loaded with drugs are a promising alternative to

influence the permeability of active compounds. Prediction of the ability of these loaded-nanoparticles to increase the permeation of low-permeant active compounds through BBB as early as possible in research and development thus remains an important challenge.

Parallel Artificial Membrane Permeability Assay (PAMPA) has been developed to fast predict passive permeation through biological membranes. This technique lies on a liquid artificial membrane separating a donor and an acceptor compartments. In this way, different biological barriers can be modelled depending on the nature of the liquid membrane immobilized [1] [2] [3]. BBB permeability has already been predicted with PAMPA using a phospholipidic membrane, allowing the ranking of the compounds into classes [2]. Recently we developed a new full-organic artificial membrane to predict passive cerebral penetration avoiding the well-known limitations of biological material [4]. In this study, permeability of nanoparticles loaded with a reference-set of compounds has been tested with PAMPA in order to evaluate the ability of the technique to provide information on passive diffusion of such complex combination.

REFERENCES

1. Wohnsland F.; Faller B. *J. Med. Chem.* 2001, *44*, 923-930
2. Di L.; Kerns E. H.; Fan K.; McConnell O. J.; Carter G. T. *Eur. J. Med. Chem.* 2003, *38*, 223-232
3. Ottaviani G.; Martel S.; Carrupt P. A. *J. Med. Chem.* 2006, *49*, 3948-3954
4. Passeleu Le Bourdonnec C., Boccard J., Rudaz S., Carrupt P.A., Martel S. In preparation, 2010.

POSTER COMMUNICATIONS – PC.322

RATIONAL DEVELOPMENT OF SELECTIVE ALLOSTERIC INHIBITORS OF BCR-ABL FOR TREATMENT OF PHILADELPHIA POSITIVE CML

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Philadelphia chromosome (Ph) is recognized as the cytogenetic hallmark of most chronic myelogenous leukemia (CML) and some of acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) patients. Philadelphia chromosome originated in

the reciprocal t(9;22) chromosomal translocation. The fusion of the breakpoint cluster region (Bcr) gene on chromosome 22 with the Abelson kinase (Abl) gene on chromosome 9 creates the Bcr-Abl oncoprotein. The constitutively active fused Bcr-Abl tyrosine kinase was recognized as the initial inducer of the leukemogenesis in blood cells. There is an increased interest in Academia and Industry to focus on developing selective Bcr-Abl inhibitors. Most of the Bcr-Abl inhibitors developed *ad hoc* (Imatinib, Nilotinib, and Dasatinib) are ATP competitors that bind either to the ATP-binding cleft or nearby. However, approximately one third of Imatinib-treated patients suffer cardiotoxicity or irresponsiveness due to resistance associated with Abl kinase domain mutations. To overcome those drawbacks, we aim at targeting leukemia cancer cells with novel molecular entities that bind selectively to alternative binding sites that are different than the ATP-cleft. The discovery that N-terminal myristated Abl is docked in a capping mechanism that inserts the myristate moiety into a hydrophobic "myristoyl binding pocket" (MBP) in an autoregulatory manner of Abl sparked the idea of developing myristate analogues as allosteric inhibitors of Bcr-Abl. Benefitted by advanced techniques of computational chemistry, sets of potential myristate analogues were examined and proposed for further examination. Those were synthesized, purified and characterized, and screened for their activity in a cell-based auto-phosphorylation and colonogenicity potential in a Bcr-Abl positive leukemia cells. Initial *in vitro* results demonstrate the usefulness of this approach, most probably due to the MPB being a binding site for allosteric inhibitors of Bcr-Abl. Additionally; some of the new derivatives are shown to override the "gatekeeper" mutation T315I. Further optimization studies are being performed.

REFERENCES

1. Quintás-Cardama A, Kantarjian H & Cortes J *Flying under the radar: the new wave of BCR-ABL inhibitors* Nature Reviews Drug Discovery October 2007 (6) 834-848.
2. Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD. *Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia*. Nature Reviews Cancer May 2007, 7(5) 345-56.

POSTER COMMUNICATIONS – PC.323

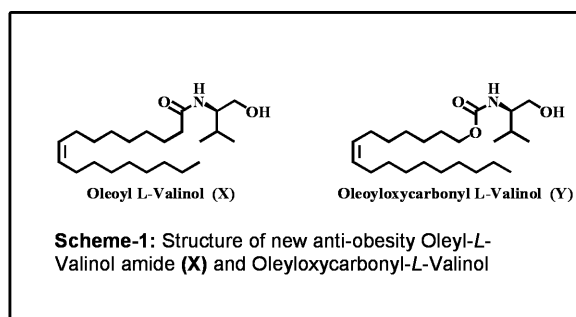
DEVELOPMENT OF NOVEL ANTI-OBESITY AGENTS THAT MODULATE COGNITIVE FUNCTIONS

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A series of novel compounds encompassing natural fatty acid and their corresponding amino and alcohols derivatives were conjugated with substituted amino ethanol. The compounds were purified and characterized. The fatty acid residue included saturated alkyl chains as well as *mono*-, *di*-, *tri*- and *tetra*- conjugated double bonds. Fatty acids and their corresponding amino and alcohols alkyl chain were in the ranges of 16 to 20 carbon atoms. The linkage to different substituted amino ethanol was implemented via -C(O)-NH-; -NHC(O)-; -O-C(O)-NH-; and -NH-C(O)-NH-linkers. The biological activity of these compounds was evaluated *in vivo*. Sabra mice were assigned at random to different groups of 10 mice. The mice were fed ad libitum, body weight and food consumption were monitored during the experiment. In the second week mice were evaluated for their cognitive function using the eight arm maze 15 min following compounds administration during 5 days.



Two leading compounds [Oleyl-L-Valinol amide (**X**) and Oleyloxycarbonyl-L-Valinol amide (**Y**; **Scheme-1**) showed significant inhibition of weight gain (about 10%) short time after administration of 1mg/kg ($p < 0.001$, 0.01 respectively). The effect of both compounds lasted for 15 days and was associated with decreased food consumption ($p < 0.05$) increased activity ($p < 0.001$ $p < 0.01$) and improvement in cognitive function ($p < 0.05$) with no change in neurological score. This was associated in the hypothalamus with decreased 2-Arachidonoyl Glycerol (2-AG) levels ($p < 0.05$) and increased NE and 5-HT levels ($p < 0.05$). Both compounds improved cognitive function which was related to increase in NE levels ($p < 0.05$) in the hippocampus. Compound-X enhanced POMC expression in the hypothalamus with no change in CB1, TRPV1 or 5HT1A expression. In contrast to oleylethanolamide (OEA), MDS revealed that (**X**) does not bind in higher affinity to either of CB-1, -2, FAAH, TRPV-1 nor PPAR α . Substantial evidence indicates that (**X**) exerts its anti-obese and cognitive effects through a distinct mechanism that is assumed to involve activating of pro-opiomelanocortin (POMC). MDS revealed that (**Y**) does not bind in higher affinity to either of CB-1, -2, TRPV-1 or PPAR α but exert its activity on FAAH. Compound-X and Y resembles the effect of moderate caloric restriction (to 60%) which is beneficial and they produce weight reduction by affecting both energy intake and expenditure.

The results presented in this study underline the importance of the region-isomers (*Z*- vs. *E*) of the lipophilic tail and the stereo-center (*L* vs. *D*) in the amino alcohol segment.

REFERENCES

1. Oveisi F, Gaetani S, Eng KT, Piomelli D. *Oleylethanolamide inhibits food intake in free-feeding rats after oral administration* Pharmacol Res. 2004 May; 49(5):461-6.
2. Lo Verme J, Gaetani S, Fu J, Oveisi F, Burton K, Piomelli D. *Regulation of food intake by oleylethanolamide*. Cell Mol Life Sci. 2005 Mar; 62(6):708-16.

POSTER COMMUNICATIONS – PC.324

NEW PIPERIDINE-BASED RENIN INHIBITORS

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The Renin-Angiotensin-Aldosterone System (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.

Starting from known diazabicyclononene¹ and tetrahydropyridine² derivatives, the development of a new class of piperidines as renin inhibitors is reported. Using substituents previously optimized to fill the renin S3 sub-pocket in a different series,³ highly potent piperidine based inhibitors were obtained. Further introduction of polar groups at the 4-position of the piperidine moiety allowed to discover new inhibitors with good pharmacological activities in a double transgenic rat model.

REFERENCES

1. Bezençon et al. *J. Med. Chem.* **2009**, 52, 3689.
2. Remeň et al. *Bioorg. Med. Chem. Letters*, **2009**, 19, 6762.
3. Chen et al. *Bioorg. Med. Chem. Lett*, manuscript submitted.

POSTER COMMUNICATIONS – PC.325

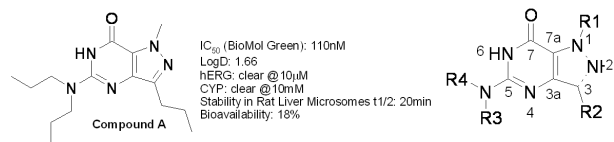
A NOVEL SERIES OF POTENT PDE1A INHIBITORS

Christel Menet, Benoit Schmitt, Nuria Merayo, Stephen Fletcher, Graeme Dykes, Nolwenn Jouannigot, Katja Conrath, Luc Nelles, Sonia Dupont, Sara Musch, An Van de Poel, Wendy Laenen, Marc De Raeymaeker

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Cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) are key second messenger molecules in cells, synthesized by guanylyl and adenylyl cyclases. By playing a role as 'relays' in signal transduction pathways, these molecules are key to controlling normal and pathological cell responses. Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that hydrolyze cyclic nucleotides and thereby control the cellular levels of these second messengers. To date, 21 mammalian PDE genes have been cloned and classified on the basis of sequence homology and biochemical properties into 11 families (PDE1-PDE11).¹⁻³ Because of their key role in cellular signaling, several PDE family members have been explored for therapeutic potential. Some data point to a role of PDEs in chondrocyte biology and it has been suggested that PDE4 is involved in cartilage catabolic events.⁴ The physiological role of the PDE1 family is less well understood, however, the focus has been primarily on PDE1B and PDE1C.⁵⁻⁶ Our research indicated that PDE1A played a role in chondrocyte biology and a program was initiated to identify selective PDE1A inhibitors to further explore this finding.

In this poster, we describe the design and synthesis of novel PDE1A ligands by a scaffold hopping approach. 5-Amino-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one was decorated with a variety of substituents. SAR studies based on the initial lead compound A (Scheme 1) were performed, which led to potent PDE1A inhibitors. Selectivity against a variety of PDE isoforms will be shown together with activity in a cellular cAMP assay. Finally, results obtained with compounds from the series showing protective effects in an *ex vivo* bovine explant model will be presented.



(Scheme 1)

REFERENCES

1. Soderling, S. H.; Beavo, J. A. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin. Cell Biol.* **2000**, 12, 174-179.
2. Conti, M.; Jin, S.-L. C. The molecular biology of cyclic nucleotide phosphodiesterases. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, 63, 1-38.
3. Mehats, C.; Andersen, C. B.; Filopanti, M.; Jin, S.-L. C.; Conti, M. Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends. Endocrinol. Metab.* **2002**, 13, 29-35.
4. Tenor H et al Phosphodiesterase isoenzyme families in human osteoarthritis chondrocytes-functional importance of Phosphodiesterase 4. *Br J Pharmacol.* (2002) 135:609-18
5. Goraya TA, Cooper DM. (2005) Ca²⁺ calmodulin-dependent phosphodiesterase (PDE1) : current perspectives. *Cell Signal.* 17: 789-97
6. Kakkar R, Raju RV, Sharma RK. (1999) Calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE1). *Cell Mol Life Sci.* 55: 1164-86

POSTER COMMUNICATIONS – PC.326

NOVEL SYMMETRICAL BIS-QUATERNARY CHOLINE KINASE INHIBITORS AS ANTICANCER AGENTS

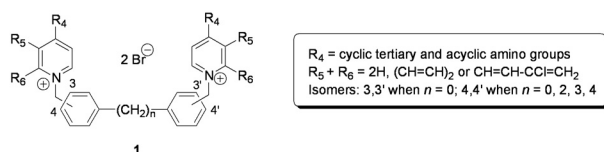
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Choline is an essential anabolic substrate for the synthesis of phospholipids. Choline kinase (ChoK) phosphorylates choline to phosphocholine (PCho) that serves as a precursor for the production of phosphatidylcholine, the major phospholipid constituent of membranes and substrate for the synthesis of lipid signaling molecules. Nuclear magnetic resonance (NMR)-based metabolomic studies of human tumors have identified a marked increase in the intracellular concentration of PCho relative to normal tissues.¹

ChoK is overexpressed in human breast, lung, colorectal, and prostate tumors, a finding that suggests the involvement of this enzyme in carcinogenesis.

With the aim of obtaining new structure-activity relationships and the study in depth of the structural parameters that define the ChoK inhibitory and antiproliferative activities, the synthesis of a new set of compounds (**1**) is proposed (Figure). These compounds are tested in an *ex vivo* system using human ChoK as a target, and the antiproliferative activity is analysed on HT-29 cultured cells by measuring the number of cells after 6 days of incubation with the compound, relative to control, non-treated cells.²



Figure

ChoK inhibitors provide evidence that the blockade of PCho is a valid strategy for the development of new anticancer agents, opening a new avenue for the development of antitumour drugs with a novel mechanism of action. The screening of new derivatives may improve currently available compounds and widen their therapeutic margin to facilitate their entry into clinical studies.

REFERENCES

1. A. Yalcin; B. Clem; S. Makoni; A. Clem; K. Nelson; J. Thornburg; D. Siow; A.N. Lane; S.E. Brock; U. Goswami; J.W. Eaton; S. Telang; J. Chesney. Selective inhibition of choline kinase simultaneously attenuates MAPK and PI3K/AKT signaling. *Oncogene*, **2010**, 29, 139-149.

2. M.C. Núñez, A. Conejo-García, R.M. Sánchez-Martín, M.A. Gallo, A. Espinosa, J.M. Campos. QSAR as a Tool for the Development of Potent Antiproliferative Agents by Inhibition of Choline Kinase. *Current Computer-Aided Drug Design*, **2007**, 3, 297-312.

POSTER COMMUNICATIONS – PC.327

ALZHEIMER'S DISEASE: IDENTIFICATION OF BACE1 INHIBITORS USING FRAGMENT BASED DYNAMIC LIGATION SCREENING (DLS)

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Recently, dynamic ligation screening (DLS) has been introduced as a fragment-based method for drug discovery [1]. The approach combines target-assisted formation of the inhibitory species and detection of reversibly ligated fragments in a functional assay through their competition with a fluorogenic enzyme substrate [2] or via a binding assay employing fluorescence polarization. The approach has been applied to several cysteine protease targets and has demonstrated to provide non peptide inhibitors through iteratively reversed DLS starting using from peptides [3].

Herein, the extension of DLS to aspartic proteases is described. For this purpose, β -secretase (BACE1) (aspartyl protease) was selected as a protein target to develop, optimize and validate this approach. BACE1 plays a key role in Alzheimer's disease (AD) because it cleaves amyloid precursor protein to produce the amyloid β_{1-42} peptide fragments, principal components of senile plaques [4]. The poor ability of the existing BACE1 inhibitors to cross the blood-brain-barrier has made the development of specific BACE1 inhibitors one of the most active areas in drug discovery for AD [5,6].

First, a fluorogenic substrate and directing probe were designed and optimized and their kinetic and inhibitory parameters (V_{max} , K_M , k_{cat}/K_M , IC_{50} , respectively) were determined. On the other hand, assay conditions like buffer, enzyme, concentrations of substrate, enzyme and directing probe or incubation time were optimized. The developed method was adapted to high throughput screening (HTS) format using 384 microtiter plates and diverse amine libraries assembled by employing a maximum common substructure (MCS) concept were screened. Apparent K_i values of the dynamic ligation products were determined. Fragment hits were validated by synthesis and testing of the corresponding irreversible ligation products. One fragment has been identified as BACE1 inhibitor, being a good starting point towards for iteration with the aim to develop higher affinity inhibitors.

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REFERENCES

- Schmidt, M. F.; Rademann, J. *Trends Biotechnol* **2009**, 27 (9), 512-521.
- Schmidt, M. F.; El-Dahshan, A.; Keller, S.; Rademann, *Angew. Chem. Int. Ed.* **2009**, 48, 6346-6349.
- Schmidt, M. F.; Isidro-Lobet, A.; Lisurek, M.; El-Dahshan, A.; Tan, J.; Higenfeld, R.; Rademann, J. *Angew Chem Int Ed Engl.* **2008**, 47 (17), 3275-3278.
- Willem, M.; Lammisch, S.; Haass, C. *Semi. Cell. Dev. Biol.* **2009**, 20 (2), 175-182.
- Silvestri, R. *Med. Res. Rev.* **2009**, 29 (2), 295-333.
- Huang, W. H.; Sheng, R.; Hu, Y. Z. *Curr. Med. Chem.* **2009**, 16 (14), 1806-1820.

POSTER COMMUNICATIONS – PC.328

CHOLESTANE DERIVATIVES AS ANTITUMOR AND ANTIANGIOGENIC DRUGS

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Cholestane derivatives and their possible use for prevention, inhibition and treating of proliferative and angiogenic disease in mammals will be described. The growth of new blood vessels in animals is essential for organism growth¹ as well as for growth of solid tumors and metastasis². Endothelial cells are the main player for angiogenesis³ and could be a target for an antiangiogenic therapy because they are nontransformed and are easily accessible to treating with achievable doses of antiangiogenic agents, and are also unlikely to acquire drug resistance⁴. The vascularization plays a crucial role in cell nutrition and oxygen distribution. Tumor angiogenesis targeting in the sense of discovery of novel potentially drugs includes inhibition of proteolytic enzymes that break down the extracellular matrix surrounding existing capillaries, inhibition of endothelial cell proliferation, migration and enhancement of tumor endothelial cell apoptosis. Potent angiogenic inhibitors capable of blocking tumour growth appear to have potential for development of novel generations of anticancer drugs.

Antiangiogenic activity has been described in some steroids, e.g., medroxyprogesteron, however with only small clinical activity; 2-methoxiestradiol has been found to inhibit proliferation, migration and invasion of endothelial cells *in vitro* as well as having antiangiogenic effects in certain *in vivo* tumor models; another

examples is squalamin etc. However, small attention has been devoted in this respect to cholestane derivatives. We have found that they are suitable for inhibition of different tumor cell lines in micromolar concentrations with minimal effects on normal cells. We shall present that treatment with cholestane derivatives induces cytotoxicity and growth inhibition of breast and prostate carcinoma cells and further that they are capable to inhibit angiogenesis in tumors and therefore that they can be used as antimetastatic and apoptotic drugs, hence as anticancer drugs.

ACKNOWLEDGMENT

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REFERENCES

- e.g. Folkman et al., 1974, *Adv Cancer Res.*, 19(0):331-358; Folkman et al., 1992, *J Biol Chem.*, 267(16):10931-10934; Carmeliet, 2005, *Nature*, 438 (7070):932-936.
- e.g. Bhat and Singh, 2008, *Food Chem Toxicol*, 46:1334-1345; Kesisis et al., 2007, *Curr Pharm Des.*, 13(27):2795-2809; Risau, 1997, *Nature*, 386(6626):671-674.
- Bagley et al., 2003, *Cancer Res.*, 63(18):5866-5873).
- Bhat and Singh, 2008, *Food Chem Toxicol*, 46:1334-1345.

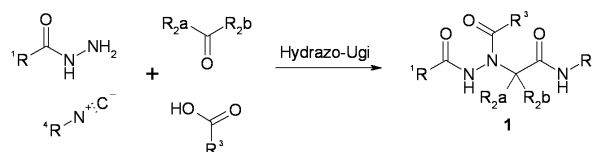
POSTER COMMUNICATIONS – PC.329

NOVEL MOTIFS FOR PEPTIDOMIMETIC DESIGN EMERGING FROM HYDRAZO-UGI REACTION

Mikhail Krasavin,* Ekaterina Lakontseva and Vladislav Parchinsky

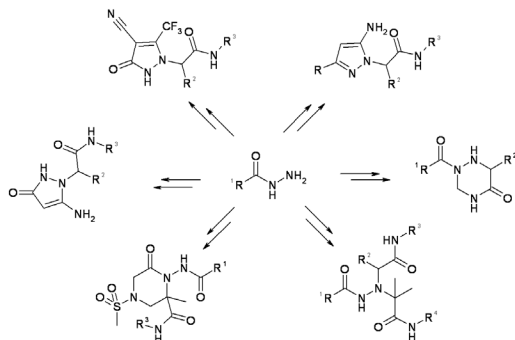
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Isocyanide-based multi-component reactions have served as a rich source of novel peptidomimetics. We have recently revisited a relatively underexplored variant of the Ugi reaction in which the classical amine component is replaced with hydrazine (more specifically, N-acylhydrazine) termed hydrazo-Ugi reaction.



While the resulting hydrazinopeptide-like structures **1** themselves are useful motifs for designing pseudopeptide replacements for bioactive peptides and introducing secondary structure bias known as hydrazino turn, broader applications of the hydrazo-Ugi reaction in elaborating more complex peptidomimetics and heterocycles still remain to be explored.

We have actively investigated the avenues for using the hydrazo-Ugi reaction in accessing new peptidomimetic chemical space and have identified several reliable directions in that respect, as briefly outlined below.



These chemistries will be presented in further detail along with some associated profiles on peptidergic biological targets.

POSTER COMMUNICATIONS – PC.330

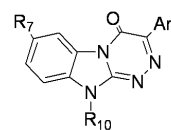
NEUROPROTECTIVE EFFECT OF NOVEL SELECTIVE A_{2A} ADENOSINE RECEPTOR ANTAGONISTS: 3-ARYL-[1,2,4]TRIAZINO[4,3-*a*]BENZIMIDAZOL-4(10*H*)-ONES (ATBIS)

Pugliesi I.,¹ Taliani S.,¹ Salerno S.,¹ Simorini F.,¹ La Motta C.,¹ Marini A.M.,¹ Da Settimo F.,¹ Cosconati S.,² Marinelli L.,² Cosimelli B.,² Greco G.,² Novellino E.,² Scatena A.,³ Fornai F.,³ Paparelli A.,³ Trincavelli M.L.,⁴ Martini C.⁴

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Adenosine is a physiological nucleoside which plays an important role in many patho-physiological conditions in the central nervous system (CNS), as well as in peripheral organs and tissues through modulation of specific membrane G protein-coupled receptors (ARs), currently classified into A_1 , A_{2A} , A_{2B} , and A_3 subtypes.^[1]

A_{2A} ARs occur mainly on neurons in the striatum and on cholinergic interneurons, but they are also found in the nucleus accumbens, olfactory tubercle, hippocampus and cerebral cortex. Recent evidences showed that A_{2A} ARs may modulate a number of cellular processes affecting neuronal cell death in a broad spectrum of brain injury. A_{2A} ARs resulted up-regulated in noxious brain conditions and their blockade confers robust brain neuroprotection. As this effect is exerted without observable peripheral effects, there is an increasing interest in the development of selective A_{2A} AR antagonists as novel protective agents in neurodegenerative diseases.^[2]



ATBI I

In recent years, we have described a series of 3-aryl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-ones (ATBIs) **I** as potent adenosine receptors antagonists.^[3,4] In this study, using **I** as lead compound, we have synthesized a novel series of ligands featuring modified substituents at the 10 position, combined with an appropriate aromatic ring at the 3-position. Synthesis, biological evaluation and structure-activity relationships for these new derivatives ATBI **I** will be presented.

Finally, the most promising A_{2A} AR ATBI antagonist was evaluated for its neuroprotective effect on PC12 cells treated with dopamine neurotoxins: MPP⁺ or methamphetamine. We found a neuroprotective effect consisting of full prevention of neurotoxin-induced cell loss, as measured by combining a variety of staining techniques. These latter results, which were confirmed by combining neurotoxic models, each owing a different mechanism of action, offer a promising perspective for such a compound as a valuable tool to protect dopamine neurons.

REFERENCES

1. Fredholm et al, *Pharmacological Reviews* 2001, 53 (4), 527–552.
2. Stone, T. W.; Ceruti, S.; Abbracchio, M. P. *Handb. Exp. Pharmacol.* 2009, 193, 535–587.
3. F. Da Settimo, et al. *J. Med. Chem.* 2001, 44, 316–327.
4. F. Da Settimo, et al. *DDR* 2004, 63, 1–7.

POSTER COMMUNICATIONS – PC.331

DEVELOPMENT OF NEW DERIVATIVES OF AMINOGLYCOSIDES WITH ENHANCED SUPPRESSION OF DISEASES-CAUSING NONSENSE MUTATIONS

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Many human genetic diseases, such as cystic fibrosis (CF), Usher syndrome (US), Duchenne muscular dystrophy (DMD), Hurler syndrome (HS) and several types of cancer result from nonsense mutations that cause premature termination of the protein synthesis. For many of those diseases there is presently no effective treatment. Recent studies have shown that some aminoglycoside antibiotics have the ability to impose the mammalian ribosome to read past a false-stop signal and generate full-length functional proteins. However, high toxicity of those drugs in

humans limits their therapeutic use. To date, no efforts have been made to optimize aminoglycosides activity as stop codon readthrough inducers.

The main objective of this research is to develop novel aminoglycoside variants that will have efficient termination suppression activity, and at the same time will have reduced toxicity against mammalian cells.

Towards these ends, series of new derivatives of paromomycin were designed, synthesized and their ability to readthrough stop codon mutations was examined in both *in vitro* and *ex vivo* mammalian systems. Our most recent discovery was the pseudotrisaccharide **3** (Figure 1), which showed several folds greater suppression activity and far lower toxicity than those of gentamicin and paromomycin [1]. This finding placed compound **3** on the top among all the natural and/or synthetic aminoglycosides reported to date for the potential use in translational therapy. In order to further improve readthrough efficacy of **3** here we will discuss on the design, synthesis and evaluation of a series of new derivatives of aminoglycosides that differ from the previous leads by the presence of a chiral C6'-methyl group on the glucosamine ring. Our results indicate that addition of both the (*R*)-C6'-methyl and the AHB group at N-1 position significantly improve the readthrough efficiency. The observed structure-activity relationship in new compounds are discussed on the basis of their binding affinity to human rRNA A-site, inhibition of protein translation in prokaryotic and eukaryotic systems, and their *in vitro* and *ex vivo* ability to readthrough premature stop codon contexts derived from a series of different genes that underline CF, US, DMD and HS genetic diseases [2].

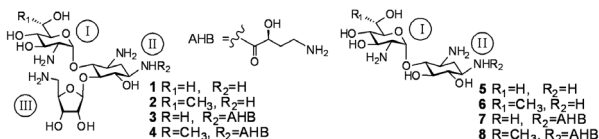


Figure 1. Novel aminoglycoside readthrough inducers.

REFERENCES

1. I.Nudelman, A.Rebibo-Sabah, M.Cherniavsky, V.Belakhov, M.Hainrichson, F.Chen, J.Schacht, D.S.Pilch, T.Ben-Yosef, and T.Baasov. – *J. Med. Chem.*, 52, No. 9, 2836-2845 (2009).
2. I.Nudelman, D.Glikin, B.Smolkin, M.Hainrichson, V.Belakhov, and T.Baasov. – *Bioorg. Med. Chem.*, submitted, 2010.

POSTER COMMUNICATIONS – PC.332

MOLECULAR IMAGING AGENTS FOR EGFR BASED ON THE 4-ANILINOQUINAZOLINE SCAFFOLD

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Patients presenting subclasses of cancers that overexpress EGFR or HER2, two members of the ErbB family of growth factor receptors, have poorer prognoses, with higher risks of disease recurrence and metastatic events. Despite FDA approval of small molecule EGFR/HER2 inhibitors, the widespread use of these therapeutics has been hampered by an inability to identify subpopulations sensitive to anti-EGFR/HER2 therapy. This project addresses this issue through the design and synthesis of PET and SPECT imaging agents specific to these subclasses of cancer, by ligating the characteristic 4-anilinoquinazoline inhibitor scaffold to a linker bearing an appropriate radioisotope. The modular synthetic approach employed produces a more readily optimized series of molecular probes, while strategic placement of the imaging modality in solvent-accessible space minimizes the negative effect of its presence on the binding of the radiotracer in the active sites of the receptors.

Supported by DOE Award ER647823 Susan B Komen Foundation grant BCTR65906.

POSTER COMMUNICATIONS – PC.333

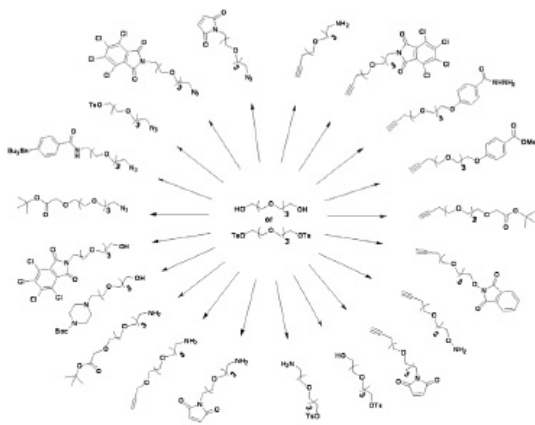
NANOMEDICINAL CHEMISTRY: SYNTHESIS OF HETEROBIFUNCTIONAL LINKERS FOR POLYVALENT MULTIFUNCTIONAL NANOPARTICLES

Robert N. Hanson, Kinh Luan Dao, J. Adam Hendricks, Marshall Brennan, Anton Kozhushnyan, Victoria Ronga, Helen Trinh Pham and Teresa Dunstan

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As part of our program in developing polyvalent multifunctional nanoparticles it was necessary to prepare new linkers for selective ligation of appropriate terminal groups. In this study we describe our approaches for preparing specific heterobifunctional oligoethylene glycol (OEG) linkers. These linkers serve as modular units that allow us to introduce small molecule targeting groups, imaging moieties, chemotherapeutic drugs or combinations thereof onto a nanoparticulate surface by using chemo-orthogonal ligation chemistry. We will describe several

examples of modular functionalized nanoparticle assembly using this approach. Preliminary data suggest that our versatile assortment of heterobifunctional linkers has the potential for developing multiply targeted therapeutic agents and disease selective drug delivery systems.



Supported by DoD PRCP and DOE ER64823

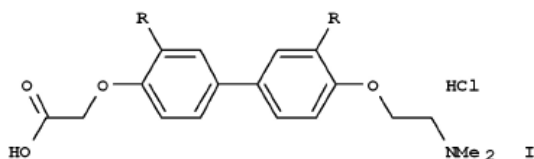
POSTER COMMUNICATIONS – PC.334

SUBSTITUTED 4,4'-BIS(OXY)BIPHENYL PROTEOMIMETICS AS INHIBITORS OF ESTROGEN RECEPTOR-ALPHA CO-ACTIVATOR BINDING

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Significant interest has focused on the development of small molecules that can inhibit the binding of coactivator proteins to nuclear receptors as a means of modulating hormonal function. Proteomimetics, small molecules that mimic peptidyl structures offer one approach to this problem. We have designed and prepared a small series of novel 4,4'-bis(oxy)biphenyl derivatives using a modular, convergent strategy. The compounds offer the potential for exploring chemical diversity within the biphenyl scaffold as well as at the termini. Synthetic methods and preliminary results will be discussed.



Supported by DoD PCRP.

POSTER COMMUNICATIONS – PC.335

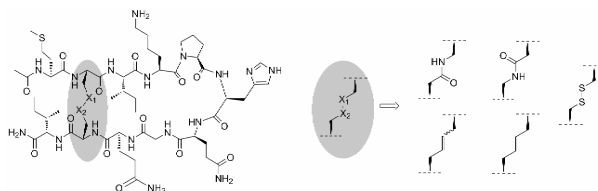
A NEW APPROACH TOWARDS INHIBITION OF ANGIOGENESIS: CYCLIC PEPTIDE ANALOGUES OF VEGF₈₁₋₉₁ FRAGMENT

M. Isabel García-Aranda,¹ Yasmina Mirassou,² Benoit Gautier,³ Nicolas Inguibert,³ Michel Vidal,³ Mercedes Martín Martínez,¹ M. Ángeles Jiménez,² M. Teresa García-López,¹ M. Jesús Pérez de Vega,¹ M. Rosario González-Muñiz.¹

¹ Instituto de Química Médica-CSIC, Juan de la Cierva, 3, 28006 Madrid; ² Instituto de Química Física Rocasolano-CSIC, Serrano 119, 28006 Madrid and ³ L. de pharmacochimie moleculaire et cellulaire, INSERM U 648, 45 rue de Saints Péres 75270 Paris.

The inhibition of the interaction between VEGF and its receptors constitutes a valuable approach in the search of new antiangiogenic agents, due to the importance of this interaction in the triggering of the angiogenesis process. Taking into account the key role of VEGF₈₁₋₉₁ fragment for the VEGF interaction with its receptors,^{1,2} and aiming to preserve its β -hairpin conformation, new cyclic peptide derivatives were designed restricting the conformation in order to fix the β -hairpin structure. Compounds with bridges of different nature were prepared by introduction of the adequate modifications in two positions of the sequence, trying to preserve the residues described as key for the interaction. The influence of these modifications, not only in the structure but also in the biological activity, was studied. Additionally, considering that the β -turn of the native VEGF₈₁₋₉₁ fragment is slightly distorted, due to the presence of an extra amino acid residue, two series of decapeptide analogues were also prepared, just by removing the residue Glu⁸⁷ or Gly⁸⁸. On the other hand, incorporation of an α -MePro, which is known to stabilize β -turn conformations, instead of the Pro⁸⁵ residue of the native fragment was also performed. This will avoid *cis/trans* rotamery and would help in the stabilization of the hairpin.

In this communication we will comment on the synthesis, NMR conformational analysis, and binding affinities for the Flt-1 receptor of the proposed VEGF₈₁₋₉₁ peptide derivatives.



REFERENCES

- Keyt, et al. J. Biol. Chem. 1996, 271, 5638-5646.
- Muller, et al. Proc. Natl. Acad. Sci.. 1997, 94, 7192-7197.

POSTER COMMUNICATIONS – PC.336

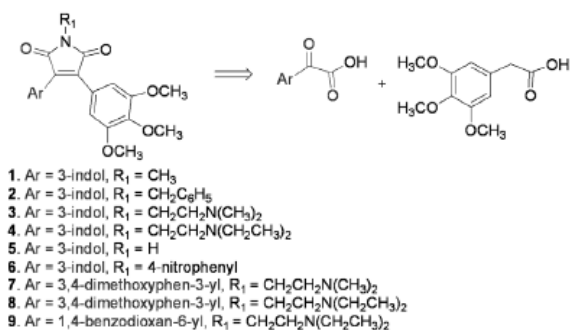
DESIGN, SYNTHESIS AND ANTICANCER PROPERTIES OF NEW CDKS INHIBITORS

Giovanni Casula,¹ Ruben Francisco,¹ Salvatore Plescia,² Maria Grazia Cusimano,² M. Dolors Pujol^{1*}

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Hartwell, L. H., Hunt, R. T. and Nurse, P. M. won the 2001 Nobel Prize in Medicine for their description of cyclin-dependent kinase mechanisms and its role in the regulation of the cell cycle. CDKs are considered a potential target for anti-cancer treatment, these kinases phosphorylated the retinoblastoma (Rb) tumor suppressor protein.¹ The phosphorylated Rb causes the release of members of the transcription factors, E2F proteins. It is noteworthy that inhibition of CDK4 results in hyperphosphorylation of Rb and restricts the passage only to G₁ phase, whereas CDK2 regulates G₁ and also S phases. Malumbres and col. have reported that knockdown of CDK4 in mammary tumor cells prevents tumor formation.² These results suggest that selective inhibition of CDK4 could be a more valuable mechanism to cancer treatment than that of CDK2.3

An efficient approach to the synthesis of unsymmetrical 3,4-diarylmaleimides has been developed including a Perkin condensation between the arylacetic acid and the corresponding arylketoacid followed by reaction with aminoalkylamines (Scheme 1).



Scheme 1

In this report we describe several maleimides designed to explore the pharmacophore and their effect on cytotoxic activity and CDK selectivity. These compounds were found to be interesting such as antitumor possessing good antiproliferative activity against human carcinoma cell lines. Several of these compounds showed inhibition of pRb phosphorylation and arrest tumor cells at the G₁ phase. Structure–activity relationships were also investigated.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Minister of Science and Technology (CTQ2007-60614/BQU) for the financial support.

REFERENCES

1. Sandhu, C.; Slingerland, J. *Cancer Detect. Prev.* **2000**, *24*, 107-112.
2. Ortega, S.; Malumbres, M.; Barbacid, M. *Biochim Biophys. Acta. Rev. Cancer* **2002**, *1602*, 73-87.
3. Malumbres, M.; Barbacid, M. *Cancer Cell* **2006**, *9*, 2-8.

POSTER COMMUNICATIONS – PC.337

PREPARATION OF NEW SELFOTEL STRUCTURAL ANALOGUES TO TREAT TINNITUS

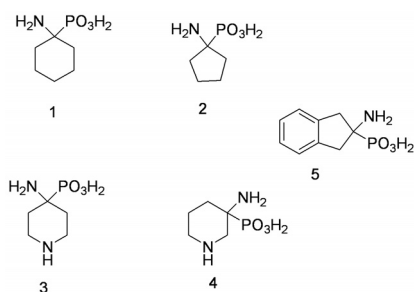
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Nowadays, tinnitus - an ailment of the auditory system - starts to be the public health problem. It afflicts about 10 % of Western Europe society [1]. Although some alleviating agents exist, there is no convincing treatment available. Lately, it has been found that tinnitus is connected with activity of N-methyl-D-aspartic acid and glutamate receptors [2].

Organophosphorus compounds are effective pharmacological drugs. Furthermore, it is known that they have also biological activity as NMDA and glutamate antagonists [3].

Herein, we present cyclic aminophosphonates. Their synthesis is based on Oleksyszyn-Soroka reaction [4]. These compounds (Pic. 1) can be potential NMDA and glutamate antagonists.



Pic. 1

REFERENCES

1. Coles R.R.A., *J. Laryngol. Otol.*, **1984**, *9*, 195-202.
2. Ruel J., Chabbert C., Nouvian R., Bendris R., Eybalin M., Leger C.L., Bourien J., Mersel M., Puel J.L., *J. Neurosci.*, **2008**, *28*, 7313-7323.

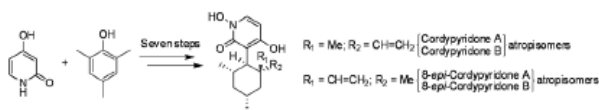
- Lejczak B., Kafarski P., *Top Heterocycl Chem*, **2009**, 20, 31-63, Springer Berlin-Heidelberg.
- Ziora Z., Kafarski P., *Phosphorus, Sulfur and Silicon*, **2009**, 184, 1047-1053.

POSTER COMMUNICATIONS – PC.338

THE TOTAL SYNTHESIS OF (±)-CORDYPYRIDONE AND RELATED COMPOUNDS AS ANTIMALARIAL AND ANTIBACTERIAL AGENTS

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The family of natural products containing the *N*-hydroxypyridone motif are of significant interest for total synthesis due to their compact structure, pronounced biological activities and possible modes of action. Cordypyridone A and B were selected as targets due to their antimalarial activity against *Plasmodium falciparum*,¹ antibacterial activity against *Staphylococcus aureus*² and the ability of cordypyridone A to induce erythropoietin gene expression in human cells.³ This work details the total synthesis of cordypyridone A and B⁴ and demonstrates the generality of the methodology as applied to the total synthesis of an additional natural product, pyridoxatin, and other *N*-hydroxypyridone analogues as antimalarial and antibacterial agents.

REFERENCES

- Isaka M. *et al. J. Org. Chem.* 2001, 66, 4803.
- Clardy J. *et al. Org. Lett.* 2002, 4, 671.
- Houck D. J. *Nat. Prod.* 1999, 62, 397.
- Jones, I. L.; Moore, F. K.; Chai, C. L. L. *Org. Lett.* **2009**, 11, 5526.

POSTER COMMUNICATIONS – PC.340

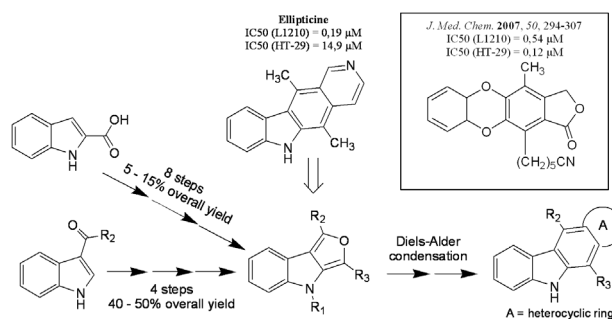
DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF NEW TETRACYCLIC COMPOUNDS RELATED TO ELLIPTICINE

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Ellipticine is a natural product isolated from *Ochrosia elliptica* with significant cytotoxic activity by several mechanisms of action. Its tetracyclic structure exhibits antineoplastic activity against several human cancer cell lines. The initial isolation of ellipticine was closely followed by its first synthesis. Since then, many other syntheses have been reported by Gribble and col. and other authors and its structure has been widely modified in order to obtain more efficacious, selective and less toxic new compounds.

Our group had previously reported a series of ellipticine analogues including 1,4-denzodioxin nucleus. Recently, we designed a new series of condensed indoles with anticancer activity. Herein, we present two new alternative pathways to prepare the intermediate isobenzofuroindole starting from either indole-2-carboxylic acid (8 steps) or 3-indolylalkanones (4 steps) (see scheme below).



Some interesting steps (involving protection of indole, directed *ortho*-metallation and cyclization) had been studied and developed in our laboratory. These methods lead us to the intermediate diene, which reacts with the corresponding dienophiles to obtain the desired compounds. Other attempts starting from indole-3-carboxylic acid or 3-indolylacetone nitrile were unsuccessful. The total synthesis and the biological activity of several compounds will be reported.

ACKNOWLEDGMENTS

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REFERENCES

- G. Gribble, M. Sautnier, M. Sibi, J. Obaza-Nutaitis et al. *J. Org. Chem.* **1984**, 49, 4518-4523.
- C. Bozzo, N. Mur, P. Constants, M. D. Pujol. *Eur. J. Org. Chem.* **2009**, 13, 2174-78.
- M. Romero, P. Renard, D. Caignard, G. Atassi, J. Solans, M. D. Pujol. *J. Med. Chem.* **2007**, 50, 294-307.

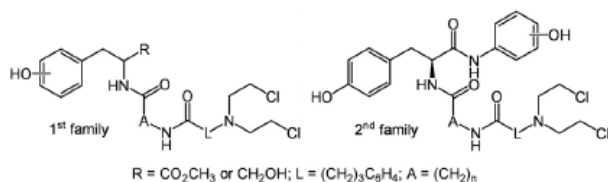
POSTER COMMUNICATIONS – PC.341

DEVELOPMENT OF TYROSINE-CHLORAMBUCIL HYBRIDS SHOWING ACTIVITY AGAINST BREAST AND OVARIAN CANCERS

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In our search for anticancer agents with improved selectivity towards breast and ovarian cancers,¹⁻³ we have synthesized two families of amino acid-linked nitrogen mustard hybrids. The new hybrids are designed to mimic 17 β -estradiol, the female sex hormone, in order to bind to the estrogen receptor. The first family is composed of tyrosine (*ortho*-, *meta*- or *para*-) methyl ester or tyrosinol moiety linked to chlorambucil. For the second family, *para*-tyrosine was transformed into several propionamide derivatives. Afterwards, chlorambucil was linked to the propionamides either directly or via a tether chain leading to various tyrosine-nitrogen mustard hybrids. The phenol group of the tyrosine moiety is used as a potential bioisosteric function of the 17 β -estradiol phenol group. The hybrids were made using two distinct synthetic methodologies which will be described and compared in terms of their efficiency. MTT assays on several breast and ovarian cancer cell lines show that the novel compounds generally possess higher biological activities when compared to chlorambucil. They are up to 9 times more powerful than the parent drug. The cytotoxic activity of these tyrosine-chlorambucil hybrids could advantageously be used to provide compounds with anticancer activity against hormone-dependent female cancers. Molecular modeling shows the possible interactions of the hybrids with the estrogen receptor alpha.



REFERENCES

1. A. Gupta, P. Saha, C. Descôteaux, V. Leblanc, É. Asselin and G. Bérubé. Design, synthesis and biological evaluation of estradiol-chlorambucil hybrids as anticancer agents. *Bioorganic & Medicinal Chemistry Letters*, **20**, 1614-1618 (2010).
2. C. Van Themsche, S. Parent, V. Leblanc, C. Descôteaux, A.-M. Simard, G. Bérubé and É. Asselin. VP-128, a novel estradiol-platinum(II) hybrid with selective antitumour activity towards hormone-dependent breast cancer cells *in vivo*. *Endocrine-Related Cancer*, **16** (4), 1185-1195 (2009).
3. C. Descôteaux, V. Leblanc, G. Bélanger, S. Parent, É. Asselin and G. Bérubé. Improved Synthesis of Unique Estradiol-Linked Platinum(II) Complexes Showing Potent Cytocidal Activity and Affinity for the Estrogen Receptor Alpha and Beta. *Steroids*, **73**, 1077-1089 (2008).

POSTER COMMUNICATIONS – PC.342

ECOSCALE, A METRIC TOOL TO ASSESS THE SCALABILITY OF A REACTION EARLY IN DRUG DEVELOPMENT

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In the pharmaceutical industry, it is important to advance promising drug candidates as soon as possible through the pipeline of drug discovery. One way to achieve this goal is to find out as early as possible when the originally developed medicinal protocol is no longer adequate (mainly for cost and safety issues) and a new protocol needs to be developed for larger quantities. This underlines the importance in early phase drug discovery of the use of synthetic protocols that are easily scalable.

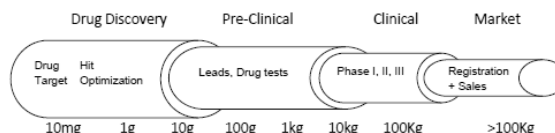


Figure 1: A reaction with a high score on the EcoScale is likely suitable for multi kg synthesis.

To facilitate the selection of adequate protocols, a new transparent and user-friendly metric system, the EcoScale,^{1,2} was developed to assess the scale-up potential of a laboratory scale reaction. For every reaction protocol this EcoScale can be calculated based on 6 basic reaction parameters:

- Yield
- Price of the reaction components
- Safety of the reagents
- Technical Setup
- Temperature and time of the reaction
- Workup and Purification.

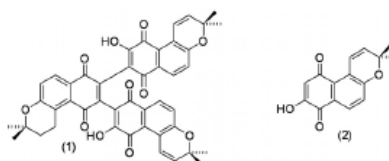
The EcoScale is designed by first defining an ideal reaction which has a maximum value of 100 and then subtracting penalty points of a relative weight for 6 important parameters. The result is a number on a scale from 0 to 100. A value higher than 75 is a good protocol, a value between 75 and 50 is acceptable and below 50 is described as a poor protocol. The flexibility of the system is such that the penalty points can be adapted to the preferences and needs of the chemist or organization.

The power of the EcoScale is that it can be used in a time efficient way in early stage drug development to select reaction pro-

protocols that are more suitable for scale up which finally results in a gain of time and money.

REFERENCES

1. EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters. Koen Van Aken, Lucjan Strekowski, Luc Patiny Beilstein Journal of Organic Chemistry **2006**, 2 No. 3.
2. The EcoScale as a Post-reaction Analysis Tool to Select Reaction Conditions for Scale-up Koen Van Aken, Lucjan Strekowski, Luc Patiny Handbook of Green Chemistry, Oxford University Press, **2010** (in press).
3. For on-line calculation of the EcoScale see <http://www.ecoscale.org>



REFERENCES

1. Jonathon A V Coates, E D Jones, S Cox, Ian T Crosby, David G Bourke, Tyrone P Jeynes. PCT Int. Appl. **2005** WO2005095376
2. Ian T Crosby, Mark L Rose, Maree P Collis, Paula J de Bruyn, Philip L C Keep, Alan D Robertson, Aust J Chem **2008**, 61, 768-784.

POSTER COMMUNICATIONS – PC.343

DISCOVERY OF A NOVEL NON-NUCLEOSIDE INHIBITOR OF HEPATITIS B VIRUS (HBV)

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We will present the discovery and development of a non-nucleoside inhibitor of Hepatitis B Virus.¹

Hepatitis B virus (HBV) is one of the most prevalent viral infections of humans, it attacks the liver and can cause both acute and chronic disease. The WHO estimated 350 million chronic carriers and that about 2 billion people have been infected. An estimated 600,000 person die each year of HBV associated illnesses. HBV causes a potentially life-threatening liver infection and is a major global health problem and the most serious type of hepatitis. Current therapies include; the biological agent Interferon, and the nucleoside analogues Lamivudine, Adefovir dipivoxil, Ribavirin. There is no small molecule non nucleoside inhibitor of HBV.

During our work on conocuvone derived naphthoquinone trimers for the treatment of HIV,² we discovered a potent inhibitor (1) of Hepatitis B Virus in an antiviral selectivity screen. During attempts to resynthesise (1) for proof of concept studies, we altered the synthesis in order reduced side reactions and difficult to remove by-products. As a result we discovered the small molecule (2) that also was a potent inhibitor of HBV. The development of (2) as an agent to treat HBV infections will be presented.

POSTER COMMUNICATIONS – PC.344

NOVEL CHEMICAL APPROACH TO GENERATE DIVERSE DEPSIPEPTIDES DERIVATED FROM THE NATURAL PRODUCT 'A54556 COMPLEX' FOR ANTIBIOTIC SCREENING

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Over the past decades, more and more depsipeptides including acylated depsipeptides were separated and identified from terrestrial microorganisms and marine organisms. Generally, naturally occurring acylated depsipeptides contain unusual amino acids and building blocks. Most of them will offer a new frontier in both synthetic organic chemistry and biological activities. In particular, unique unusual structures will offer the challenge of exploitation of novel synthetic methods, reactions, reagents, catalysts, etc. However, practically, it is rather difficult to isolate a larger amount of naturally occurring acylated depsipeptides because of minute constituents in organisms, difficulty of collection of organisms, and resistance of laboratory culturing. Thus, the limits of the quantity preclude the precise structure determination as well as clarification of detailed biological activities. In fact, the proposed structures of many cyclic peptides have been revised by synthetic works. Thus, the total synthesis is still playing a final means for the structure determination of naturally occurring acylated depsipeptides just like several decades ago. Furthermore, the efficient large-scale production of naturally occurring acylated depsipeptides by synthesis will offer an opportunity to investigate their biological activities in detail. One of the characteristic features of cyclic peptides will be their conformational rigidity and stability in vivo, in contrast to their linear counterparts. In addition, unusual amino acid and non-amino acid moieties of naturally occurring acylated depsipeptides will offer the lead structures of new biologically useful compounds. On the basis of our previous work on total solid-phase synthesis of cyclic peptides, we establish and develop a totally novel chemical approach to synthesize diverse depsipeptides derivat-

ed from the natural product 'A54556 complex' by on-resin head-to-tail cyclization. This powerful and universal total solid-phase synthesis strategy will be applicable to the parallel preparation or one-bead one-peptide (OBOP) library of naturally occurring acylated depsipeptides and the artificial mimic derivatives for systematic research into the structure-activity relationship (SAR) of potent lead antibiotic compounds.

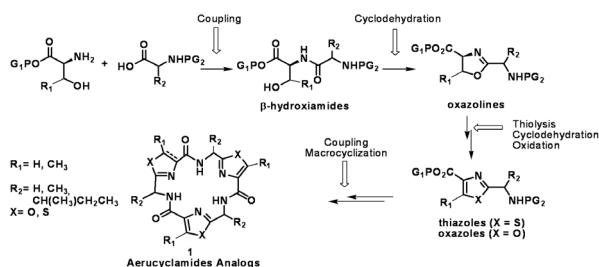
Key words: total solid-phase synthesis; acylated depsipeptides; A54556 complex; lead antibiotic compound; high through-put screening

ACKNOWLEDGMENT

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REFERENCES

1. Michel, K.H. & Kastner, R.E. **US patent** 4492650 (1985).
2. Berthold Hinzen, Siegfried Raddatz, Holger Paulsen, et al. **ChemMedChem** 2006, 1, 689–693.
3. Heike Brotz-Oesterhelt, Dieter Beyer, Hein-Peter Kroll, et al. **Nat. Med.** 2005, 11(10): 1082-1087.



Scheme 1

ACKNOWLEDGMENTS

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REFERENCES

1. a) Portmann, C.; Blom, J. F.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2008**, 71, 1193. b) Portmann, C., Blom, J. F., Kaiser, M., Brun, R., Jüttner, F. Gademann, K. *J. Nat. Prod.* **2008**, 71, 1891.
2. a) Mahler, G.; Serra, G.; Dematteis, S.; Saldaña, J.; Domínguez, L.; Manta, E. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1309. b) Sellanes, D.; Scarone, L.; Mahler, G.; Manta, E.; Baz, A.; Dematteis, S.; Saldaña, J.; Domínguez, L.; Serra, G. *Drug Design Discovery* **2006**, 3, 625. c) Sellanes, D.; Manta, E.; Serra, G. *Tetrahedron Letters*, **2007**, 48, 1827. d) Scarone, L.; Fajardo, J.; Saldaña, J.; Domínguez, L.; Espósito, P.; Dematteis, S.; Wipf, P.; Manta, E.; Serra, G. *Letters in Drug Design and Discovery*, **2009**, 6, 413.

POSTER COMMUNICATIONS – PC.346

APPROACH TO THE SYNTHESIS OF AEURUCYCLAMIDES ANALOGS AS POTENTIAL ANTIPARASITIC DRUGS

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Aerucyclamides A, B, C and D were isolated from the cyanobacteria *Microcystis aeruginosa* PCC 7806. This compound class encompasses hexacyclopeptides alternating in hydrophobic and hydrophilic (Ser, Thr, and Cys) amino acids. The side chains of these polar amino acids are heterocyclized to form oxazole or thiazole rings or their reduced derivatives.

Aerucyclamide B, shows submicromolar (0.7 μM) activity against *Plasmodium falciparum* and Aerucyclamide C is the most active of them against *T. brucei rhodesiense*.¹

As part of our search for candidates for antiparasitic new drugs,² we embarked in the synthesis of Aerucyclamides analogs. In the present work, we present a convergent strategy that involves the coupling between hydrophobic and hydrophilic amino acids, thiolysis, cyclodehydration/ oxidation processes and coupling between different heterocycles fragments. (Scheme 1)

POSTER COMMUNICATIONS – PC.347

DISCOVERY OF NOVEL TIRAZOLONE MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITORS DERIVED FROM MITRATAPIDE USING SOLID- AND SOLUTION-PARALLEL SYNTHESIS

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Microsomal Triglyceride Transfer Protein (MTP) is a lipid transfer protein that is involved in the assembly of very low density lipoproteins (VLDL) in the liver and chylomicrons in the gut wall. Genetic loss of functional MTP is the basis of the human recessive disorder called abetalipoproteinemia. This disease results from an inability of both the liver and intestine to secrete respectively VLDL and chylomicrons leading to extremely low plasma lipid levels and malabsorption of dietary fat. In recent studies it has been shown that MTP inhibitors lower blood lipids with potential use in hyperlipidemia, obesity and atherosclerosis. Previously, we identified R103757 as a novel inhibitor of MTP ($\text{IC}_{50} = 6 \text{ nM}$) and demonstrated that this compound inhibits ApoB secretion of the human hepatoma cell line HepG2 ($\text{IC}_{50} = 26 \text{ nM}$). After oral dosing up to

POSTER COMMUNICATIONS

POSTER COMMUNICATIONS – PC.003

ANTITUBERCULAR CONSTITUENTS FROM AMPALAYA (*MOMORDICA CHARANTIA* LINN.)

Bella G. Panlilio^{1,2}, *Scott Franzblau*³, *Wen, Zhang Qing*⁴,
*Richomme, Pascual*⁵, *Gehle, Dietmar*⁶, *Kouam, Simeon*⁶,
*Krohn, Karsten*⁶ and *Alicia M. Aguinaldo*⁷.

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An earlier study on the crude alcoholic extract of the leaves of *Momordica charantia* Linn. showed inhibition of the growth of *Mycobacterium tuberculosis* H₃₇Rv at 100 µg.ml⁻¹ (94%) and at 1000 µg.ml⁻¹ (100%). To determine if *M. charantia* would exhibit a wide range of inhibitory activities against *M. tuberculosis* H₃₇Rv with respect to plant parts, location, age, and variety, the crude alcoholic extracts were tested using the Microplate Alamar Blue Assay. The results showed that the various parts of *M. charantia* have different inhibitory activities against *M. tuberculosis* H₃₇Rv with leaves generally exhibiting the highest inhibitory activity, ranging from 48% to 100% at 100 µg.ml⁻¹.

M. charantia species from selected locations in Luzon likewise exhibited different inhibitory activities against *M. tuberculosis* H₃₇Rv with species from Bicol showing the highest percent inhibition, 100%, for the leaves, fruits and stems. The antitubercular activity of the leaves of the cultivated variety was higher (99%) compared to that of the wild variety (66%). On the other hand, the percent inhibition of the fruits of wild *M. charantia* (90%), was higher than the cultivated variety (66%). The difference between the antitubercular activity of the young (98%) and mature (99%) leaves of cultivated *M. charantia* is minimal.

Two compounds were isolated from the cultivated leaves of *M. charantia* and their structures were elucidated through spectroscopic studies. One of the compounds, is a new triterpene aldehyde (3ß,7ß,25-trihydroxycucurbita-5,23(Z)-dien-30-al) with an

antiubercular activity against *Mtb* H₃₇Rv 35 % inhibition (128 µg.ml⁻¹) and 29% inhibition (64 µg.ml⁻¹) (Figure 1); and the other one is a known phenolic compound, 2,4-bis(dimethyl benzyl) phenol, with 100 % inhibition against *Mtb* H₃₇Rv (100 µg.ml⁻¹) (Figure 2). A number of compounds were isolated and identified from the bioactive sub fractions using GC-MS analysis.

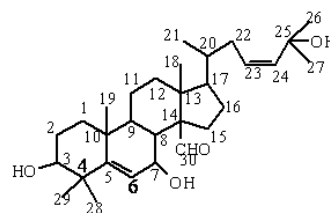


Figure 1

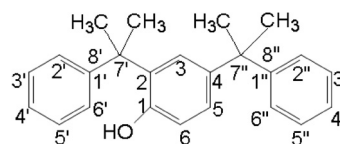


Figure 2

POSTER COMMUNICATIONS – PC.005

SYNTHESIS AND PRECLINICAL PROFILING OF NOVEL HYDROXAMIC ACIDS AS HISTONE DEACETYLASE INHIBITORS

*Stefania Gagliardi*¹, *Andrea Colombo*¹, *Florian Thaler*^{2,3}, *Antonello Mai*⁴, *Raffaella Amici*^{2,3}, *Chiara Bigogno*¹, *Roberto Boggio*⁵, *Anna Cappa*^{2,5}, *Simone Carrara*¹, *Tiziana Cataudella*⁵, *Fulvia Fusar*⁵, *Eleonora Gianti*^{2,3}, *Maurizio Moroni*¹, *Davide Munari*⁵, *Gilles Pain*^{3,8}, *Nickolas Regalia*¹, *Luca Sartori*^{2,5}, *Stefania Vultaggio*⁵, *Giulio Dondio*¹, *Saverio Minucci*^{6,7}, *Ciro Mercurio*⁵, *Mario Varasi*^{2,5}

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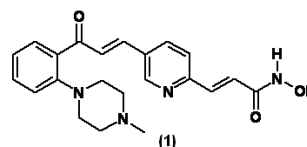
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The histone deacetylases (HDACs) are able to regulate gene expression and histone deacetylase inhibitors (HDACi) emerged as a new class of agents in the treatment of cancer as well as other human disorders such as neurodegenerative diseases.¹ SAHA (Suberoylanilide hydroxamic acid), one of the early HDAC inhibitor discovered by Breslow and colleagues,² was approved by FDA in October 2006 for the treatment of patients with cutaneous T-cell Lymphoma (CTCL).³ Dozens of other inhibitors with different structures are now in various stages of clinical trial.

In the present investigation, we describe the synthesis, the biological evaluation and preclinical profiling of compounds derived from the expansion of N-hydroxy-arylacrylamide scaffold:

- N-Hydroxy-3-phenyl-2-propenamide and N-hydroxy-3-(pyridin-2-yl)-2-propenamide as core structures containing a phenylloxopropenyl moiety, either unsubstituted or substituted by a 4-methylpiperazin-1-yl or 4-methylpiperazin-1-ylmethyl group;
- N-Hydroxyacrylamide-propenylamido derivatives.

The compounds were evaluated for their ability to inhibit nuclear extract HDACs as well as for their *in vitro* antiproliferative activity. Moreover, their metabolic stability in human and mouse microsomes and solubility was studied. Selected compounds were further characterized by *in vivo* pharmacokinetic experiments. The compounds showed a remarkable stability *in vivo*, also compared to hydroxamic acid HDAC inhibitors already entered in clinical trials. One representative compound (**1**) was submitted to an *in vivo* efficacy experiment and showed a significant antitumor activity in a human colon carcinoma xenograft model.



REFERENCES

1. Sleiman, S.F.; Basso, M.; Manishi, L.; Kozikowski, A.P.; Donhoe, M.E.; Langley, B.; Ratan, R.R. *Expert Opin Investig Drug* **2009**, *18*, 573-574
2. Breslow, R.; Marks, P. A.; Jursic, B.. WO 93/07148, April 15, 1993
3. (a) Grant, S.; Easley, C.; Kirkpatrick, P. *Nature Rev. Drug Discovery* **2007**, *6*, 21-22; (b) Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T. *Cancer Research* **2000**, *60*, 5165-5170

POSTER COMMUNICATIONS – PC.006

DISCOVERY OF NOVEL ORALLY ACTIVE MGLUR5 NEGATIVE ALLOSTERIC MODULATOR GSK2210875

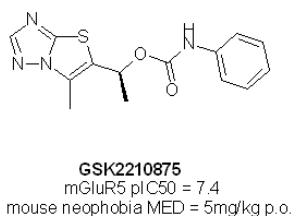
Steve P Watson, Olivier Lorthioir, Pier D'Alessandro, Michela Tessari, Maria Pilla, Michela Andreoli, Sonia Delle-Fratte, Adelheid Roth, Fiona McKay

GlaxoSmithKline, Neurosciences Centre of Excellence for Drug Discovery, New Frontiers Science Park, Harlow (UK), and Medicine Research Centre, Verona (Italy)

This lecture will report the discovery of the novel orally active mGluR5 negative allosteric modulator / antagonist GSK2210875.

This novel MPEP competitive chemotype was identified by high throughput screening and demonstrates intriguing and complex structure activity relationships. Optimisation of receptor potency, oral exposure, and brain free fraction allowed identification of compounds which could achieve receptor occupancy of >90% required for activity in *in vivo* models. A focus on polar compounds (logD <2.5) of modest potency, but high free brain exposure, led to the identification of GSK2210875 which demonstrates an oral minimum effective dose (MED) of 5mg/kg in the mouse neophobia model.

The relationships between this chemotype, with its unique SAR, and other known mGluR5 negative modulators will be outlined.



POSTER COMMUNICATIONS – PC.007

STRUCTURE BASED DISCOVERY AND RATIONAL DESIGN OF FIRST-IN-CLASS INHIBITORS OF LEDGF/p75-INTEGRASE INTERACTION

Voet AR, Christ F, Marchand A, Nicolet S, Strelkov S, Debyser Z and De Maeyer M.*

AIDS, caused by the HIV virus is a worldwide spread disease. Until today there is no cure, however with a combination of drugs to halt the replication of the virus and curing opportunistic infections, infected people can be treated. This is the so called highly active anti retroviral therapy (HAART). Due to the easily occurring mutations into the HIV genome, resistance readily arises against the currently used antiretroviral agents. This resistance calls for the development of new compounds, and more importantly, new antiretroviral strategies.

A new potential strategy is the inhibition of the integration process. During this step of the replication cycle, the viral genome is integrated into the human genome. This complex step is carried out by the viral integrase enzyme during several consecutive steps, each one of these steps being a potential pharmaceutical target. LEDGF/p75 was discovered as a cellular cofactor of the integrase enzyme^[1] and following validation, appeared to be essential for the viral replication cycle. Thus inhibition of the integrase-LEDGF/p75 interaction was proven to be a new and interesting strategy to threat HIV infected people.^[2-3]

Historically, this type of interactions (protein-protein interactions), were considered as challenging but recent successes have proven the feasibility. Therefore we initiated the search for integrase-LEDGF/p75 interaction inhibitors using computer-aided drug design methods.

An exploratory study on published small molecule protein-protein interaction inhibitors gained knowledge directly applicable in our research. Analysis of the chemical space showed that usage of 3 filter descriptors is enough to discard uninteresting molecules (molecules without the capacity to inhibit protein-protein interactions).

In a second part of the computational research we showed that small molecules, which competitively inhibit protein-protein interactions, mimic partially the protein-protein interface. Thus a pharmacophore can be constructed from the protein-protein interface for the virtual discovery of an inhibitor of this protein-protein interaction.

These insights were applied to the HIV-1 integrase - LEDGF/p75 interaction. In an initial virtual screening setup the molecules were filtered according to the protein-protein interaction inhibition capacity according to the descriptors. In the next step the molecules were selected based on a pharmacophore model which was constructed out of all available integrase X-ray structures, of which the integrase CCD - LEDGF/p75 IBD structure is the most important.^[4]

Further selection was based on the results of molecular docking simulations. The inhibitory activity of the first molecules was experimentally tested in an ALPHA-screen assay. The molecules which showed some activity were optimized, leading to the discovery of novel compounds with *in vivo* inhibitory activity. Using X-ray crystallography and biochemical assays, the binding mode and mechanism of action of the compounds was validated. Furthermore the crystal structures were used for structure based optimization of new compounds and the discovery of new classes of inhibitory compounds. Currently the most potent inhibitors show an *in vivo* IC₅₀ activity of 55nM.

We can conclude that during this collaborative research (KULeuven Laboratories for Biomolecular Modelling, for Molecular Virology and Gene therapy, for Biocrystallization and the Centre for Drug Design and Discovery (CD3)) a new antiretroviral compound class was discovered and optimized using computational and rational drug design strategies. In this study the rational modeling approach was a leading and essential part of the discovery phase.

These compounds are promising for future pharmaceutical optimizations to be used in the clinic as new antiretroviral agents.^[5]

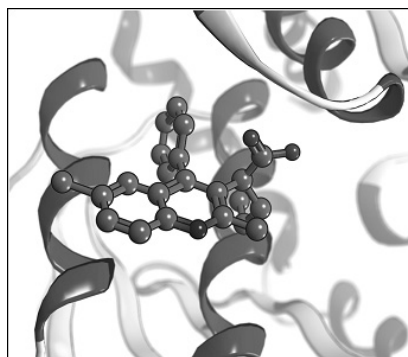


Figure: Crystallography was used to validate the binding mode of discovered inhibitors. Insights in the interactions of the ligand-protein complex allowed for rational design of optimized inhibitors.

REFERENCES

- Cherepanov P, *et al.* HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem.* 2003;278(1):372-81.
- Vandekerckhove L, *et al.* Transient and stable knockdown of the integrase cofactor LEDGF/p75 reveals its role in the replication cycle of human immunodeficiency virus. *J Virol.* 2006 Feb;80(4):1886-96.
- De Rijck J, *et al.* Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. *J Virol.* 2006 Dec;80(23):11498-509

- Cherepanov P, *et al.* Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proc Natl Acad Sci U S A.* 2005 Nov 29;102(48):17308-13
- Christ F, Voet AR, Marchand A, *et al.* Design of first-in-class inhibitors of LEDGF/p75-integrase interaction and HIV replication. *Nature Chemical Biology (accepted for publication)*

POSTER COMMUNICATIONS – PC.008

NEW N-[X-(Y-METHOXY-6,11-DIHYDROINDOLO[1,2-B]ISOQUINOLIN-11-YL)ALKYL]ALKANAMIDES: SYNTHESIS AND MELATONINERGIC POTENCY

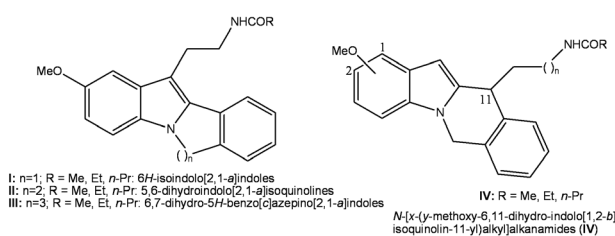
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Melatonin (*N*-acetyl 5-methoxytryptamine), a hormone synthesized and released from the pineal gland at night, has been shown to have a hypnotic action in animals and humans and there has been considerable recent interest in the therapeutic potential of melatonin analogues as hypnotics and as agents for restoring circadian rhythms disturbed by jet-lag, shiftwork and aging.¹

The physiological actions of melatonin in regulating seasonal and circadian rhythms are thought to be mediated through a family of specific, high affinity, G-protein-coupled cell membrane receptors. Two receptor subtypes (MT1 and MT2) have been cloned in mammals² which, when expressed in host cells, show the general pharmacological characteristics of native melatonin receptors.

Notwithstanding the renewed interest in melatonin and its actions in recent years, the pharmacology of melatonin is in its infancy. In a series of studies we have sought to understand how melatonin binds to and activates its receptor and to use the knowledge gained to design subtype specific receptor agonists and antagonists, which will be useful tools for defining the full physiological and pathophysiological role of this hormone. In 2000 we published on the synthesis and biology of compounds **I-III** (*vide infra*), and found that increasing the carbon chain length between *N*-1 of indole and the 2-phenyl group from *n* = 1 through *n* = 3 leads to a fairly regular decrease in the binding affinity, but, remarkably, when *n* = 3, it converts these compounds from melatonin agonists to antagonists.³



As an extension of this work, we synthesised analogues **IV**, in which the side chain of **I-III** was translocated from C3 (indole numbering) to C11 and the 2-phenyl ring moved one carbon atom further away. The evaluation of the biological action of the new analogues is currently underway.

ACKNOWLEDGMENT

Professor Tsotinis is indebted to the Pharmaceutical Company SERVIER-France for financial support (Programme: 70/3/9764).

REFERENCES

- Delagrangé, P.; Guardiola-Lemaitre, B. *Clin. Neuropharmacol.* **1997**, 20, 482-5102.
- Reppert, S.M.; Godson, C.; Mahle, C.D.; Weaver, D.R.; Slaugenhaupt, S.A.; Gusella, J.F. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, 92, 8734-8738.
- Faust, R.; Garratt, P.J.; Jones, R.; Yeh, L.-K.; Tsotinis, A.; Panoussopoulou, M.; Calogeropoulou, T.; Teh, M.-T.; Sugden, D. *J. Med. Chem.*, **2000**, 43, 1050-1061.

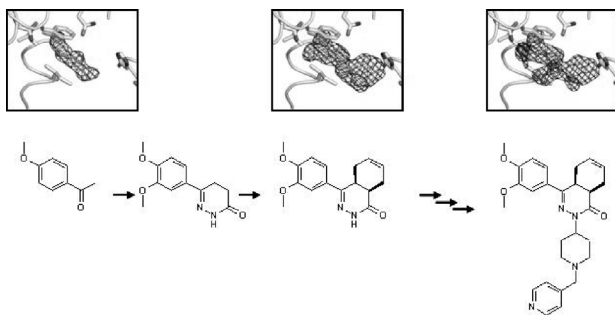
POSTER COMMUNICATIONS – PC.009

FRAGMENT-BASED DESIGN OF PDE4 INHIBITORS

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Fragment-Based Lead Generation has matured over the last couple of years into a viable methodology to generate tractable lead structures against a variety of target enzymes and receptors. Here we report on a structure-guided medicinal chemistry campaign towards PDE4 inhibitors, employing a fragment-based lead generation approach with a strong emphasis laid on a number of methodological aspects. We considered phosphodiesterase 4 (PDE4) as an ideal enzyme since it emerged as an attractive target for therapeutic intervention in inflammatory and immune disorders, such as asthma, rheumatoid arthritis, or COPD. In addition, highly active PDE4 inhibitors have been described. From a methodological point of view, we were mainly interested in probing the limits towards smallest fragments that can be identified by an in-house developed site-specific binding assay and can be successfully crystallized in complex with the target enzyme. For that purpose, smallest possible fragments based on a retro-engineering of the pyridazinone scaffold were designed and submitted to a fragment-screening and fragment crystallization campaign. Differently substituted acetophenones were identified as smallest fragments that bound site-specifically to the adenine-recognition site of PDE4. Unexpectedly, first qualitative structure-activity relationships emerged already at this initial process step.



Fragment Evolution Strategy from initial seed fragments (left) to complex lead compounds (right). Inhibitors are shown in the binding site with their electron densities as determined by protein crystallography (top).

Para-methoxy-acetophenone derivatives were successively evolved over several iterative cycles of successive design and synthesis towards a fully elaborated lead structure exhibiting sub-nanomolar PDE4 inhibitory activity. Each round of optimization was supported by structural biology, alongside with biophysical, and biochemical investigations. For optimal efficiency a synthetic route was designed that could easily be adapted for the preparation of a large variety of analogs. Special emphasis was laid on developing a detailed structural understanding of the enthalpic and entropic aspects that contribute to the total free energy of binding of the seed fragments, as well as intermediates taken from the fragment evolution strategy. We consider an in-depth analysis of thermodynamic and kinetic binding attributes as key for an efficient decision making process at the outset of and within any lead finding and optimization campaign.

POSTER COMMUNICATIONS – PC.010

NEW FLUORINE CONTAINING AMINO ACIDS AS LABELS TO STUDY ANTIMICROBIAL PEPTIDES BY SOLID STATE ^{19}F NMR

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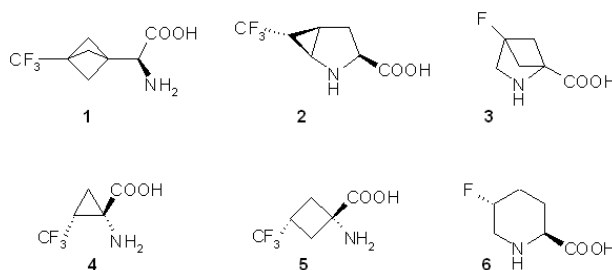
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Solid state ^{19}F -NMR is a powerful method to study membrane-active peptides, as it can reveal their conformation, orientation and dynamics when embedded in a lipid bilayer.¹ For this purpose the peptide of interest has to be selectively labeled with a suitable ^{19}F -containing amino acid at several different positions. The resulting battery of singly ^{19}F -labeled analogues then has to

be tested for full functional integrity, before they are reconstituted into lipid bilayer samples for the solid state ^{19}F -NMR analysis.

The main limitation of this approach, however, lies in the poor repertoire of suitable ^{19}F -labels, as only very few of the known ^{19}F -containing amino acid can serve as proper ^{19}F -labels. Therefore, we have rationally designed and synthesized compounds **1** and **2**, to serve as proper ^{19}F -labels for the substitution of Leu, Ile, Val, Ala and Pro, respectively, in peptides.²⁻⁶ These novel amino acids have been successfully used to study the structure and dynamic behaviour of the antimicrobial peptides GS, PGLa and cell penetrating peptide SAP in model membranes by ^{19}F -NMR.

Also, the new potential ^{19}F -labels **3-6** have been synthesized.⁷⁻⁹ Validation of their utility in peptide studies is in progress.



REFERENCES

1. A. S. Ulrich, **Prog. NMR Spectr.** 46 (2005) 1-21.
2. P. K. Mykhailiuk, S. Afonin, A. N. Chernega, E. B. Rusanov, M. Platonov, G. Dubinina, M. Berditsch, A. S. Ulrich, I. V. Komarov, **Angew. Chem.** 118 (2006) 5787-5789; **Angew. Chem. Int. Ed.** 45 (2006) 5659-5661.
3. P. K. Mykhailiuk, N. M. Voievoda, S. Afonin, A. S. Ulrich, I. V. Komarov, **J. Fluor. Chem.** 131 (2010) 217-220.
4. S. Afonin, P. K. Mykhailiuk, I. V. Komarov, A. S. Ulrich, **J. Pept. Sci.** 13 (2007) 614-623.
5. P. K. Mykhailiuk, S. Afonin, G. V. Palamarchuk, O. V. Shishkin, A. S. Ulrich, I. V. Komarov, **Angew. Chem.** 47 (2008) 5849-5851; **Angew. Chem. Int. Ed.** 47 (2008) 5765-5767.
6. P. K. Mykhailiuk, S. Afonin, A. S. Ulrich, I. V. Komarov, **Synthesis** 11 (2008) 1757-1760.
7. A. N. Tkachenko, D. S. Radchenko, P. K. Mykhailiuk, O. O. Grygorenko, I. V. Komarov, **Org. Lett.** 11 (2009) 5674-5676.
8. O. S. Artamonov, P. K. Mykhailiuk, D. M. Volochnyuk, I. V. Komarov, **Synthesis** 42 (2010) 443-446.
9. D. Radchenko, P. K. Mykhailiuk, A. Bezudnyy, I. Komarov, **Synlett** 11 (2009) 1827-1829.

POSTER COMMUNICATIONS – PC.011

THE IMPORTANCE OF DRUG-TARGET BINDING KINETICS FOR DRUG EFFICACY - WITHOUT ON YOU ARE OFF!

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Binding strength, affinity (K_D) can be broken down into kinetic constants that reflect drug-target recognition (the association rate constant, k_{on}) and the stability of the resulting complex (dissociation rate constant, k_{off}). The relationship between these properties can be described as $K_D = k_{off}/k_{on}$. The off-rate has been recognized for some time as a key parameter for understanding pharmacokinetic and dynamic (PKPD) properties of drugs¹⁻³. A slow off-rate can prolong the therapeutic effect both by tight binding and by "hiding" the drug from enzymatic degradation and clearance by keeping it out of the circulation. High off-rate selectivity can also minimize side effects caused by off-target binding.

The importance of on-rate is less recognized, however, and even (erroneously) thought to be constant (diffusion rate limited) for small molecules. In fact, the combination of available drug concentration and the on-rate is the driving force to get the drug into the binding site of the target protein. If the on-rate is too low in relation to its bioavailability, the drug will never bind to the target, and affinity/dissociation rate is then a false indication of the potency of the drug.

In a lead series, k_{on} and k_{off} can vary by several orders of magnitude even when compounds have similar affinities. It is very important, therefore, to measure and understand the binding kinetic pattern of lead series and the effect this might have on the PK/PD properties of the scaffolds. Association rate is concentration driven ($M^{-1}s^{-1}$) and can therefore be compensated by good ADME properties that give a high concentration of available drug. When the bioavailability is low, compounds with rapid on-rates might still do the job. A rapid on-rate is also preferred when a rapid effect is desired. If the on-rate is low in relation to free concentration, then an increase in affinity driven by improved complex stability (even by several orders of magnitude) does not increase the number of blocked target binding sites. If the limiting factor is relatively rapid clearance, then a drug with a slow off-rate might be preferred therapeutically. Dissociation rate is concentration independent (s^{-1}) and can only be influenced by structural changes to the lead compound. Structure-activity-relationship (SAR) studies^{4,5} can be improved by plotting the structures of the lead series in an $k_{on}/k_{off}/K_D$ -map.

In this lecture, I will discuss the effects of different on/off-rate combinations in relation to the bioavailable concentration of leads and how this influences binding to the target protein and the residence time of the drug in the binding site. Examples will be given to highlight the importance of on-rate – Without "on" there is no "off"!

REFERENCES

1. Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov.* 2006;5(9):730-9
2. Gabrielsson J, Hugues D, Gillberg, P-G, Bredberg U, Benthem B, Duker G. Early integration of pharmacokinetic and dynamic reasoning is essential for optimal development of lead compounds: strategic considerations. *Drug Discovery Today*, 2009;14(7/8):58-372.
3. Andersson K, Löfås S, Karlsson R, Hämmäläinen MD. Label-free kinetics binding data as a decisive element in drug discovery. *Expert opinion on drug discovery* 2006;1(5):439-446.
4. Markgren P-O, Schaal W, Hämmäläinen MD, Karlén A, Hallberg A, Samuelsson B, Danielson UH. Relationships between structure and interaction kinetics for HIV-1 protease inhibitors, *J Med Chem.* 2002;45:5430-9.
5. Huber W. A new strategy for improved secondary screening and lead optimization using high-resolution SPR characterization of compound-target interactions. *J Mol Recognit.* 2005;8(4):273-81.

POSTER COMMUNICATIONS – PC.013

SELECTIVE INHIBITORS OF MMP-12, A TARGET IN INFLAMMATION : FROM CHEMISTRY TO THERAPEUTIC APPLICATIONS

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Following the disappointment of the clinical trials of early broad-spectrum synthetic inhibitors of matrix metalloproteinase (MMPs), the field is now resurgent focusing on the development of inhibitors able to fully discriminate between the different members of the MMP family. MMP-12 has been involved in the development of several human pathologies like atherosclerosis, aneurysm and COPD, observations justifying the development of selective MMP-12 inhibitors.

Extremely potent inhibitors of MMP-12 have been developed by the screening of phosphinic peptide libraries. In order to understand the molecular determinants responsible for the observed inhibitor selectivity, X-ray, isothermal titration microcalorimetry, NMR and MMP-12 mutant studies have been performed. Altogether, these new data open new vistas for developing selective inhibitors towards other MMPs. The *in vivo* activity of one MMP-12 selective inhibitor has been evaluated in mouse model of atherosclerosis. Inhibitor treatment was observed to affect both plaque initiation and progression at multiple sites, thus MMP-12 specific inhibition may prove useful for the treatment of progressive atherosclerosis in human.

POSTER COMMUNICATIONS – PC.014

POTENT, SELECTIVE AND PLASMA-STABLE INHIBITORS OF THE PSD-95/NMDA RECEPTOR INTERACTION

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The protein-protein interaction between the *N*-methyl-D-aspartate (NMDA) receptor and the intracellular scaffolding protein postsynaptic density protein-95 (PSD-95) is a potential target for treatment of ischemic brain diseases. Extensive activation of the NMDA receptor by glutamate, as seen in excitotoxicity, plays a key role in several brain diseases. However, development of drugs that directly modulate the NMDA receptor has been difficult.¹ Instead, inhibition of the ternary complex of the NMDA receptor, neuronal nitric oxide synthase (nNOS), and PSD-95 attenuates glutamate induced cell death by impairing the functional link between Ca²⁺ entry and NO production without affecting vital synaptic transmission. Thus, PSD-95 constitutes a promising target for neuroprotective drugs. Uncoupling of PSD-95 and the NMDA receptor subunit, GluN2B, has previously been achieved by a 20-mer peptide (Tat-N2B), which mimics the C-terminal of GluN2B and thereby inhibits the nNOS-NMDA receptor linkage by binding to two protein domains of PSD-95 – the PDZ1 and PDZ2 domains. This peptide is currently in clinical trials as a potential treatment of stroke.²

We have identified two novel types of compounds as potent inhibitors of the PSD-95/NMDA receptor interaction: *N*-alkylated tetrapeptides³ and dimeric ligands⁴, respectively. In the first approach a compound was identified that is 20-fold more potent towards PSD-95 than the clinical candidate Tat-N2B.¹ In the other approach, two peptide ligands have been dimerized whereby extremely potent compounds were generated with affinities as low as 10 nM – approximately 1000-fold more potent than the peptide in clinical development. The two series were optimized for human blood plasma-stability and is currently undergoing investigations focusing on membrane permeability and biological activity.

REFERENCES

1. F. Gardoni, M. Di Luca, *Eur. J. Pharmacol.* 2006, 545, 2.
2. M. Aarts, Y. Liu, L. Liu, S. Besshoh, M. Arundine, J.W. Gurd, Y. T. Wang, M.W. Salter, M. Tymianski, *Science* 2002, 298, 846.
3. A. Bach, C. N. Chi, T. B. Olsen, S.W. Pedersen, M. U. Røder, G. F. Pang, R. P. Clausen, P. Jemth, K. Strømgaard, *J. Med. Chem.* 2008, 51, 6450.

4. A. Bach, C. N. Chi, G. F. Pang, L. Olsen, A. S. Kristensen, P. Jemth, and K. Strømgaard, *Angew. Chem. Int. Ed.* 2009, 48, 9685.

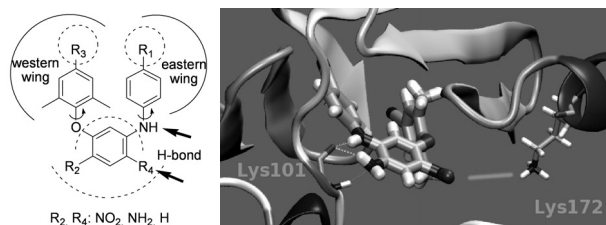
POSTER COMMUNICATIONS – PC.015

DIARYLANILINE DERIVATIVES AS A NOVEL CLASS OF HIV-1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Lan Xie^{1*}, Bingjie Qin,¹ Xingtao Tian,¹ Xingkai Jiang,¹ Hong Lu,³ Florent Barbault,⁴ Li Huang,⁵ Keduo Qian,² Rong Huang,² Chin-Ho Chen,⁵ Shibo Jiang,³ Kuo-Hsiung Lee²

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Structure-based drug design and isosteric replacement were applied to the synthesis of diarylaniline (*N*,5-diarylbenzene-1,2-diamine) derivatives, which were evaluated against wild-type HIV-1 and drug-resistant viral strains. The most promising compound **37** showed significant EC₅₀ values of 0.003–0.032 μM against wild-type HIV-1, which were better than that of TMC125 in the same assay, and EC₅₀ values of 0.005–0.604 μM against several drug-resistant strains. Current results also revealed important structure-activity relationship (SAR) conclusions for diarylanilines and strongly support our hypothesis that an NH₂ group on the central benzene ring *ortho* to the aniline moiety is crucial for interaction with K101 of the NNRTI binding site in HIV-1 RT, likely by forming H-bonds with K101. Furthermore, molecular modeling studies with MM/GBSA technology demonstrated the rationality of our hypothesis. Overall, the results in this study were promising for the discovery of diarylaniline derivatives as a novel class of next-generation HIV-1 NNRTI agents.



37, R₁ = R₃ = CN, R₂ = NO₂, R₄ = NH₂

EC₅₀ 0.003 μM (WT), 0.005–0.604 μM (MDR strains)

ACKNOWLEDGMENTS

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China, awarded to L. Xie, as well as US NIH grants awarded to K. H. Lee (AI33066), S. Jiang (AI46221), and C. H. Chen (AI65310).

POSTER COMMUNICATIONS – PC.016

A PHOTOACTIVABLE PROBE TO IDENTIFY PROTEINS IMPLIED IN IODIDE TRANSPORT

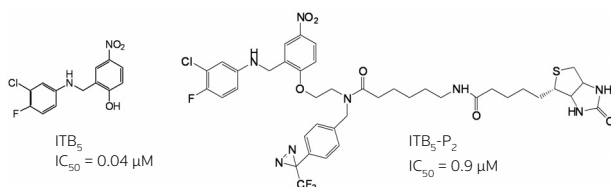
Fanny Waltz, Lucie Pilette, Sarah Bregant, Yves Ambroise

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The Na⁺/I⁻ symporter (NIS) is a membrane glycoprotein essentially found in thyroid cells which is responsible for plasmatic iodide uptake into the cytoplasm¹. This transport is at the basis of an emerging approach using radioiodide for cancer diagnosis and treatment. However, cancer cells naturally or artificially overexpressing the NIS present in many cases a greatly reduced activity of this protein. A better understanding of the still poorly defined post-traductionnal regulation mechanisms of the NIS could consequently allow us to reinduce the activity of the NIS in these cells and to open new prospects for the treatment of thyroid and non-thyroid cancers.

To study these mechanisms, our laboratory set up a direct chemical genetics strategy whose first step was to look for small organic molecules being able to inhibit iodide transport. After a high-throughput screening, 10 inhibitors were identified with IC₅₀ values in the submicromolar range². The second step consists in isolating the target proteins of these molecules. The considered method, namely affinity photolabelling, is based on the use of photoactivable analogues which can covalently bind their target proteins under light irradiation. These analogues are then coupled to a molecule allowing for the isolation of the complex formed by affinity chromatography.

We started with our most active inhibitor (ITB₅). The study of the structure-activity relationship of this compound allowed to design and to synthesize a probe (ITB₅-P₂) which contains a photoactivable group and a biotin molecule and which displays a satisfactory inhibitory activity (IC₅₀ = 0.9 μM). Preliminary experiments gave promising results. The photolysis of ITB₅-P₂ in the presence of living cells has led to the specific labelling of a single protein (molecular weight ~ 25 kDa). These results as well as the different methods currently tested to isolate and identify this protein will be presented.



REFERENCES

1. Dai G, Levy O, Amzel L.M, Carrasco N : **The mediator of thyroidal iodide accumulation : the sodium/iodide symporter**. *Handbook of biological physics*. Elsevier Press : Amsterdam, 1996, 15-22.
2. Lecat-Guillet N, Goulven M, Lopez R, Pourcher T, Rousseau B, Ambroise Y : **Small-Molecule Inhibitors of Sodium Iodide Symporter Function**. *ChemBiochem* 2008, **9**:889-895

POSTER COMMUNICATIONS – PC.017

NUCLEOPHILIC SUBSTITUTION AT THE C3a TERTIARY CARBON OF THE BROMOPYRROLOINDOLE SCAFFOLD

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The hexahydropyrrolo[2,3-b]indole scaffold is a common structural element of a variety of natural products that possesses a wide range of biological activities (**Fig. 1**).¹ This molecular framework is, therefore, particularly attractive for the preparation of compound libraries for medicinal chemistry programmes. Furthermore, alkaloids containing the hexahydropyrrolo[2,3-b]indole molecular framework have been identified as potent and selective protein-protein interaction (PPI) inhibitors.² In the modern drug discovery era, targeting PPIs offers the opportunity to identify more selective drugs.

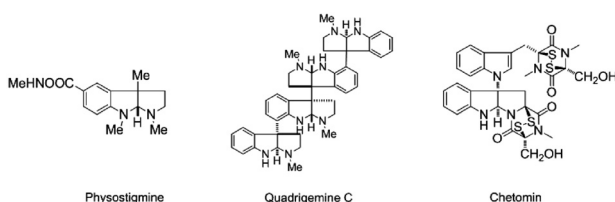
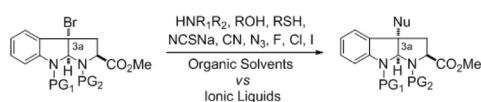


Figure 1. Examples of natural products containing the pyrroloindole molecular framework.

To date, the striking architecture of these pyrroloindole-based alkaloids has generated considerable interest within the synthetic community.³ For the preparation of compound libraries, functionalisation at the C3a position is critical and, although explored for total syntheses, the scope at present remains limited.⁴ Progress on synthetic studies to nucleophilic substitution at C3a to introduce a broader range of groups will be presented (**Scheme 1**).



Scheme 1. Nucleophilic substitution at C3a.

REFERENCES

1. (a) Hino, T.; Nakagawa, M. *Alkaloids* **1988**, 34, 1-75. (b) Anthoni, U.; Christophersen, C.; Nielsen, P. H. *Alkaloids* **1999**, 13, 163-236.
2. Zinzalla, G.; Thurston, D.E. *Future Med. Chem.* **2009**, 1, 65.
3. Crich, D.; Banerjee, A. *Acc. Chem. Res.* **2007**, 40, 151 – 161.
4. Espejo, V. R.; Rainier, J.D. *J. Am. Chem. Soc.* **2008**, 39, 12894-12895.

POSTER COMMUNICATIONS – PC.018

STRUCTURE–MEMBRANE INTERACTIVITY
RELATIONSHIP OF LOCAL ANAESTHETICS
ASSOCIATED WITH THEIR STEREOSELECTIVE
CARDIOTOXICITY

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Local anaesthetics display cardiotoxic capacities by an accidental intravenous injection or an absolute overdose. *S*(–)-Bupivacaine and *S*(–)-ropivacaine have been reported to be less toxic than their *R*(+)-enantiomeric counterparts and racemic mixture [1]. In mechanistic pharmacotoxicology, local anaesthetics interact not only with cardiomyocyte channels and receptors but also with membrane lipids to modify the physicochemical properties of lipid bilayers [2,3]. In order to find a clue to understand the cardiotoxic stereoselectivity, we studied the structure–membrane interactivity relationship of local anaesthetic stereoisomers with attention to chiral membrane components.

Liposomal membranes were prepared with cardiolipin, phospholipids and cholesterol of varying compositions, and cardiomyocyte-mimetic membranes with a mixture of 40 mol% cholesterol, 10 mol% cardiolipin, 47 mol% different phospholipids and 3 mol% sphingomyelin [3]. Bupivacaine and ropivacaine (*S*(–), *R*(+)-enantiomers and racemate) were reacted with these membrane preparations at the cardiotoxically relevant concentration of 200 μM [4], and then their membrane interactivities were comparatively determined by measuring fluorescence polarisation [5].

All the tested drugs interacted with lipid bilayers to increase the membrane fluidity. Despite having two chiral centres, cardiolipin was not effective in discriminating the induced membrane fluidisation between *S*(–)- and *R*(+)-enantiomers. In contrast, increasing cholesterol (5–45 mol%) in membranes produced the different membrane interactions of *S*(–)-, racemic and *R*(+)-anaesthetics, but not in the absence of cholesterol. When lipid bilayers contained 40 mol% and more cholesterol, local anaesthetics fluidised such membranes with the potency being *S*(–) < racemic < *R*(+)-bupivacaine and being *S*(–) < *R*(+)-ropivacaine.

Bupivacaine (racemate), levobupivacaine (*S*(–)-enantiomer) and ropivacaine (*S*(–)-enantiomer) interacted with cardiomyocyte-mimetic membranes in the decreasing order of intensity. The rank order of membrane interactivity agreed with that of cardiotoxicity [1]. Their opposite absolute configurations allow enantiomers to be discriminated by the interaction with another chiral molecule. The enantioselectivity was increased with elevating the molar ratio of cholesterol in membranes. Membrane cholesterol is responsible for the enantiomeric discrimination because it has higher chirality compared with the glycerol backbone of phospholipids including cardiolipin. The stereoselective membrane interactivity determined by cholesterol may be associated with the stereoselective cardiotoxicity of local anaesthetics.

The stereostructure–membrane interactivity relationship characterised in the present study supports the development and clinical use of *S*(–)-enantiomers to reduce the toxic effects of piperidine local anaesthetics on cardiovascular systems.

REFERENCES

1. Heavner JE. *Reg Anesth Pain Med*, **27**: 545–555, 2002.
2. Önyüksel H, Sethi V, Weinberg GL, Dudeja PK, Rubinstein I. *Chem Biol Interact*, **169**: 154–159, 2007.
3. Tsuchiya H, Ueno T, Mizogami M, Takakura K. *Chem Biol Interact*, **183**: 19–24, 2010.
4. Groban L, Deal DD, Vernon JC, James RL, Butterworth J. *Anesth Analg*, **92**: 37–43, 2001.
5. Tsuchiya H, Mizogami M, Takakura K. *J Chromatogr A*, **1073**: 303–308, 2005.

POSTER COMMUNICATIONS – PC.019

DESIGN, SYNTHESIS AND BIOLOGICAL
EVALUATION OF SPIRO [CHROMANE-2,4'-
PIPERIDINE] HYDROXAMIC ACID
DERIVATIVES AS STRUCTURALLY NOVEL
HISTONE DEACETYLASE (HDAC) INHIBITORS

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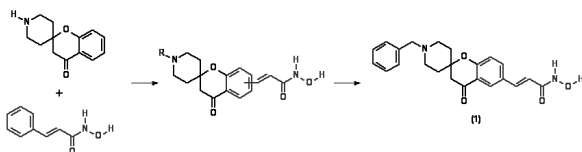
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The post-translational acetylation status of chromatin is regulated by the competing activities of two enzymatic classes: histone

acetyl transferases (HATs) and histone deacetylases (HDACs). Acetylation of histones is generally associated with gene activation; conversely, deacetylation is associated with gene silencing, including a decrease in the expression of tumor suppressor genes¹. Inhibition of HDAC activity can reverse this epigenetic silencing making histone deacetylases (HDACs) an attractive anticancer target. SAHA (suberoylanilide hydroxamic acid), the first HDAC inhibitor approved by the FDA in 2006 for the treatment of cutaneous T-cell lymphoma (CTCL), validates this approach for the treatment of cancer and several other HDAC inhibitors are currently at different stages of clinical development².

In the present study, we report the design, synthesis and biological evaluation of a new series of spiro[chromane-2,4'-piperidine] hydroxamic acid derivatives. We sought to combine a 'privileged scaffold', spiro[chromane-2,4'-piperidine], with minimal structures able to inhibit histone deacetylase enzymes³. The compounds were evaluated for their ability to inhibit nuclear extract HDACs as well as for their in vitro antiproliferative activity on different tumor cell lines. This work resulted in the discovery of spirocycle derivative (**1**) that showed good oral bioavailability and tumor growth inhibition in a HCT116 murine xenograft model.



REFERENCES

1. J. E. Bolden, M. J. Peart, R. W. Johnstone *Nat. Rev. Drug Discov.* **2006**, *5*, 769-784.
2. A. A. Lane, B. A. Chabner. *Journal of Clinical Oncology* **2009**, *27*, 5459-5468.
3. F. Thaler, A. Colombo, A. Mai, R. Amici, C. Bigogno R. Boggio, A. Cappa, S. Carrara, T. Cataudella, F. Fusar, E. Gianti, S.J. di Ventimiglia, M. Moroni, D. Munari, G. Pain, N. Regalia, L. Sartori, S. Vultaggio, G. Dondio, S. Gagliardi, S. Minucci, C. Mercurio, M Varasi, M., *Journal of Medicinal Chemistry* **2010**, *53*, 822-839.

POSTER COMMUNICATIONS – PC.020

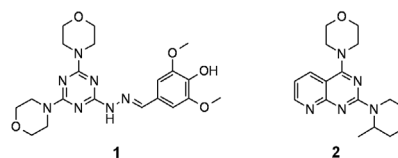
THE IDENTIFICATION OF CLINICAL CANDIDATE, AZD8055: A POTENT, SELECTIVE SMALL MOLECULE INHIBITOR OF mTOR KINASE

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The mammalian target of rapamycin (mTOR) is a key target in the development of antitumour therapies¹. Activated by growth factor/mitogenic stimulation activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway mTOR is a central

regulator of cell growth and proliferation. This PI3K-Akt-mTOR pathway is one of the most frequently dysregulated pathways in cancer². The known mTOR inhibitor Rapamycin and its analogues (such as Everolimus and Temsirolimus) bind to the FKBP12/rapamycin complex binding domain (FRB), resulting in suppression of signalling to the downstream targets p70S6K and 4E-BP1³. However, it has recently been shown that mTOR can exist in an alternative, rapamycin insensitive, complex that signals to Akt⁴. Therefore, it is proposed that a compound directly targeting the kinase domain of mTOR would inhibit signalling through both the rapamycin sensitive complex (mTORC1) and the rapamycin insensitive complex (mTORC2) and as such the compound would exhibit a different spectrum of activity compared with rapamycin.



Two alternative approaches to identify selective inhibitors of the mTOR kinase domain resulted in the identification of two distinct lead series, (1) and (2)⁵. The identification and optimisation of these series will be described culminating in the discovery of AZD8055, which has been shown to be a potent and selective inhibitor of both complexes of mTOR (mTORC1 and mTORC2) and demonstrates dose dependant tumour growth inhibition in xenograft studies. AZD8055 is currently undergoing clinical evaluation as a potential cancer therapy.

REFERENCES

1. Huang, S.; Houghton, P. J. *Curr Opin Pharmacol.* **2003**, *3*, 371.
2. Yap, T.A.; Garrett, M.D.; Walton, M.I.; Raynaud, F.; de Bono, J.S.; Workman, P. *Curr. Opin. Pharmacol.* **2008**, *8*, 393.
3. Fingar, D.C.; Salama, S.; Tsou, C.; Harlow, E.; Blenis, J. *Genes Dev.* **2002**, *16*, 1472.
4. Sarbassov, D.D.; Ali, S.M.; Sabatini, D.M. *Curr. Opin. Cell Biol.* **2005**, *17*, 596.
5. Menear, K. A; *et al*, *Bioorg. Med. chem. Lett.* **2009**, *19*, 5898; Malagu, K.; *et al*, *Bioorg. Med. chem. Lett.* **2009**, *19*, 5950.

POSTER COMMUNICATIONS – PC.021

ARYL PIPERAZINE AND PYRROLIDINE AS ANTIMALARIAL AGENTS SYNTHESIS AND INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

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The 2009 Word Malaria Report indicated that 108 countries showed endemic malaria¹. The search for novel drugs with inexpensive and easy synthetic methods against specific parasites is an important goal for antimalarial drug discovery.

Taking into account the antimalarial activity shown by aminealcohol derivatives², piperazine and pyrrolidine derivatives have been developed and evaluated as growth inhibitors of Chloroquine-resistant *Plasmodium falciparum* strains (FCR-3).

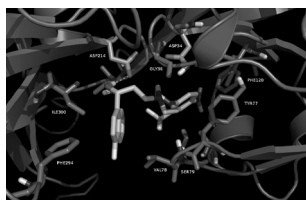
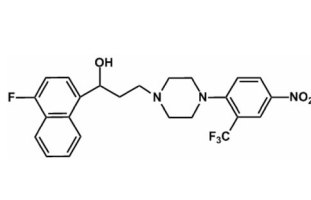
Five of the aryl alcohol assayed inhibited the parasite growth in 50% at a concentration <10 µM.

These studies have demonstrated a direct relationship between antiplasmodial activity and the presence of aryl alcohol, a four carbon aliphatic chain and a fluorine atom in the aryl alcohol.

Complementary toxicity studies have shown low toxicity for the most active compound (Fig. 1a). This compound is 20 to 40 times more active in FCR-3 strain against tumoural cells.

In spite of a favourable physical chemical profile of intestinal absorption, the low solubility by oral administration reduces *in vivo* assay activity.

Finally, computational and docking studies (Fig. 1b) have developed the compound affinity for *Plasmodium* enzyme, Plasmepsin II. This fact suggests an alternative action mechanism, different from Chloroquine.



Figures 1a, 1b: 1-(4-fluoronaphthyl)-3-[4-(4-nitro-2-trifluoromethylphenyl) piperazin-1-yl]propan-1-ol.

REFERENCES

1. 2009 Word Malaria Report. www.who.int.
2. Pérez-Silanes, L., Berrade, R.N., García-Sánchez, A., Mendoza, S., Galiano, B., Martín-P.Solórzano, J.J., Nogal-Ruiz, A.R., Martínez-Fernández, I., Aldana, Monge. A., 2009. New 1-aryl-3-substituted propanol derivatives as antimalarial agents. *Molecules* 14, 4120-4135.

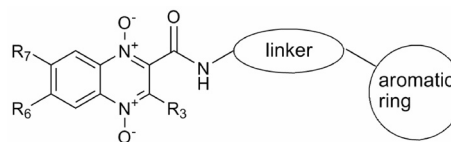
POSTER COMMUNICATIONS – PC.022

QUINOXALINE-2-CARBOXAMIDE 1,4-DI-N-OXIDE DERIVATIVES AS ANTI-TUBERCULOSIS AGENTS: SYNTHESIS, BIOLOGICAL EVALUATION AND ELECTROCHEMICAL STUDIES

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The global epidemiology of TB, particularly MDR-TB and XDR-TB, is unknown and the true magnitude of the problem is most likely underestimated, thereby encouraging the search for new anti-tuberculosis agents¹. As a continuation of our research in quinoxaline 1,4-di-N-oxide and with the aim of establishing structural requirements for the design of new anti-tuberculosis drugs, seventy-nine quinoxaline-2-carboxamide 1,4-di-N-oxide derivatives were prepared according to the following structure^{2,3}:



All of the synthesized compounds were evaluated for *in vitro* anti-tuberculosis activity following the screening program for the discovery of new drugs for the treatment of tuberculosis carried out by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF).

To gain insight into the mechanism of action and based on the results, twenty-six derivatives were selected in order to study the redox properties of these substances, using cyclic voltammetry and first derivative cyclic voltammetry in DMF with TBAP as supporting electrolyte. For all of the compounds studied, two cyclic voltammetric waves were observed during reduction.

The influence of molecular structure on reduction potential is addressed and it can be said that some relationships exist between reduction potential and reported antimicrobial activity. For the compounds in the present study, electrochemical reduction in DMF is consistent with reduction of the N-oxide functionality for forming a radical anion⁴. The second reduction wave observed could be due to the formation of the dianion, or to reduction of the product formed from a chemical step involving the radical anion⁵. The latter is supported by the irreversibility of the first reduction wave. The exact mechanism, however, was not further investigated.

This work has been carried out with the financial support of the PIUNA project from University of Navarra. We wish to express our gratitude to the TAACF for the evaluation of the anti-tuberculosis activity through. E. M. is indebted to the La Rioja Government for a grant.

REFERENCES

1. Global TB Control WHO REPORT 2009. www.who.int/tb/publications/global_report/2009/pdf/full_report.pdf
2. S. Ancizu, E. Moreno, et al., Bioorg. Med. Chem. (2010) doi:10.1016/j.bmc.2010.02.024
3. E. Moreno, S. Ancizu, et al., Eur. J. Med. Chem. *sent*
4. P. W. Crawford, R. G. Scamehorn, et al., Chem.-Biol. Interactions 60, (1986), 67.
5. K. R. Barqawi and M. A. Atfah, Electrochim. Acta 32, (1987), 597.

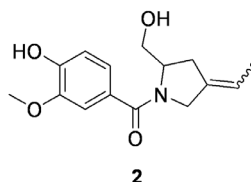
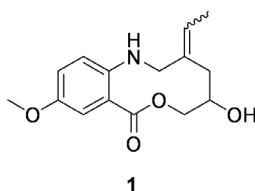
POSTER COMMUNICATIONS – PC.023

SYNTHESIS AND STRUCTURE DETERMINATION OF CH0035

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CH0035, was isolated from the extract of the marine Actinomycete BL-54, and presented cytotoxicity against three human tumor cell lines: A459, HT-29 and MDA-231. Its structure was determined by NMR spectroscopy and it is based in a 10 membered lactone with an exocyclic double bond fused with an aromatic ring (**1**). Synthesis was used at this stage to confirm the structure of CH0035.



Our synthetic results using two alternative routes will be discussed. Both routes converged in one intermediate that was converted by Wittig olefination and macrocyclisation to the proposed structure **1**. The main goal of our synthesis is the alkylation of a weakly nucleophilic aniline by this two different methods, which are based on a reductive amination and on a nucleophilic substitution.

The synthetic product was compared with the isolated natural product and the chemical shifts were slightly different. As a con-

sequence a new proposal for the structure were mandatory. The new proposed structure should have similar predicted chemical shifts, and the main difference between the former and the latter should be the connectivity between the aromatic system and the alkyl chain. A new structure was proposed, consisting on a pyrrolidine ring with an exocyclic double bond linked to an aromatic ring by an amide bond (**2**). The new synthesis will be also discussed as well as the comparison of both structures.

POSTER COMMUNICATIONS – PC.024

POST-COLUMN AFFINITY TECHNOLOGIES FOR LIGAND SCREENING IN MIXTURES FOR THE ACETYLCHOLINE BINDING PROTEIN

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The Acetylcholine Binding Protein (AChBP) is considered an excellent analog for the ligand-binding domain of neuronal nicotinic Acetylcholine Receptors. Its stability and solubility in water allowed the development of an on-line bioaffinity analysis technology. For this, a tracer ligand which displays enhanced fluorescence in the active site of AChBP was designed and synthesized. Evaluation and optimization was performed in convenient microplate reader format and subsequently transferred to the on-line format. The high reproducibility of the system led to the possibility of estimating the affinity of ligands injected in one known concentration. Furthermore, the strength of the on-line bioaffinity analysis system was highlighted with the analysis of biologically active mixtures in gradient HPLC mode with parallel high resolution mass spectrometry (MS) for compound identification.

Since the on-line technology relies on fast post-column incubation times, it cannot efficiently analyze ligands with low a k_{on} . Therefore, a bioaffinity analysis system allowing long post-column incubation times was developed. It is centralized around a contact-less post-column spotter technology capable of rapidly and accurately depositing HPLC eluent onto another platform (e.g. a 1536 microtiter well plate). The technology overcomes the hurdle of traditional low resolution fractionation strategies by tailoring the fractionation frequency to chromatographic peaks.

During the spotting process, biochemical assay constituents were simultaneously mixed in and spotted with HPLC eluent in 1536 microtiter well plates. The strength and possibilities of the spotter were demonstrated by running a post-column at-line spotted AChBP bioaffinity assay with 2 second fractions, each comprising only ~300 nl of eluent from the HPLC separation. Bioaffinities of ligands (also with low a k_{on}) in mixtures could be matched with their identities by correlating reconstructed bioaffinity chromatograms after plate reader analysis with parallel MS chromatograms.

POSTER COMMUNICATIONS – PC.026

SYNTHESIS AND SUBSEQUENT NMR STUDIES OF TRKA BINDERS TOWARDS A NOVEL THERAPEUTIC TO TREAT ALZHEIMER'S DISEASE

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The loss of cholinergic neurons in the basal forebrain has been observed in Alzheimer's disease, resulting in cognitive decline. Such cholinergic neurons are dependent on nerve growth factor (NGF) for survival. NGF binds to the TrkA receptor, dimerises two of these receptors and activates intracellular signalling¹. The fact that NGF is involved in the neuronal survival makes it extremely attractive therapeutically. Administration of NGF has been shown to have therapeutic potential, however in practical terms it would require invasive and expensive surgery². It would be more desirable to design a small orally administered drug able to mimic the way in which NGF binds to its receptor and its effects *in vivo*³.



The crystal structure of NGF dimerising two TrkA receptors⁴.

We have previously prepared a series of small molecule binders which can bind the TrkA receptor and have more recently identified a small molecule capable of binding the TrkA receptor and stimulating downstream signalling *in vitro*. This compound will act as our lead compound for developing an orally available TrkA agonist in treating Alzheimer's disease.

The synthesis of our small molecule binders is currently being explored. HSQC NMR is also acting as a powerful tool in eluci-

dating structure-activity relationships and in the design of our binders. The results of this ongoing work will be presented.

REFERENCES

1. Jing, S., Tapley, P. And Barbacid, M., *Neuron*, **1992**, 9, 1067-79.
2. Tuszyński, M. H., Thal. L., Pay. M., Salmon, D. P. *et al*, *Nat. Med.*, **2005**, 11, 551-5.
3. Dawbarn, D., Fahey, M., Watson, J., Tyler, S., Shoemark, D., Sessions, R., Zhang, R., Brady, L., Willis, C., Allen, S. J., *Biochem. Soc. Trans.*, **2006**, 34, 587-90.
4. Wiesmann, C., Ultsch, M. H., Bass, S. H., de Vos, A. M., *Nature*, **1999**, 401, 184-8.

POSTER COMMUNICATIONS – PC.027

MOLECULAR FRAGMENTS AS PROBES FOR STRUCTURE SELECTIVITY RELATIONSHIP ANALYSIS

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Target selectivity or specificity has long been considered a hallmark of biological recognition processes. However, the use of computational methodologies to study and predict the selectivity of target-ligand interactions is still in its infancy. We have introduced molecular fragment-based approaches to analyze target selectivity. Hierarchical fragmentation and knowledge-based design strategies were applied to systematically extract well-defined molecular fragments from different compound sets that could serve as molecular selectivity markers. Furthermore, a new type of atom environment descriptors were designed to distinguish compounds having different target selectivity profiles. These descriptors, termed Bonded Atom Pairs (BAPs), represent conceptually new atom pair descriptors with high chemical information content. BAPs combine different types of structural information including element type, hybridization states, aliphatic/aromatic character, and cyclic/acyclic structural motifs. When applied to molecular selectivity analysis, BAPs and conventional atom pair descriptors were found to capture complementary structural information. Moreover, BAPs accurately distinguished between compounds having different selectivity and hence extend the current repertoire of topological molecular descriptors.

REFERENCES

1. Ahmed H. E. A, Vogt M & Bajorath J. Design and evaluation of bonded atom pair descriptors. *J. Chem. Inf. Model.*, in press.
2. Ahmed H. E. A. and Bajorath J. Methods for computer-aided chemical biology. Part 5: Rationalizing the selectivity of cathepsin inhibitors on the basis of molecular fragments and topological feature distributions. *Chem. Biol. Drug Des.*, 74:129-141, 2009.

POSTER COMMUNICATIONS – PC.028

BENZOAZEPINONES AS MODULATORS OF γ -SECRETASE

Christian Fischer, Susan Zultanski, Hua Zhou, Joey Methot, Colby Brown, Elizabeth Kelley, Sanjiv Shah, Bethany Hughes, Christopher Moxham, Nathan Bays, Bo Pan, Zhenhua Wu, Lily Moy, Jonathan Cruz, Deborah Walker, George Nikov, Sujal Deshmukh, Thomas Miller, Benito Munoz, Mark Goulet, Mark Shearman

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which in 2009 affected over 5 million patients in the US and more than 25 million worldwide¹. Current standard of care relies on symptomatic treatment, which only provides short-term stabilization and to date no disease modifying therapy has been clinically validated. The widely accepted amyloid hypothesis describes oligomers of $A\beta$, specifically $A\beta_{42}$, as the neurotoxic species leading to neurodegeneration and ultimately AD². γ -Secretase modulators (GSMs) are a novel therapeutic approach for the treatment of AD. GSMs modulate APP cleavage by γ -secretase (Figure 1) to decrease $A\beta_{42}$, increase $A\beta_{38}$ whilst not affecting the cleavage of other substrates, such as Notch (Figure 2)³.

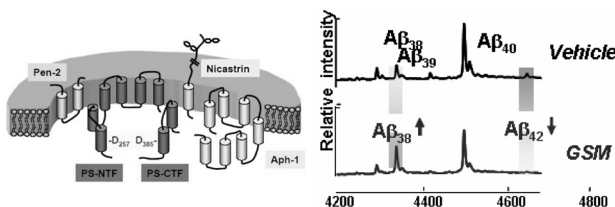
Figure1: γ -Secretase complex

Figure 2: GSM mode of action

This poster will discuss the medicinal chemistry strategy on triazole GSMs, specifically our recent advances in the benzoazepinone series (Figure 3)⁴. Detailed SAR studies and the medicinal chemistry lead optimization strategy will be presented. This effort has led to the identification of several potent and selective GSMs which we present herein detailing their *in vitro* and *in vivo* properties.

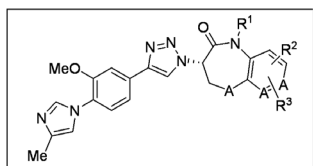


Figure 3: Benzoazepinone GSM

REFERENCES

1. Alzheimer's Association 2009 Alzheimer's disease facts and figures http://www.alz.org/national/documents/report_alzfactsfigures2009.pdf
2. Imbimbo, B. P. *Curr. Top. Med. Chem.* **2008**, 8, 54.
3. Peretto, I.; La Porta, E. *Curr. Top. Med. Chem.* **2008**, 8, 38.
4. Fischer, C.; Munoz, B.; Zultanski, S.; Methot, J.; Zhou, H.; Brown, W. C. *PCT Int. Appl.* (2008), WO 2008156580.

POSTER COMMUNICATIONS – PC.030

ANTIMALARIAL 4(1H)PYRIDONES

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4(1H)Pyridones (Fig 1) are novel potent and selective inhibitors of Plasmodium electron transport chain at the cytochrome bc1 level (Complex III). Although Plasmodium makes ATP predominantly by anaerobic fermentation in the cytoplasm, its mitochondria plays additional roles as the cellular compartment where essential reactions take place, such as, for example, reactions involved in heme and pyrimidine biosynthesis, the latter being the only source of pyrimidines in Plasmodium species. In summary, the presence of an intact mitochondria, energised by a functioning electron transport chain is likely to be essential for the parasite.

Atovaquone (GSK, Malarone®) and antimalarial 4(1H)Pyridones display the same pattern of inhibition of cytochrome bc1, providing strong circumstantial evidence that the site of action of 4(1H)Pyridones is complex III. However, as 4(1H)Pyridones are active against Atovaquone-resistant strains, their binding site in complex III is not identical to that of Atovaquone, but it could still be in cytochrome bc1.

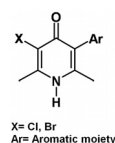


Fig 1

POSTER COMMUNICATIONS – **PC.031****RATIONAL DESIGN, SYNTHESIS AND MECHANISM OF ACTION OF NOVEL ANTIDIABETIC ETHOXYBENZO-THIAZOL DERIVATIVES***Ella Meltzer- Mats¹, Shlomo Sasson² and Arie Gruzman¹*

¹Division of Medicinal Chemistry, Department of Chemistry, Faculty of Exact Science, Bar Ilan University, Ramat Gan, Israel. ² Department of Pharmacology, Institute for Drug Studies, Faculty of Medicine, Hebrew University of Jerusalem, Israel.

Type 2 diabetes has been defined as a worldwide epidemic by the World Health Organization. The WHO estimated that more than 220 million people worldwide have diabetes. Over time this disease can damage the heart, blood vessels, eyes, kidneys, nerves and cause death. Type 2 diabetes is a chronic disease with a major pathophysiological symptom: decreasing glucose utilization in peripheral tissues such as muscle and fat due to insulin resistance. Modern antidiabetic drug therapy aims at a strict regulation of glucose homeostasis. However, monotherapy and combined therapy with oral agents often fail to achieve near-normoglycemia; hence the frequent need for insulin treatment. Therefore, the search for novel antidiabetic drugs is intense. Recently, we design and synthesized new line of compounds (ethoxybenzo-thiazol derivatives) with potential antidiabetic activity. According to our preliminary results these compounds increased the rate of glucose transport and utilization in peripheral tissues, most notably in skeletal muscle, which is the main consumer of glucose. Stimulation of intracellular glucose transport was induced via activation (phosphorylation) of key enzyme in energy metabolism: 5'-AMP-activated protein kinase (AMPK), since in its active form, it induces glucose transporter-4 (GLUT4) translocation to the plasma membrane of insulin sensitive cells and thus increases glucose influx in a non-insulin-dependent manner. This mechanism is distinct from insulin action, therefore it can be targeted by ethoxybenzo-thiazol derivatives that exert their effect even in the face of severe insulin resistance, such as found in obese type 2 diabetic patients.

POSTER COMMUNICATIONS – **PC.032****DEVELOPMENT OF NEW DRUGS FOR TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)***Omer Green and Arie Gruzman*

Division of Medicinal Chemistry, Department of Chemistry, Faculty of Exact Science, Bar Ilan University, Ramat Gan, Israel

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive muscle weakness and

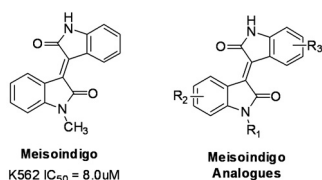
reflective loss of upper and lower motor neurons, predominantly in the spinal cord. Tremendous advances have been made in understanding the pathogenesis of ALS. However, these advances have not led to significant results in the development of an effective drug therapy. Unfortunately, beside riluzole which can prolong the life of ALS patients for an estimated three months, no other compounds have yet to have been developed. Thus, the development of anti-ALS drugs remains urgent. Mutations in the SOD1 are most common mutations that lead to familial ALS. The formation of SOD1-rich inclusions in the spinal cord is an early and prominent feature of SOD1-linked familial ALS in human patients and animal models of this disease. These inclusions have been shown to consist of SOD1-rich fibrils, suggesting that the conversion of soluble SOD1 into amyloid fibrils may play an important role in the etiology of familial ALS. SOD1 is also present in the inclusions found in spinal cords of sporadic ALS patients. SOD1 is degraded by at least two pathways in lysosomes and in the proteasome. Quick and sufficient clearance of mutant SOD1 reduces its motor neuron toxicity. Therefore, it has become clear, that drugs that prevent aggregation of proteins (especially SOD-1) in motor neurons or induce their appropriate clearance may be beneficial for treating ALS patients in general, and in particular patients with SOD-1 linked familial ALS. In search for compounds that will be able to prevent aggregation and accelerate degradation of misfolded SOD1 ALS affected motor neurons, we have found that the chemical chaperone trimethylamine N-oxide (TMAO) stimulates degradation of the disease-linked species of SOD1 by activation of its Cathepsin D cleavage. TMAO per se has unfavorable pharmacokinetic parameters to become a useful drug: the concentrations required to achieve its biological effect are rather high, TMAO reaches all organelles in the cell non-selectively, when we are interested in selective targeting in lysosomes, where Cathepsin D is localized. We, therefore, aimed at synthesizing potent lysosome targeted TMAO derivatives with favorable pharmacokinetic parameters.

POSTER COMMUNICATIONS – **PC.033****LEAD OPTIMIZATION STUDY OF MEISOINDIGO AGAINST CHRONIC MYELOGENOUS LEUKEMIA***Xi-Kai Wee, Clement Jun-Wen Ong, Mei-Lin Go*

Department of Pharmacy, Faculty of Science, National University of Singapore. 18 Science Drive 4, Singapore 117543.

Meisoindigo (1-methyl-isoidindigo) has been used as a Chinese medication for chronic myelogenous leukemia (CML) for many years¹. Meisoindigo was developed from a lead optimization effort based on a structurally similar compound named indirubin.² Indirubin is the active entity of the traditional Chinese herbal prescription "Danggui-Longhui Wan" for the treatment of chronic myelogenous leukemia². We investigated lead optimization efforts on the meisoindigo scaffold to probe structure activity relationships. A series of functionalized isoidindigos was synthesized by a modified acid-catalyzed aldol condensation

between substituted isatins and oxindoles assisted by microwave irradiation³. The compounds were then evaluated for their anti-proliferative activity on chronic myelogenous leukemic lymphoblast K562 cell line³. While meisindigo (IC_{50} = 8.0 μ M) contains a methyl group at position 1, a range of substituents was tolerated at this position, giving rise to compounds with close to 5 fold improvement in anti-proliferative activities. For instance, 1-phenpropyl-isoidindigo gave an antiproliferative IC_{50} value of 1.8 μ M. Meisindigo analogs with substitutions on the aromatic rings of the scaffold were also evaluated. Substitution at position 6' was found to be the most promising with the 6'-methoxy analogue of meisindigo being the most active (IC_{50} = 3.2 μ M). Substitution at the 7'-position generally led to poorer solubility while substitution on other positions did not yield any analogue that is better than meisindigo. Of the 65 compounds investigated, the most promising compound identified to date is more than 15 fold more potent than meisindigo as an anti-proliferative agent on K562 cells. A screen for kinase inhibitory activity of selected compounds showed that they did not target cyclin dependent kinases (CDKs) as previously thought³, although several receptor tyrosine kinases were possible targets.



REFERENCES

- Xiao, Z., Hao, Y., Liu, B. and Qian, L., *Leuk. Lymphoma*. **2002**, 43, 1763-1768.
- Xiao, Z., Hao, Y. *Indirubin, the red shade of indigo*. **2006**, 20, 203-208.
- Wee, X.K., Yeo, W.K., Zhang, B., Tan, V.B.C., Lim, K.M., Tay, T.E., Go, M.L. *Bioorg. Med. Chem.* **2009**, 17, 7562-7571.

POSTER COMMUNICATIONS – PC.034

AMINOPHOSPHOLIPID GLYCATION. STUDY OF THE REACTIVITY OF 2-AMINOETHYLPHENETHYLPHOSPHATE WITH GLYCATING COMPOUNDS

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Non-enzymatic glycation of biomolecules has been implicated in the pathophysiology of aging and diabetes¹. In most studies of glycation, the amino components have been proteins, amino acids or peptides². Several studies have demonstrated that carbohydrates can also modify aminophospholipids by reacting

with their amino groups both *in vivo*³ and *in vitro*⁴. Such reactions have been suggested to promote lipid oxidation as a result of glycation. As aminophospholipids are important components of cell membranes, their glycation could have a significant effect on membrane permeability and fluidity⁵. The complex structure of natural aminophospholipids makes it difficult to study the chemical changes that accompany their glycation. For that reason, in the present work, we have synthesised 2-aminoethylphenethylphosphate, a low molecular weight analogue of natural phosphatidylethanolamines⁶ and compared its glycation with that of a peptide (Ac-Phe-Lys) and pyridoxamine, an inhibitor of formation of advanced glycation and lipoxidation end-products⁷. We have conducted the kinetic study, under physiological pH and temperature conditions, of the reactions of 2-aminoethylphenethylphosphate with various glycating carbonyl compounds (acetol, arabinose, and glucose) by using high performance liquid chromatography-UV/Vis. The formation rate constants for Schiff bases of the aminophospholipid analogue and the carbonyl compounds are of the same order of magnitude as those determined for the Schiff bases of the peptide Ac-Phe-Lys⁸. However, the values obtained for Schiff bases of pyridoxamine are five times greater than that for the aminophospholipid analogue that could explain pyridoxamine ability to compete with amino residues in aminophospholipids glycation.

REFERENCES

- Thorpe S R, and Baynes J W *Drugs Aging*, 1996, 2, 69-77.
- Horvat S, and Jakas A J. *Peptide Sci.* 2004, 10, 119-137.
- Nakagawa K, Oak J H, Higuchi O, Tsuzuki T, Oikawa S, Otani H, Mune M, Cai H and Miyazawa T J. *Lipid Res.* 2005, 46, 2514-2524.
- Oak J; Nakagawa K; and Miyazawa T. *FEBS Lett.* 2000, 481, 26-30.
- Levi V, Villamil A M, Castello P R, Rossi J P, and Gonzalez-Flecha F L *Biochem. J.* 2008, 416, 145-152.
- Breitling-Utzmann C M, Unger A, Friedl D A, and Lederer M O *Arch. Biochem. Biophys.* 2001, 391, 245-254.
- Onorato J M, Jenkins A J, Thorpe S R, and Baynes J W *J Biol Chem* 2000, 275, 21177-21184.
- Adrover M, Vilanova B, Frau J, Muñoz F, and Donoso J *Amino Acids* 2009, 36, 437-448.

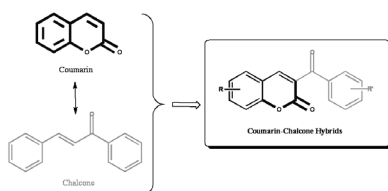
POSTER COMMUNICATIONS – PC.035

RATIONAL DESIGN AND SYNTHESIS OF COUMARIN-CHALCONE DERIVATIVES AS CHEMOTHERAPEUTIC AGENTS

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Chemotherapy, in its most general sense, is the treatment of diseases by chemicals especially by killing micro-organisms or cancerous cells. Nowadays are known a wide range of different chemotherapeutic agents. One of our aims in the last years has been the development of new tools and methodologies for drug discovery. Following this aim we designed hybrid molecules coming from two natural occurring compounds: coumarin and chalcones. Both separated compounds have well known pharmacological activities such as antibacterial antitumor, anti-inflammatory, antithrombotic, cardio protectors or enzymatic inhibitors^{1,2}.



Including the double bond of the chalcone moiety in a coumarinic structure we will be able to obtain compounds whose scaffold will include both pharmacophores, with a semi-rigid chalcone. In the 3-benzoylcoumarin scaffold can be introduced atoms, groups or fragments of very different nature in variable number and position. With the aim of selecting the most appropriated series of compounds in order to make the synthesis and the pharmacological evaluation, we pretend to help us by using rational design based on QSAR methodologies.

Having got good QSAR models for different chemotherapeutic activities, even also multi-target models, we use them for doing a virtual screening and also for improving the pharmacological profile of a lead compound. According to the QSAR models, we selected a series of compounds and we have developed simple, efficient and generalized procedures to synthesize them using a Knoevenagel type reaction as key step³. All results will be further discussed and showed in our communication.

REFERENCES

1. Ni, L.; Meng, C. Q. and Sikorski, J. A., *Expert Opin. Ther. Patents*, **2004**, 14(12), 1669.
2. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L. and Uriarte, E., *Current Med. Chem.*, **2005**, 12, 887
3. Frédéric, R.; Rovert, S.; Charlier, C.; Ruyck, J. de; Wouters, J.; Pirote, B.; Marsereel, B. and Pochet, L., *J. Med. Chem.*, **2005**, 48, 7592

POSTER COMMUNICATIONS – PC.036

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY FROM DIFFERENT SAMPLES OF MEDICINAL PLANT *GALPHIMIA GLAUCA* CAV. AND STRUCTURAL ELUCIDATION USING SPECTROPHOTOMETRY AND HPLC-ESI-MS

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According to epidemiological studies, availability and consumption of antioxidants in plants is closely related to the prevention of various degenerative diseases. Phenolic compounds are attracting considerable interest in the field of chemistry, food and medicine due to their promising antioxidant potential and other useful functions. Thryallis [*Galphimia glauca*, (Cav.) Malpighiaceae] [Syn. *Thryallis glauca* (Poir.) O. Kuntze], commonly known as "calderona amarilla", has been widely used in Mexican traditional medicine and homoeopathic preparations. Pharmacological investigations have reported sedative, (CNS) depressant, anticonvulsant, antiasthmatic, antiallergic properties, hypothermic activity, barbiturate potentiation, protection against strychnine- and leptazol-induced convulsions properties. As different antioxidants can act in vivo through different mechanisms, single method for determination of total antioxidant capacity is insufficient [1].

The present investigations deal with the determination of total antioxidant capacity (TAC), total polyphenolic content (TPC) and further structural identification of the major constituents from samples of *Galphimia glauca*, after fractionating extracts (microwave assisted extraction in 50 % ethanol) using polyamide column, to yield six different fractions following a modified protocol [2]. Three sensitive, speedy, and specific methodologies (DPPH [3], FRAP [1], TEAC [4]) have been adopted for the assessment of TAC and Folin Ciocalteu's method [5] was used for the determination of TPC.

Percent inhibition and IC 50, FRAP [Fe⁺² g/g sample], TEAC [trolox g/g sample], AEAC [Ascorbic acid g/g sample] values, calculated using ascorbic acid, trolox and FeSO₄ as standards, revealed that fraction 3 and 4 in all samples proved to be most potent antioxidants in comparison to others. A directly proportional relationship between TPC and TAC was found to exist. All three methods used to determine TAC values exhibited same tendency, showing that the mechanism of action of these antioxidants was nearly same. Mass spectrometric investigations showed that fractions rich in TPC and consequently most potent antioxidants, consisted of multiple galloyl derivatives of quinic acid and oligomers/polymers of flavanols.

REFERENCES

1. Nicoletta Pellegrinim, Mauro Serafini (2003) J. Nutr. 133: 2812-2819.
2. Ulla Svedström, (2006) J. of Chromatogr. A. 1112: 103-111.
3. Rajbir Singh et al. (2007) Food Chemistry, 103: 505-511.
4. Susan Cheung and Joseph Tai (2005) Oncology Reports, 14: 1331-1336.
5. Singleton and Rossi (1965) Am. J. Enol. Vitic. 16: 144

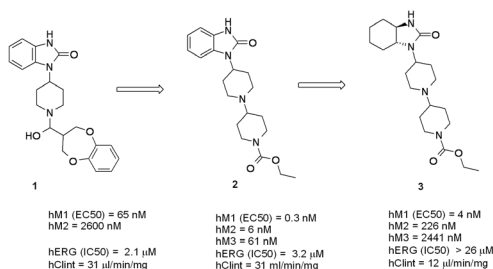
POSTER COMMUNICATIONS – PC.037

DISCOVERY AND PRECLINICAL EVALUATION OF M1 SELECTIVE AGONISTS

Vijayaratnam Santhakumar, Yun-Xing Cheng, Jennifer Laird, Shujuan Jin, Xuehong Luo, Nektaria Markoglou, Mehrnaz Pousaraf, and Mirek Tomaszewski

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M1 agonists have therapeutic potential as cognitive enhancing agents and disease modifying agents for Alzheimer's disease as demonstrated by preclinical and clinical studies. 3-(4-piperidyl)-1H-benzimidazol-2-ones (example: compound 1) were identified as M1 selective agonists by high throughput screening. Replacement of the aromatic benzimidazolone core (compound 2) structure with the aliphatic octahydro benzimidazolone core (compound 3) improved selectivity against M2 and reduced hERG activity. SAR analysis showed that aryl alkyl groups and amide groups at the piperidine ring improve selectivity against M2 but aryl alkyl groups increase hERG activity and amide groups increase efflux. Removal of the hydrogen bond donor at the benzimidazolone ring reduced efflux however resulted in reduced metabolic stability or reduced selectivity against M2. Introduction of quaternary methyl group at the piperidine ring and replacement of ethyl carbamate group with isopropyl carbamate improve M2 selectivity and hERG profiles. Four candidates suitable for pre-clinical evaluation of in vivo efficacy and safety profiles were identified.



POSTER COMMUNICATIONS – PC.038

DE NOVO DESIGN AND SYNTHESIS OF POTENTIAL CXCR4 INHIBITORS

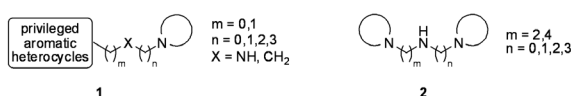
Laia Ros-Blanco¹, Violeta I. Pérez-Nueno¹, Llorenç Nieto¹, Maria Pau Mena², José A. Esté², Bonaventura Clotet², Jordi Teixidó¹, José I. Borrell¹

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HIV cell fusion and entry coreceptors CXCR4 and CCR5 have been validated as targets for therapeutic intervention¹. Nowadays, there is one entry inhibitor (Maraviroc, Pfizer) and one fusion inhibitor (Enfuvirtide, Roche Pharmaceuticals) available for the treatment of HIV infection. However, none of them inhibits CXCR4 coreceptor. Our group previously described a combinatorial library of analogues of the currently most active *lead* AMD3100, whose polinitrogenated building block consisted of a nitrogen-containing heterocycle (piperidine, piperazine, morpholine and pyrrolidine among others), a (CH₂)_n (n = 0, 1, 2, 3) spacer and a terminal amine group. Library selection led to the identification of a *lead* with nanomolar activity against HIV².

Looking for new potential CXCR4 inhibitors, a *de novo* structure based drug design was carried out with LUDI package from Discovery Studio³, using a 3D homology model of the coreceptor⁴ (built from the available template structure of bovine rhodopsin, since no CXCR4 crystal structure exists), to obtain a new virtual library of compounds. LUDI interaction sites were generated on CXCR4 binding pocket according to the key binding residues (Asp171, Asp262, Glu288), pointed by directed mutagenesis⁵. This approach yielded a total of 200 new ligands, based on the 20 highest-ranked scaffolds from standard calculations. The most relevant fragments of the resulting library were "privileged" nitrogen-containing aromatic cycles (like indole and iminodibenzyl), connected by spacers with difficult synthetic feasibility. For this reason, a more synthetically accessible library of compounds was designed, focusing on both polinitrogenated building blocks previously described by our group and *de novo* selected fragments (and their commercial availability). Complex spacers were substituted by hydrocarbonated chains. Virtual screening approaches (docking, pharmacophore modelling, shape matching and similarity searches) were performed to select the best potential active ligands to be synthesized and tested. Final structures **1** and **2** can be obtained by reductive amination or by secondary amine alkylation, amide synthesis and reduction.

Synthetic strategies and anti-HIV activity of selected new potential inhibitors will be presented.



REFERENCES

1. Bleul *et al.*, *Nature* **1996**, 382, 829-833; Deng *et al.*, *Nature* **1996**, 381, 661-666
2. Pettersson *et al.*, *ChemMedChem* **2008**, 3, 1549-57
3. LUDI, Discovery Studio, Version 2.5, Accelrys Inc., **2009**
4. Pérez-Nueno *et al.*, *J.Chem.Inf.Model.* **2008**, 48, 509-533
5. Zhou *et al.*, *J.Biol.Chem.* **2001**, 276, 42826-42833

POSTER COMMUNICATIONS – PC.039

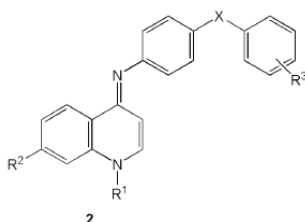
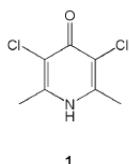
SYNTHESIS AND SAR OF QUINOLONIMINES AS POTENTIAL ANTIMALARIALS

Tiago Rodrigues¹, Jiri Gut², Philip J. Rosenthal², Rui Moreira¹, Rita C. Guedes¹, Daniel dos Santos¹, Francisca Lopes¹

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Malaria is a serious global health problem, with devastating social and economic consequences in countries where the disease is endemic, due to the rapid emergence and spread of parasites that are resistant to well-established antimalarial drugs [1]. Although continued attempts to develop a vaccine for malaria are ongoing, drugs continue to be the only treatment option [2]. As a result, there is an urgent need for novel drugs, preferably acting on underexploited parasite targets in order to delay or overcome the selection of clinical resistance [3,4].

Cytochrome *bc_L*, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [5]. Clopidol, **1**, is a known *bc_L* complex inhibitors and has been taken as a lead for structural optimization [6]. Based in the 4(1*H*)-pyridone scaffold we recently designed and synthesized potential isosters containing the pyridin-4 amines core [7,8]. Now we report the synthesis of related quinolin-4 amines, **2**. These were obtained in good or excellent yields and their activity found to be in the low or sub-micromolar range against *Plasmodium falciparum* W2 strain. A docking study was also carried out to predict the major interactions of the ligand with the binding pocket. SAR will be discussed.



REFERENCES

1. Martinelli, A. *et al.*, *Mini-Rev Med Chem* **2008**, 8, 201-212
2. Nwaka S, *et al.*, *Nat Rev drug Discov* **2006**, 5, 941-955
3. Arav-Boger, R. *et al.*, *Annu Rev Pharmacol Toxicol* **2005**, 45, 565-585

4. Fidock, D.A. *et al.*, *Trends Parasitol* **2008**, 24, 537-544
5. Rodrigues, T. *et al.*, *Curr Med Chem* **2010**, 17, 929-956
6. Yeates, C. L. *et al.*, *J Med Chem* **2008**, 51, 2845-2852
7. Rodrigues, T. *et al.*, *Int J Quantum Chem* **2010**, accepted
8. Rodrigues, T. *et al.*, *Bioorg Med Chem Lett* **2009**, 19, 3476-3480

POSTER COMMUNICATIONS – PC.040

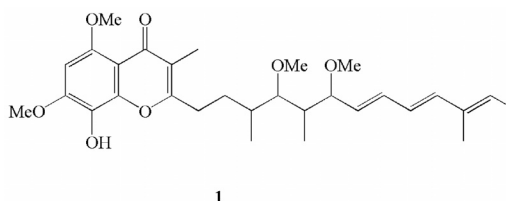
SYNTHESIS AND SAR OF FLAVONES AS POTENTIAL ANTIMALARIAL SCAFFOLD

Tiago Rodrigues¹, Jiri Gut², Philip J. Rosenthal², Rui Moreira¹, Rita C. Guedes¹, Daniel dos Santos¹, Francisca Lopes¹

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Malaria is an infectious disease with serious social and economic repercussions mainly in endemic areas. The rapid emergence and spread of parasites that are resistant to well-established antimalarial drugs poses a great challenge in finding efficacious chemotherapeutic agents [1]. Drugs continue to be the only treatment option, despite ongoing attempts to develop a vaccine for malaria [2]. As a result, there is an urgent need for novel drugs, preferably acting on underexploited parasite targets in order to delay or overcome the selection of clinical resistance [3,4].

Cytochrome *bc_L*, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [5]. Stigmatellin, **1**, is an antibiotic presenting the flavone core and significant antimalarial activity through inhibition of the mitochondrial electron transport chain [5]. Here we report the synthesis of a series of flavones through a one-pot Baker-Venkataraman procedure. These displayed antimalarial activity against the *Plasmodium falciparum* W2 strain in the micromolar range. The SAR and future directions will be discussed.



REFERENCES

1. Martinelli, A. *et al.*, *Mini-Rev Med Chem* **2008**, 8, 201-212
2. Nwaka S, *et al.*, *Nat Rev Drug Discov* **2006**, 5, 941-955
3. Arav-Boger, R. *et al.*, *Annu Rev Pharmacol Toxicol* **2005**, 45, 565-585
4. Fidock, D.A. *et al.*, *Trends Parasitol* **2008**, 24, 537-544
5. Rodrigues, T. *et al.*, *Curr Med Chem* **2010**, 17, 929-956

POSTER COMMUNICATIONS – PC.042

SULFONATES OF N-TRIAZINYLAMMONIUM SALTS AS A NEW COUPLING REAGENTS FOR AMYLIN SYNTHESIS

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Activation of carboxylic acids, for the formation of amide or ester bonds, is a key step in the synthesis of a large number of bioorganic molecules, particularly in the case of peptide synthesis. Recently, systematic exploration of structural diversity offered by incorporation of non-coded amino acids into the peptide chain has been developed into one of the most efficient strategies used in designing new analogues of pharmaceutically active peptides, inspired the search for the new, more versatile and general activation reagents and procedures. This demand is of the prime importance in the case of so-called "difficult couplings" involving strongly hindered amino acids or peptide sequences coiled into non-reactive conformations.

In the search for improved coupling reagents we found that triazine based "superactive esters" superior to the most popular coupling reagents which are based on the chemistry of the benzotriazole or azabenzotriazole ring system [1]. Taking advantage of the modular structure of triazine reagents allowing the fine tuning of their properties, we attempted to develop the new family of them by treatment of 2-chloro-4,6-dimethoxy-1,3,5-triazine with salts of N-methylmorpholine, N-methylpiperidine and N-methylpyrrolidine with toluene-4-sulfonic acid, methanesulfonic acid, trifluoromethanesulfonic acid and camphorosulfonic acid respectively. We found all of them useful for activation of carboxylic components and peptide synthesis in solution and SPPS using all basic N-protecting groups: Z-, Boc-, and Fmoc-. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium toluene-4-sulfonate selected after screening experiments as most versatile coupling reagent was used in SPPS synthesis of Islet Amyloid Polypeptide – amylin and their analogues with α , α -amino acids replacing L-amino acids residues in the native structure.

REFERENCES

1. a) König, W.; and Geiger, R.; Chem. Ber. 1970, 103, 788-798; b) Carpino, L. A. J. Am. Chem. Soc. 1993, 115, 4397-4398; c) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Tetrahedron Lett. 1975, 16(14), 1219-1222; d) Coste, J.; Le-Nguyen, B.; Castro, B. Tetrahedron Lett., 1990, 31, 205-208; e) Carpino, L.A.; Imazumi, H.; El-Faham, A.; Ferrer, F.J.; Zhang, C.; Lee, Y.; Foxman, B.M.; Henklein, P.; Hanay, C.; Mugge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. Angew. Chem. Int. Ed. 2002, 41, 441-445; f) Li, P.; Xu, J. C. J. Chem. Soc., Perkin 2, 2001, 113-120. g) Carpino, L. A.; Henklein, P.; Foxman, B. M.; Abdelmoty, I.; Costisella, B.; Wray, V.; Domke, T.; El-Faham, A.; Muegge, C. J. Org. Chem. **2001**, 66, 5245-5247.

POSTER COMMUNICATIONS – PC.043

SYNTHESIS OF N,N'-DISUBSTITUTED-2-(4-(2-AMINOETHYL)-PHENYL)ETHYLAMINES AS A POTENTIAL CXCR4 CORECEPTOR INHIBITORS

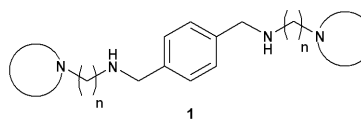
Raimon Puig de la Bellacasa¹, María Pau Mena²,
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HIV cell fusion and entry have been validated as targets for therapeutic intervention against infection^[1]. Nowadays, FDA have approved Enfuvirtide (Hoffmann-La Roche & Trimeris) as a fusion inhibitor and Maraviroc (Pfizer) as a CCR5 entry coreceptor antagonist.

Bicyclams were the first low-molecular-weight compounds to show specific interaction with CXCR4, another entry coreceptor. The most potent bicyclam was AMD3100, in which the two cyclam moieties are tethered by a 1,4-phenylenebis(methylene) bridge. It was withdrawn from clinical trials owing to its lack of oral bioavailability and cardiotoxicity.

As a part of our research in this field, we have developed a series of new non-cyclam polynitrogenated CXCR4 coreceptor inhibitors. The structure of these compounds (**1**) is constituted by two commercially available nitrogenated building blocks (consisting of a nitrogen-containing heterocyclic system, a $(CH_2)_n$ ($n = 2, 3$) spacer and a terminal amine) tethered by a 1,4-phenylenebis(methylene) bridge^[2].



These molecules were tested for anti-VIH activity and some of them present high anti-HIV activity. These good results, prompted us to explore variations around this kind of structures. Among these variations, we have designed and synthesized the corresponding next higher homologous systems. Thus, instead of using 1,4-phenylenebis(methylene) as a bridge, we have selected 1,4-phenylenebis(ethylene) to link the two amine moieties.

The subsequent anti-HIV *in vitro* testing led to the identification of some highly active compounds. In some cases the results obtained were similar to those achieved for compounds **1**.

REFERENCES

1. Bleul *et al.* Nature. **1996**, 382, 829-833; b) Deng *et al.* Nature. **1996**, 381, 661-666.
2. Pettersson *et al.* ChemMedChem. **2008**, 3 (10), 1549-1557.

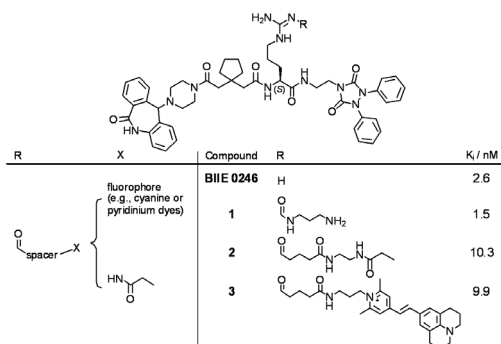
POSTER COMMUNICATIONS – PC.044

NONPEPTIDIC FLUORESCENCE- AND RADIOLIGANDS AS PHARMACOLOGICAL TOOLS FOR THE NEUROPEPTIDE Y₂ RECEPTOR

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As shown previously for argininamide-type neuropeptide Y (NPY) Y₁ (Y₁R) and Y₂ receptor (Y₂R) antagonists, the introduction of various electron-withdrawing groups into the N^G-position is well tolerated and may be used to attach radiolabels or fluorescent dyes^[1-3]. Based on the (S)-argininamide BIIIE 0246^[4], the first highly potent and selective nonpeptidic Y₂R antagonist, we synthesized N^G-substituted acylguanidine bioisosters as fluorescence- and radioligands. The functionalization of BIIIE 0246 with different ω-amino acyl substituents yielded precursors for the preparation of variously labeled Y₂R antagonists. Binding affinities and functional activities were investigated in a flow cytometric binding assay and in a spectrofluorimetric Ca²⁺ assay^[5], respectively. The new substances are highly potent Y₂R antagonists comparable or even superior to the parent compound BIIIE 0246. The high affinity fluorescence ligands proved to be applicable in confocal laser scanning microscopy. Additionally, due to Y₂R selectivity, these fluorescent nonpeptides are suitable to replace the universal NPY receptor ligand Cy5-NPY in flow cytometric binding studies. [³H]-labeled argininamide-type Y₂R selective antagonists can be used instead of radio-labeled NPY derivatives in radioligand binding studies or for the quantification of Y₂R's, and should be applicable to autoradiography.



REFERENCES

1. Keller, M., et al., *J. Med. Chem.* **2008**, 51, 8168-8172.
2. Schneider, E., et al., *ChemBioChem* **2007**, 8, 1981-1988.
3. Brennauer, A., PhD thesis, University of Regensburg, **2006**.
4. Doods, H., et al., *Eur. J. Pharmacol.* **1999**, 384, R 3-5.
5. Ziemek, R., et al., *Eur. J. Pharmacol.* **2006**, 551, 10-18.

POSTER COMMUNICATIONS – PC.046

SYNTHESIS OF NEW MORPHOLINE DERIVATIVES OF PHENCYCLIDINE WITH MORE ANALGESIC EFFECTS

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Phencyclidine (1-(1-phenylcyclohexyl) piperidine, PCP, **I**) has shown analgesic effects. Some of its derivatives have been synthesized and their biological properties have been studied. In this research, (1-[1-(4-methylphenyl) (cyclohexyl)]-4-morpholine, **II**), (1-[1-(2-methoxyphenyl) (cyclohexyl)]-4-morpholine, **III**), (1-[1-(2-methyl-4-hydroxyphenyl) (cyclohexyl)]-4-morpholine, **IV**) as three analogues of PCP with a methyl, methoxy, methyl-hydroxyl groups on the aromatic ring and morpholine instead of piperidine ring, were synthesized and their analgesic effects were examined on rats using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute chemical and chronic pain) tests and compared to PCP and control groups. As indicated in our previous works on this family [1, 2], incorporation of methyl and methoxy groups on the aromatic ring increased electron distribution and dipole moments properties because of the high electron donating character of these groups. Also incorporation of hydroxyl group on the phenyl ring and morpholine instead of piperidine ring increased the hydrophilic, polarity and solubility properties of these analogues.

The results showed that all of the new synthesized compounds were effective in acute thermal and chronic pain but not effective in acute chemical pain compared to the PCP and control groups.

Key words: Phencyclidine- Tail immersion test- Formalin test- Methyl, Methoxy, Methyl-hydroxyl morpholine derivatives of PCP.

REFERENCES

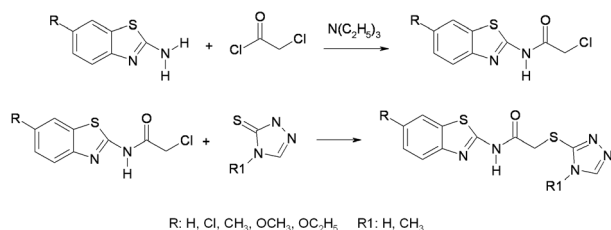
1. A. Ahmadi et al., *Arzneimittelforschung*.2009;59(4):202-206.
2. A. Ahmadi et al., *Arzneimittelforschung*.2009;59(12).

POSTER COMMUNICATIONS – PC.047

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEW BENZOTHAZOLYL-AMIDES, DERIVATIVES OF 1,2,4-TRIAZOL-3-THIONE

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The enhancement of bacterial resistance of pathogens to currently available antibiotics constitutes a serious public health threat. So, intensive efforts are underway worldwide to develop new antimicrobial agents [1]. For this purpose, ten new N-(benzothiazol-2-yl)-2-(1,2,4-triazol-3-yl-sulfonyl)acetamide derivatives were synthesized by reacting 2-chloro-N-(benzothiazol-2-yl)acetamides with 1,2,4-triazol-3-thione derivatives in acetone [2,3]. The chemical structures of the synthesized compounds were elucidated by IR, ¹H-NMR, FAB⁺-MS spectral data. The compounds were evaluated for antimicrobial activity. Promising results were observed.



REFERENCES

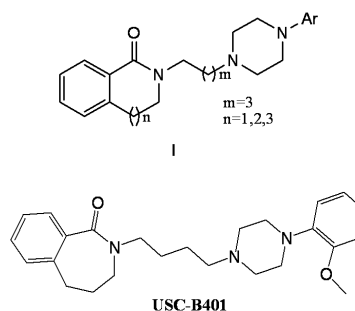
1. C. Franchini, M. Muraglia, F. Corbo, M.A. Florio, A.D. Mola, A. Rosato, R. Matucci, M. Nesi, F.V. Bambeke, C. Vitali, Arch. Pharm. Chem. Life Sci. 342, 605-613, 2009.
2. G. Turan-Zitouni, Ş. Demirayak, A. Özdemir, Z.A. Kaplancıklı, M.T. Yıldız, Eur. J. Med. Chem., 39(3), 267-272, 2004.
3. G. Turan-Zitouni, Z.A. Kaplancıklı, Ü. Uçucu, A. Özdemir, P. Chevallet, Y. Tunalı, Phosphorus, Sulfur, and Silicon and the Related Elements, 179(11), 2183-21

POSTER COMMUNICATIONS – PC.049

AROMATIC RING FUNCTIONALIZATION OF BENZOLACTAM DERIVATIVES: NEW POTENT DOPAMINE D₃ RECEPTOR LIGANDSRaquel Ortega¹, Christian F. Masaguer¹, Harald Huebner², Peter Gmeiner².¹ Department of Organic Chemistry. Faculty of Pharmacy. University of Santiago de Compostela. 15782-Santiago de Compostela, Spain.² Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, D-91052 Erlangen, Germany.

Since the discovery of the dopamine D₃ receptor by Sokoloff et al in 1990¹, intensive effort has been directed toward the development of selective ligands for this receptor to help definitively separate D₃ actions from those mediated by the D₂ receptors, in order to elucidate the function and potential therapeutic advantages of targeting D₃ receptors. Subsequent studies evidence that dopamine D₃ receptor is an interesting target in different CNS diseases such as schizophrenia^{2,3}, Parkinson⁴, drug-induced dyskinesia⁵, and drug abuse⁶.

Joining to these efforts, we have synthesized a new series (I) based on a benzolactam scaffold, which maintains three characteristic elements of many dopamine D₃ receptor antagonist: 1) an amine moiety, 2) a spacer, usually a linear alkyl chain, and 3) a hydrophobic residue, often connected through an amide bond.



Owing to the excellent affinity results of some of this compounds such as **USC-B401**⁷ together with the docking simulation studies and SAR analysis, some modifications have been made on the benzolactam moiety with the addition of different substituents. The new synthesized compounds will allow us to evaluate some of the structural requirements in this series for high affinity and selectivity binding at the D₃ receptor.

ACKNOWLEDGMENT

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REFERENCES

1. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. *Nature* **1990**, 347, 146

- Joyce, J. N.; Millan, M. J. *Drug Discov. Today* **2005**, 10, 917.
- Richtand, N. M.; Woods, S. C.; Berger, S. P.; Strakowski, S. M. *Neurosci. Biobehav. Rev.* **2001**, 25, 427.
- Joyce, J. N.; Ryoo, H. L.; Beach, T. B.; Caviness, J. N.; Stacy, M.; Gurevich, E. V.; Reiser, M.; Adler, C. H. *Brain Res.* **2002**, 955, 138.
- Bezard, E.; Ferry, S.; Mach, U.; Stark, H.; Leriche, L.; Boraud, T.; Gross, C.; Sokoloff, P. *Nat. Med.* **2003**, 9, 762.
- Heidbreder, C. A.; Gardner, E. L.; Xi, Z.-X.; Thanos, P. K.; Mugnaini, M.; Hagan, J. J.; Ashby, C. R. *Brain Res. Rev.* **2005**, 49, 77.
- Ortega, R.; Raviña, E.; Masaguer, C.F.; Areias, F.; Brea, J.; Loza, M.I.; López, L.; Selent, J.; Pastor, M.; Sanz, F. *Bioorg. Med. Chem. Lett.* **2009**, 19, 1773.

POSTER COMMUNICATIONS – PC.050

SMALL QUATERNARY AChE INHIBITORS FOR MG TREATMENT – SYNTHESIS, IN VITRO SCREENING AND MOLECULAR DOCKING

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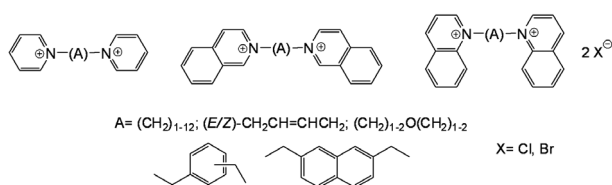
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Keywords: AChE, small inhibitor, Myasthenia gravis, synthesis, in vitro, docking

Small quaternary acetylcholinesterase (AChE) inhibitors are used (e.g. pyridostigmine bromide, neostigmine bromide, ambenonium dichloride) are used for early Myasthenia gravis treatment [1]. Their peripheral effect allows sufficient quantity of acetylcholine available for decreasing amount of AChE receptors. Their side effects including gastrointestinal effects (nausea, intestinal obstruction), increased bronchial secretion, cardiac arrhythmia or cholinergic crisis are well described and limit their dosage and long-term treatment.

Novel small quaternary inhibitors were designed as peripherally acting compounds (pyridinium, quinolinium and isoquinolinium analogues) [2,3]. The *in vitro* ability was determined using IC₅₀ values and kinetic constants. Inhibitory ability towards AChE was found superior to used drugs and non-competitive mechanism of inhibition was confirmed by kinetic parameters and molecular docking.



REFERENCES

- Garcia-Carrasco, M. et al. *Autoimmun. Rev.* 2007, 6, 373.
- Musilek, K. et al. *Bioorg. Med. Chem. Lett.* 2010, 20, 1763.
- Komloova, M. et al. *Curr. Med. Chem.* 2010, in press.

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POSTER COMMUNICATIONS – PC.051

THE MULTIPLE EFFECTS OF CYP17 INHIBITORS ON PROSTATE CANCER CELLS

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Cytochrome P450 17 α -hydroxylase, C_{17,20}-lyase (CYP17) catalyses androgen biosynthesis in the human body^{1, 2} and its inhibition is an important strategy for PC treatment as a way to lower androgen levels and thus stop disease progression. The non steroidal antifungal compound ketoconazole was reported to cause gynecomastia in male patients and became the first drug to be used clinically for PC treatment. In line with ketoconazole, several steroidal and non steroidal compounds have been synthesized and evaluated as CYP17 inhibitors in the hope of finding more potent molecules with a better side effects' profile³.

Pre-clinical evaluation of several compounds designed as CYP17 inhibitors has shown that some of these compounds interact with other molecular targets such as the androgen receptor (AR) which is involved in disease progression, and might therefore bear additional interest as promiscuous agents for PC treatment³⁻⁵. We present our efforts and results in the synthesis of two series of novel androstane derivatives, generally designated as indazole derivatives and C17 2'-methylimidazole derived carbamates. Some of these compounds were found to inhibit CYP17 and also bind to the AR, blocking AR-mediated transcription^{6, 7}. Moreover, they inhibited the proliferation of the most common PC cell line models, namely LNCaP, PC-3 and LAPC4 cells.

ACKNOWLEDGMENTS

Vânia M. Moreira thanks Fundação para a Ciência e a Tecnologia for financial support (SFRH/BD/12508/2003). Jorge A. R. Salvador thanks Universidade de Coimbra for financial support.

REFERENCES

1. S. Nakajin; J. E. Shively; P. M. Yuan; P. F. Hall, Microsomal Cytochrome P-450 from Neonatal Pig Testis: Two Enzymatic Activities (17 α -Hydroxylase and C_{17,20}-Lyase) Associated with One Protein. *Biochemistry* **1981**, 20, (14), 4037-42.
2. P. F. Hall, Cytochrome P-450 C_{21sc}: One Enzyme with Two Actions: Hydroxylase and Lyase. *J. Steroid Biochem. Mol. Biol.* **1991**, 40, (4-6), 527-32.
3. V. M. Moreira; J. A. R. Salvador; T. S. Vasaitis; V. C. Njar, CYP17 Inhibitors for Prostate Cancer Treatment - An Update. *Curr. Med. Chem.* **2008**, 15, 868-899.
4. V. C. Njar; A. M. Brodie, Inhibitors of 17 α -Hydroxylase/17,20-Lyase (CYP17): Potential Agents for the Treatment of Prostate Cancer. *Curr. Pharm. Des.* **1999**, 5, (3), 163-80.
5. R. W. Hartmann; P. B. Ehmer; S. Haidar; M. Hector; J. Jose; C. D. Klein; S. B. Seidel; T. F. Sergejew; B. G. Wachall; G. A. Wachter; Y. Zhuang, Inhibition of CYP 17, a New Strategy for the Treatment of Prostate Cancer. *Arch. Pharm. Pharm. Med. Chem.* **2002**, 335, (4), 119-28.
6. V. M. Moreira; T. S. Vasaitis; V. C. Njar; J. A. R. Salvador, Synthesis and evaluation of novel 17-indazole androstene derivatives designed as CYP17 inhibitors. *Steroids* **2007**, 72, 939-948.
7. V. M. Moreira; T. S. Vasaitis; Z. Guo; V. C. Njar; J. A. R. Salvador, Synthesis of Novel C17 Steroidal Carbamates. Studies on CYP17 Action, Androgen Receptor Binding and Function, and Prostate Cancer Cell Growth. *Steroids* **2008**, 73, 1217-1227.

POSTER COMMUNICATIONS – PC.052

LACTATE DEHYDROGENASE A (LDH-A) INHIBITORS AS STARVATION AGENTS AGAINST INVASIVE HYPOXIC TUMOURS

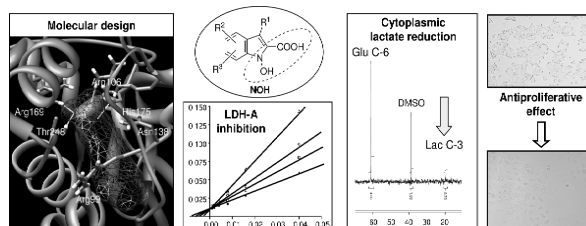
C. Granchi¹, S. Roy¹, T. Tuccinardi¹, A. Martinelli¹, M. Macchia¹, M. Lanza², L. Betti², G. Giannaccini², A. Lucacchini², L. G. León³, E. Giovannetti³, G. J. Peters³, R. Palchoudhuri⁴, P. J. Hergenrother⁴, F. Minutolo¹

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Hypoxic tumours, normally resistant to chemotherapy and radiotherapy, are often characterized by a metabolic switch ("Warburg effect") from normal oxidative phosphorylation to anaerobic glycolysis. This ensures a sufficient energy supply from glucose, even in hypoxic environments. One of the key enzymes of anaerobic glycolysis, the muscle isoform of lactate dehydrogenase (LDH-A), is over-expressed by metastatic cancer cells and is linked to the vitality of tumours in hypoxia. This enzyme constitutes a new target for anticancer agents, since its inhibition leads to a cut in energy supply to cancer, thus reducing its metastatic and invasive potential¹. LDH-A is regarded as a safe target since hereditary LDH-A deficiency causes a certain

level of myopathy only after intense anaerobic exercise, whereas it does not provoke any symptoms under ordinary circumstances². Nowadays, only a few LDH inhibitors have been developed, mainly because there was no evidence so far for health benefits deriving from this action³. Some catechol-type derivatives, originally designed as antimalarial agents, have very recently proved that LDH-A inhibition by small molecules represses tumour progression⁴.

We have discovered new carboxy-substituted N-hydroxyheterocycles (**NOH**), which are efficient and selective LDH-A inhibitors, with minimal inhibition of the heart isoform (LDH-B). Their activity in cells was confirmed by ¹³C NMR experiments measuring the reduction of the cytoplasmic lactate production in tumour cells, and they also proved to be efficient antiproliferative agents *in vitro*.



REFERENCES

1. V. R. Fantin, J. St-Pierre, P. Leder. *Cancer Cell.* **2006**, 9, 425.
2. T. Kanno, K. Sudo, M. Maekawa, Y. Nishimura, M. Ukita, K. Fukutake. *Clin. Chim. Acta* **1988**, 173, 89.
3. C. Granchi, S. Bertini, M. Macchia, F. Minutolo. *Curr. Med. Chem.* **2010**, 17, 672.
4. A. Le, C.R. Cooper, A.M. Gouw, et al. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, 107, 2037.

POSTER COMMUNICATIONS – PC.053

IMIDAZOLIUM-BASED FRAMEWORKS. II. A GENERAL COUNTERANION EXCHANGE PROCEDURE FOR ILS

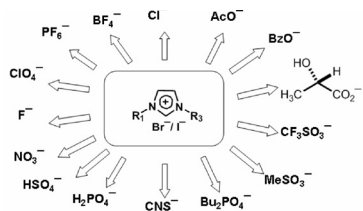
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The incorporation of imidazolium quaternary salts in a wide array of cationic and oligocationic systems situate them at the cross-roads of multidisciplinary fields in chemistry leading toward ionic liquids (ILs), anion recognition chemistry and N-heterocyclic carbenes¹. Simple imidazolium quaternary salts with a low melting point have become a green alternative to conventional organic solvents¹ with applications in the chemical industry³. Additionally, some ILs are also emerging as active pharmaceuti-

cal ingredients (APIs)⁴. Systematic anion variation could be a useful tool in the sustainable design of 'task specific ionic liquids'.

As part of our ongoing research into imidazolium-based frameworks, we recently reported an efficient and practical procedure using anion-exchange resins to obtain ionic liquids based on imidazolium salts with the selected anion counterpart quantitatively⁵.



We herein describe our current efforts directed to broadening this protocol to obtain an increased number of anions and cations, using non-aqueous media for the loading and exchange procedures.

ACKNOWLEDGMENTS

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REFERENCES

1. E. Alcalde, I. Dinarès, N. Mesquida. Imidazolium based receptors. In Topics in Heterocyclic Chemistry: Anion Complexation by Heterocycle Based Receptors. Springer-Verlag, Heidelberg, 2010. Due: November 2010.
2. T. Welton. Ionic liquids in synthesis. P. Wasserscheid and T. Welton, Eds. Wiley-VCH, Weinheim, 2008.
3. N. V. Plechkova, K. R. Seddon. Chem. Soc. Rev. 2008, 37, 123–150.
4. J. Stoimenovski, D. R. MacFarlane, K. Bica, R. D. Rogers. Pharmaceutical Research. 2010, 27, 521–526.
5. I. Dinarès, C. García de Miguel, A. Ibañez, N. Mesquida, E. Alcalde. Green Chem. 2009, 11, 1507–1510.

POSTER COMMUNICATIONS – PC.054

PDE7 INHIBITORS AS NEW POTENTIAL DRUGS FOR THE THERAPY OF SPINAL CORD INJURY

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Primary traumatic mechanical injury to the spinal cord (SCI) causes the death of a number of neurons that to date can neither be recovered nor regenerated. However, neurons continue to die for hours after SCI, and this represents a potentially avoidable event. Local inflammatory response in the injured spinal cord contributes significantly to the evolution of this secondary damage.

During the last years our group has been involved in the design, synthesis and evaluation of PDE7 inhibitors as new innovative drugs for several neurological disorders [1, 2].

Our working hypothesis is based on two different facts. Firstly, neuroinflammation is modulated by cAMP levels, thus the key role for phosphodiesterases (PDEs), which hydrolyze cAMP, is undoubtedly demonstrated. On the other hand, PDE7 is expressed simultaneously on leukocytes and on brain, highlighting the potential crucial role of PDE7 as drug target for neuroinflammation.

Here we present two chemically diverse families of PDE7 inhibitors, designed using computational techniques such as virtual screening and neuronal networks. We report their biological profile, and their efficacy in an experimental SCI model induced by the application of vascular clips (force of 24 g) to the dura via a four-level T5–T8 laminectomy.

We have selected two candidates, namely S14 and VP1.15, as PDE7 inhibitors with IC₅₀ values in the micromolar range. These compounds increase cAMP production both in macrophage and neuronal cell lines. Moreover, the inflammatory response induced by LPS treatment in both types of cells was reduced after being treated with these PDE7 inhibitors. The inflammatory response was completely reversed by treatment of the cells with the membrane-permeable inhibitor of cAMP Rp-cAMP, suggesting that the cAMP signaling pathway mediates the effects of both S14 and VP1.15 compounds.

Regarding drug like properties, compounds were able to cross the blood brain barrier using parallel artificial membranes (PAMPA) methodology. SCI in mice resulted in severe trauma characterized by edema, neutrophil infiltration, and production of a range of inflammatory mediators, tissue damage, and apoptosis. Treatment of the mice with S14 and VP1.15, two PDE7 inhibitors, significantly reduced the degree of spinal cord inflammation, tissue injury (histological score), and TNF- α , IL-6, COX-2 and iNOS expression. All these data together, led us to propose PDE7 inhibitors, and specifically S14 and VP1.15, as potential drug candidates to be further studied for the treatment of SCI.

REFERENCES

1. Castro A, Jerez MJ, Gil C, Martínez A. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: advances in the development of specific phosphodiesterase inhibitors. Med Res Rev. 2005 25:229–44.
2. Gil C, Campillo NE, Perez DI, Martínez A. PDE7 inhibitors as new drugs for neurological and inflammatory disorders. Exp. Opin. Ther. Patents 2008, 18:1127–1139

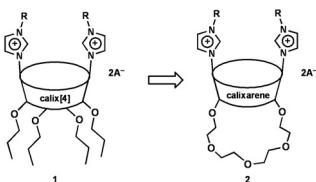
POSTER COMMUNICATIONS – PC.055

IMIDAZOLIUM-BASED FRAMEWORKS. I.
DICATIONIC SYSTEMS FOR ANION
RECOGNITION*Neus Mesquida, Anna Ibáñez and Immaculada Dinarès*

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The development of new receptors, sensors and transporters for anionic species continues to be a rich area of research due to the number of fundamental roles played by anions in biological and chemical processes. In the past few years, imidazolium functionality has gained a place among the classical anion binding functional groups and has emerged as an attractive starting point for the design of abiotic anion receptors and selective molecular sensors^{1,2}. In parallel, calixarenes are particularly attractive scaffolds for receptor development, the macrocyclic core being available in a variety of sizes, easily pre-organized into a number of topographies and readily selectively functionalized for the apposite introduction of ligands³. These qualities have given a biological perspective to the rapidly growing field of Bionanotechnology, whose aim is to develop new tools for biology, new biomaterials, selective sensors and supramolecular devices for clinical analysis, new therapeutics and smart drug delivery systems.

We recently published the anion recognition studies of **1**-type bis(imidazolium)-calixarenes⁴. Continuing our research into imidazolium-based frameworks, we herein report the binding properties of **2**-type calixarene crowns with bis(imidazolium) salts directly bonded to the upper rim and a polyether chain tethered across the lower rim.



ACKNOWLEDGMENTS

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REFERENCES

1. E. Alcalde, I. Dinarès, N. Mesquida. Imidazolium based receptors. In Topics in Heterocyclic Chemistry: Anion Complexation by Heterocycle Based Receptors. Springer-Verlag, Heildelberg, 2010. Due: November 2010.
2. C. Caltagirone, P. A. Gale. Chem. Soc. Rev. 2009, 520–563.
3. L. Baldini, A. Casnati, F. Sansone, R. Ungaro. Chem. Soc. Rev. 2007, 36, 254–266.

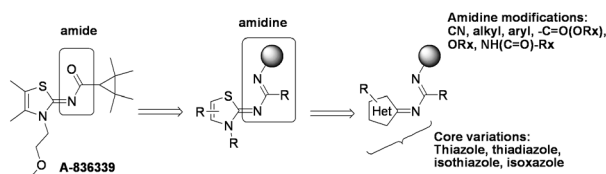
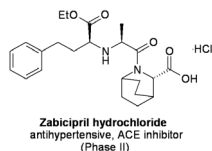
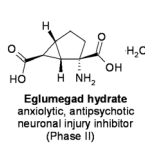
4. I. Dinarès, C. Garcia de Miguel C, N. Mesquida, E. Alcalde. J. Org. Chem. 2009, 482–485.

POSTER COMMUNICATIONS – PC.056

CONFORMATIONAL RESTRICTION IN DRUG
DESIGN: THE AMINO ACID CASE*Igor V. Komarov¹, Oleksandr O. Grygorenko^{1,2}, Dmytro S. Radchenko², Oleksiy S. Artamonov², Nataliya A. Kopylova¹, Pavel K. Mykhailiuk^{1,2}, Andrey A. Tolmachev²*

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Contemporary medicinal chemistry tends to narrow the search area of the chemical space dealing preferably with compounds which have high probability of displaying desirable pharmacokinetic and pharmacodynamic properties. The Lipinski's "Rules of Five" impose such limitations on the chemical space, defining the "drug-like" compounds. Still, the drug-like chemical space, as defined today, is very large. A recent paper on enumeration of small drug-like organic molecules containing up to 13 atoms of C, N, O, S, and Cl reported more than 970 million compounds [1]. There is an obvious need to use further limitations and concepts in drug design, narrowing the search area for new drugs. One of such concepts is decrease of conformational mobility or rigidification of drug candidate and building block molecules [2]. In principle, conformationally restricted molecules, due to their pre-organization, can be better ligands for biological targets, thus displaying more selective and pronounced biological activity, comparing to flexible analogues. We performed analysis of the literature and MDL Drug Data Report Database in order to investigate the use of a particular class of the building blocks – conformationally restricted amino acids (CRAAs) in drug design. The analysis showed that cyclic and quaternary α -amino acids are intensively used for construction of new drug candidates; however, bi- and polycyclic amino acids and their derivatives are under-represented among the actively studied compounds. Only a few compounds of this type reached clinical stage of development, for example, eglumegad and zabicipril. At the same time, bi- and polycyclic CRAAs have very large structural variability which allows fine-tuning the structure and properties of the drug candidate to the need of the design. We suggest that one of the reasons which hinder the use the bicyclic CRAAs in drug design is the lack of efficient and practical syntheses of them, and concentrated our efforts on improving the known and developing new approaches to this valuable class of building blocks. A number of methods were developed; they are based on intramolecular cyclization of different types, tandem Strecker reaction – intramolecular nucleophilic substitution, ylide addition to C=C double bonds, photochemical [2+2] cycloaddition, [3+2] dipolar cycloaddition for the construction of conformationally restricted scaffolds.



REFERENCES

- Blum, L.C.; Reymond, J.-L. *J. Am. Chem. Soc.* **2009**, 131, 8732.
- Mann A. Conformational restriction and/or steric hindrance in medicinal chemistry. In: *Practice of Medicinal Chemistry (2nd Edition)* Editor: Wermuth, C. G. Elsevier, London, **2003**, 233-250.

POSTER COMMUNICATIONS – PC.057

DESIGN, SYNTHESIS, AND INITIAL PHARMACOLOGY OF SEVERAL NEW AMIDINE CLASSES OF SELECTIVE CB₂ AGONISTS

Teodozyj Kolasa, Meena Patel, Jennifer Frost, Steven Latshaw, Xueqing Wang, Arturo Perez-Medrano, Sridhar Peddi, Tongmei Li, Bo Liu, William Carroll, Michael Dart, Anthony Daza, George Grayson, Yihong Fan, Loan Miller, Lanlan Li, Bradley Hooker, Tiffany Garrison, Odile El-Kouhen, Jill Wetter, Gin Hsieh, Betty Yao and Michael Meyer

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Both CB₁ and CB₂ cannabinoid receptor agonists have demonstrated antinociceptive properties. The clinical utility of mixed cannabinoid agonists for the treatment of pain is limited by central effects (psychoactivity, hypoactivity and hypothermia) and tolerance due to activation of CB₁ receptors. A distinct benefit of highly selective CB₂ agonists is their ability to display robust effects across a range of preclinical models of inflammatory, post-operative and neuropathic pain, without the undesirable CNS-mediated side effects associated with central CB₁ receptor activation. Clinical validation for the CB₂ target has not been achieved, and research continues to be directed toward the identification of CB₂ agonists for effective management of pathological pain in humans.

Previously we reported the pharmacological activity of the thiazolylidene amide A-836339, a selective CB₂ agonist. Replacement of the amide group in the thiazole series with a cyanoamidine led to compounds that retained high levels of CB₂ affinity and improved levels of selectivity vs. the CB₁ receptor. To expand SAR investigations around this new structural class of CB₂ agonists, additional modifications of the amidine moiety and variations of the core were desired.

Here we describe the two synthetic approaches to these various amidine compound classes. The first, which allowed access to the thiazole- and thiadiazole-based amidines, involved transformation of the amide to the corresponding thioamide followed by amination in the presence of a mercury salt. An alternate method was required for preparation of isoxazolylidene- and isothiazolylidene-cyanoamidines. The second approach was designed to prepare cyanoamidines of all core variants, and involved the reaction of the heterocycle-imine with dimethyl cyanocarbonimidodithiate. The resulting carbamimidodithiate intermediate was coupled with arylboronic acids in the presence of palladium (0) and copper (I) to afford the desired cyanoamidine analogs. A variety of amidine derivatives were synthesized and characterized in assays to evaluate cannabinoid receptor binding affinity, selectivity, and agonist activity. Several new, highly selective amidine CB₂ ligands have been identified that possess favorable PK properties and robust efficacy in animal pain models.

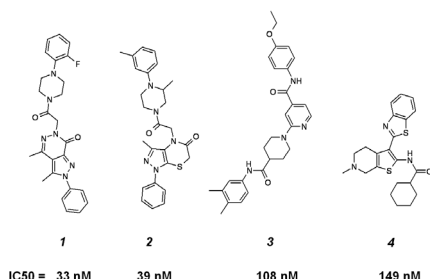
POSTER COMMUNICATIONS – PC.058

DISCOVERY AND OPTIMIZATION OF POTENT AND SELECTIVE SMO/HEDGEHOG PATHWAY INHIBITOR

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Hedgehog signalling is activated in a diverse spectrum of cancers, and its pharmacological blockade has emerged as an anti-tumour strategy. Several Hedgehog inhibitors were developed and advanced into clinical trials. So far the main mechanism for all compounds in clinical trials is an inhibition of the pathway activity on the level of activating receptor, Smoothened (Smo). We report here various new Smo/Hh pathway antagonists (e.g., hits 1-5) that were discovered by implementation of Targeted Diversity™ strategy for discovery of small molecules, inhibitors of signalling pathways. Targeted Diversity™ has been created as a universal tool for the design of high quality libraries of drug-like compounds that have been focused against multiple biological targets. These targets may be congener or 'orthogonal' (non-overlapping) and include (a) different classes of targets, (b) distinct, structurally unrelated branches of the same target class and (c) independent targets.



Development of SAR for different classes of discovered hits 1-5 led to the discovery of *CD-005-0002*, a novel potent inhibitor of the hedgehog pathway. The compound was optimized for potency, selectivity, PK and drug-like properties by modifications of the core and amide portion of the molecule. *CD-05-0002* is a potent hedgehog pathway inhibitor ($IC_{50} = 28 \text{ nM}$) that not only competes in Smo binding with bodipy-cyclopamine but also has activity on yet unknown targets below SMO level. Compound does not show any activity against a panel of 96 kinases as well as WNT and NF κ B pathways. *CD-05-0002* reduces mGluR expression in different cell-based models and produces tumor regression in MiaPaca-2 and Panc-1 mouse models.

The mechanism of action and ADME characteristics of *CD-05-0002* will be discussed.

POSTER COMMUNICATIONS – PC.059

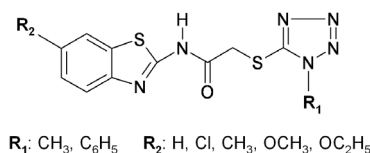
SYNTHESIS AND ANTINOCICEPTIVE ACTIVITY OF SOME ACETAMIDE DERIVATIVES

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The experimental researches for the development of safer and more effective analgesic agents are a great deal of interest for many researchers. In the chemical literature, derivatives of acetamide have been described with the antinociceptive activity [1,2].

In the present study, some acetamide derivatives were synthesized to investigate their potential antinociceptive activities. The N-(benzothiazol-2-yl)-2-(1-substituted-¹H-tetrazol-5-ylthio)acetamide derivatives were synthesized by reacting 2-(chloroacetyl)benzothiazole derivatives with tetrazol-5-thioles. The chemical structures of the compounds were elucidated by ¹H NMR and FAB+-MS spectral data and Elemental analyses. The prepared compounds were investigated for their antinociceptive activity using by hot plate and acetic acid-induced writhing tests.



REFERENCES

1. T. Önkol, D. S. Dogruer, S. Ito, M. F. Sahin, "Synthesis and Antinociceptive Activity of (5-Chloro-2-benzothiazolinon-3-yl)acetamide Derivatives", *Archiv der Pharmazie*, 333(10), 337 – 340, (2000).
2. An-Chih Chang, Alan Cowan, Akira E. Takemori, Philip S. Portoghesi, Aspartic Acid Conjugates of 2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(3-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide: Opioid Receptor Agonists with Limited Access to the Central Nervous System, *J. Med. Chem.*, 39, 4478-4482, (1996).

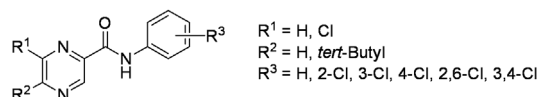
POSTER COMMUNICATIONS – PC.060

STRUCTURE-PROPERTY RELATIONSHIPS IN SERIES OF CHLORINE-SUBSTITUTED PYRAZINECARBOXANILIDES AS BIOLOGICALLY ACTIVE COMPOUNDS

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Compounds possessing -NHCO- moiety simulating a peptide bond in their molecule show a broad range of biological effects. Various ring-substituted anilides of pyrazine-2-carboxylic acid were prepared and evaluated as potential antimicrobial and herbicidal agents or abiotic elicitors [1-7]. Twenty substituted N-phenylpyrazine-2-carboxamides were analysed using the RP-HPLC method for the lipophilicity measurement. Lipophilicity is one of the most important physical properties of biologically active compounds. It influences the transport of a molecule through cellular membranes, because drugs cross biological barriers most frequently through passive transport, which strongly depends on their lipophilicity. Lipophilicity also characterizes drug solubility.



In the present study the correlation between the RP-HPLC retention parameter $\log k$ and $\log P$ data calculated in various ways is shown, and the relationships between the lipophilicity and the

chemical structure of the studied compounds are discussed. The distributive parameters π of various substituents are listed for the mentioned studied compounds. The determined π parameters of substituents can be used for describing relationships between physico-chemical properties and biological activity of prepared ring-substituted pyrazine-based compounds. Although all the discussed compounds are relatively simple structures substituted within the series only by chlorine, interesting intramolecular interactions influencing lipophilicity were observed probably due to the presence of a pyrazine ring and a carboxamide moiety.

The study was supported by MSM0021620822.

REFERENCES

1. Dolezal, M.; Vicik, R.; Miletin, M.; Kralova, K. *Chem. Pap.* **2000**, *54*, 245.
2. Dolezal, M.; Miletin, M.; Kunes, J.; Kralova, K. *Molecules* **2002**, *7*, 363.
3. Dolezal, M.; Palek, L.; Vinsova, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Molecules* **2006**, *11*, 242.
4. Dolezal, M.; Tumova, L.; Kesetovicova, D.; Tuma, J.; Kralova, K. *Molecules* **2007**, *12*, 2589.
5. Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Eur. J. Med. Chem.* **2008**, *43*, 1105.
6. Dolezal, M.; Zitko, J.; Kesetovicova, D.; Kunes, J.; Svobodova, M. *Molecules* **2009**, *14*, 4180.
7. Tumova, L.; Tuma, J.; Megusar, K.; Dolezal, M. *Molecules* **2010**, *15*, 331.

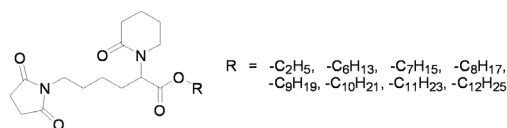
POSTER COMMUNICATIONS – PC.061

PREPARATION AND PHYSICO-CHEMICAL PROPERTIES OF ALKYL-6-(2,5-DIOXOPYRROLIDIN-1-YL)-2-(2-OXOPIPERIDIN-1-YL) HEXANOATES

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Transdermal penetration enhancers are special pharmaceutical excipients that interact with skin components to increase penetration of drugs from the topical dosage forms to blood circulation. Numerous compounds have been evaluated as penetration enhancers and many potential sites and modes of action have been identified for them [1]. This is a follow-up paper to our previous articles [2-5] and deals with synthesis, physico-chemical properties and 3D structures of below mentioned compounds derived from 6-aminohexanoic acid prepared as potential transdermal penetration enhancers.



The aim of modification of the molecule ester part with linear alkyl chains was an increase of lipophilicity and a consequent increase of penetration/carrier activities. Lipophilicity is one of the most important physical properties both for biologically active compounds and for excipients; it influences the transport of a molecule through cellular membranes. The lipophilicity (nonpolarity) of enhancers was determined and the corresponding relationship between enhancer nonpolarity and penetration enhancement potency is under further investigation.

This study was supported by the Ministry of Education of the Czech Republic MSM 6215712403.

REFERENCES

1. Williams, A.C.; Barry, B.W. *Adv. Drug Deliv. Rev.* **2004**, *56*, 603.
2. Brychtova, K.; Farsa, O.; Csollei, J. *ECSOC-12* **2008**, c005.
3. Brychtova, K.; Farsa, O.; Csollei, J. *Lett. Org. Chem.* **2009**, *6*, 25.
4. Brychtova, K.; Dittrichova, S.; Slaba, B.; Placek, L.; Opatrilova, R.; Jampilek, J.; Csollei, J. *ECSOC-13* **2009**, c010.
5. Brychtova, K.; Jampilek, J.; Opatrilova, R.; Raich, I.; Farsa, O.; Csollei, J. *Bioorg. Med. Chem.* **2010**, *18*, 73.

POSTER COMMUNICATIONS – PC.062

PLASMODIUM FALCIPARUM AMINOACYL-tRNA SYNTHETASES AS TARGETS IN THE SEARCH FOR NEW ANTIMALARIAL DRUGS

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Malaria is a wide spread infectious disease caused by the parasite Plasmodium. Every year 350 to 500 million of people are infected of which 1 to 3 million, mainly young children, die¹. Although there are a number of antimalarial drugs on the market^{2,3}, the rapid developing/developed resistance of the parasite to these drugs makes that the search for new medicines against Malaria an urgent necessity.

Aminoacyl-tRNA synthetases (aaRS) are important enzymes in the protein synthesis machinery of living organisms⁴. They catalyze the esterification of a specific amino acid or its precursor to one of all its compatible cognate tRNAs to form an aminoacyl-

tRNA in a two step process as shown in figure 1. The first step is the binding of ATP to the corresponding amino acid (AA) to form an aminoacyl-adenylate and the release of inorganic pyrophosphate (PP_i). In the second step the amino acid is transferred from the AA-AMP complex to the tRNA.



Figure 1: Two step mechanism of aaRS mediated esterification of AA with tRNA

Mephitis is a consortium of 8 research groups from 5 different countries⁵. The main goal of the consortium is to significantly advance the understanding of protein synthesis in *Plasmodium* and to contribute to the effort on drug discovery against malaria, taking the aminoacyl-tRNA synthetases of *Plasmodium falciparum* as potential drug targets. To do so, three different areas of research are covered, e.g. evolution and structural biology, molecular biology and biochemistry and drug design and discovery.

In this paper we will discuss the latest developments on the design and synthesis of new inhibitors of *Plasmodium falciparum* tRNA synthetases and related proteins.

REFERENCES

1. <http://en.wikipedia.org/wiki/Malaria>
2. M. Schlitzer, *Arch. Pharm. Chem. Life Sci.* **2008**, 341, 149-163.
3. "The Elusive Malaria Vaccine: Miracle or Mirage?" by Irwin W. Sherman, ASM Press, Washington DC, **2009**
4. M. Ibba; D. Söll *Annu. Rev. Biochem.* **2000**, 69, 617-650
5. <http://www.mephitis.eu>

POSTER COMMUNICATIONS – PC.063

DESIGNED MULTIPLE LIGANDS AS PHARMALOGICAL TOOLS TO DETECT A_{2A}-D₂ RECEPTOR HETEROMERS

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Due to a better understanding of the pathogenesis of diseases in the recent decades, drug discovery has gradually shifted from a

phenotype-based endeavor to today's single-molecular-target centered approach¹. As a result, it has become and still is aimed mainly at the discovery of small molecules able to aim a single protein target, which is thought to be responsible for a certain disease². This one-molecule, one-target approach has resulted in the discovery of a large number of successful drugs. Nevertheless, a highly selective ligand for a specific target does not always result in a highly efficient drug. This might be due to, for example, a complex mechanism of the disease in which multiple pharmacological targets play a role. One approach to overcome this problem is the use of Designed Multiple Ligands (DMLs)³⁻⁵. DMLs drugs are single molecules in which two drugs have been combined. Consequently, this molecule can interact specifically on multiple targets of relevance to a disease (targeted polypharmacology).

Recently we have published a series of DMLs which could be used as pharmacological tools in the detection of heteromized Adenosine A_{2a} (A_{2A}R) and Dopamine D₂ (D₂R) receptors⁶. Additionally, these DMLs can be potential new drug candidates against Parkinson's Disease (PD). It has been demonstrated that in the stratum the interactions between D₂ Dopamine Receptor (D₂R) and A_{2a} Adenosine Receptor (A_{2A}R) are antagonistic and have shown implications in neurological illnesses such as Parkinson's Disease (PD) and its potential treatment⁷⁻⁹.

In this poster we present the recent results obtained in the design and synthesis of new DMLs and their subsequent pharmacological characterization.

REFERENCES

1. P. T. Lansbury; *Nat. Rev. Neurosci.* **2004**, 5 (Suppl.), S51-S53.
2. S. Frantz; *Nature* **2005**, 437, 942-943.
3. R. Morphy et al.; *J. Med. Chem.* **2005**, 48(21), 6523-6543.
4. R. Morphy et al.; *Drug Disc. Today* **2004**, 9(15), 641-651.
5. A. Cavalli et al.; *J. Med. Chem.* **2008**, 51(3), 347-372.
6. A. Soriano et al.; *J. Med. Chem.* **2009**, 52(18), 5590-5602.
7. K. Fuxe et al.; *Neurology* **2003**, 61, S19-S23.
8. P. Svenningsson et al.; *Neurology* **2003**, 61, S5-S9.
9. M. Deogaonkar et al.; *Brain Res. Rev.* **2005**, 50, 156-168.

POSTER COMMUNICATIONS – PC.064

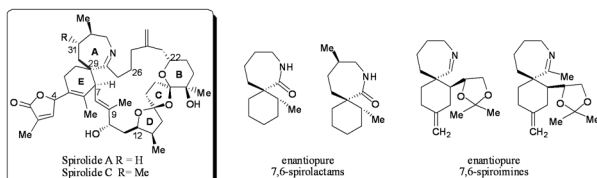
SYNTHETIC STUDIES TOWARDS THE SPIROIMINE UNIT OF THE SPIROLIDES

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The spirolide family is a class of marine toxins isolated from the digestive glands of contaminated mussels (*Mytilus edulis*), scallops (*Placopecten magellanicus*) and phytoplankton from the south-eastern coast of Nova Scotia in Canada¹. The spirolides

are metabolites of the dinoflagellate *Alexandrium ostenfeldii* and *A. peruvianum*. To date fourteen members of the spirolide family have been identified from contaminated shellfish extracts and cultures of toxic strains of *A. ostenfeldii* around the world. The spirolides are considered to be fast-acting neurotoxins due to their pronounced effects in the mouse bioassay. They are also antagonists of the nicotinic and muscarinic acetylcholine receptors as well as weak activators of L-type calcium channels, rendering these compounds possible candidates for the treatment of cardiovascular disorders². The spirolides contain a bis-spiroacetal unit, previously synthesised by the Ishihara³ and Brimble groups⁴, and an unusual 7,6-spiroimine moiety that has not yet been synthesised. The spiroimine moiety of the spirolides is thought to be the pharmacophore⁵. The first part of our studies aims to establish the quaternary centre C29 in the spirolides using an enantioselective Birch reductive alkylation. This strategy provided access to a wide range of enantiopure bicyclic spiro-lactams⁶. Unfortunately, the lactam functionality could not be converted to an imine. Subsequent, an improved retrosynthetic pathway, making use of a Diels-Alder reaction⁷, allows the construction at the same time of both C29 and C7 stereocentre in the spirolides. Further elaboration of the Diels-Alder adduct affords access to the key ketoamine or aldamine intermediate, in good overall yield. Final cyclisation of the advanced intermediate provides the first synthesis of an enantiopure 7,6-bicyclic spiroimine containing both C7 and C29 stereocentres of the spirolides. Further stability studies of the aldimine and ketimine are in progress.



REFERENCES

- Hu, T.; Curtis, J. M.; Oshima, Y.; Quilliam, M. A.; Walter, J. A.; Watson-Wright, W. M.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1995** 2159-2161.
- Guéret, S. M.; Brimble, M. A. *Mar. Drugs* **2010**, ASAP.
- Ishihara, J.; Ishizaka, T.; Suzuki, T.; Hatakeyama, S. *Tetrahedron Lett.* **2004**, 45, 7855-7858.
- Meilert, K.; Brimble, M. A. *Org. Biomol. Chem.* **2006**, 4, 2184-2192.
- Hu, T. M.; Curtis, J. M.; Walter, J. A.; Wright, J. L. C. *Tetrahedron Lett.* **1996**, 37, 7671-7674.
- Guéret, S. M.; O'Connor, P. D.; Brimble, M. A. *Org. Lett.* **2009**, 11, 963-966.
- Asano, M.; Inoue, M.; Watanabe, K.; Abe, H.; Katoh, T. *J. Org. Chem.* **2006**, 71, 6942-6951.

POSTER COMMUNICATIONS – PC.065

CANNABINOIDS AS MULTITARGET DRUGS FOR ALZHEIMER DISEASE

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Neurodegenerative diseases are an increasing public concern, being currently the eighth cause of disease burden for developed regions. According to the World Health Organization (WHO), neurodegenerative diseases will become the world's second leading cause of death by the middle of the century, overtaking cancer. This fact will have a dramatic impact in medical care, as well as important social and economic implications, unless more effective preventive procedures or treatments are available. Alzheimer's disease (AD), characterized by cortical amyloid plaques and neurofibrillary tangles is the most common cause of dementia, accounting for probably 50-75% of all dementias worldwide, followed by vascular dementia, mixed dementia, and Lewy body dementia¹.

The targeting of multiple receptors through a single drug molecule is gaining increasing acceptance. This multifunctional drug paradigm strongly challenges the widely held assumption that single-target agents are better to "dirty drugs" in therapy. Although the single-target approach currently remains the major drug discovery strategy in pharmaceutical companies, there is increasing recognition of the limitations of such an approach for complex diseases².

During the last years there are more evidences about the implications of the cannabinoid system on neurodegenerative processes such as excessive glutamate production, oxidative stress and neuroinflammation. Moreover, several evidences indicate the relation that exists between the cannabinoid and the cholinergic systems.

Regarding to this, the aim of our study has been to development a new family of multitarget drugs with cannabinoid and cholinergic properties for the treatment of neurodegenerative diseases as Alzheimer disease or Lewy body dementia. The key steps for this task have been the design of the new family of drugs using docking tools, the synthesis and the biological study of cannabinoid and cholinergic properties. Thus, we have obtained a new family of 3-indazolyl ether derivatives as innovative strategy for treatment of Alzheimer disease³.

REFERENCES

- Campillo N.E.; Páez J.A. Cannabinoid System in Neurodegeneration. New Perspectives in Alzheimer's disease. *Mini Rev. Med. Chem.* **2009**, 9 (5), 539-59

2. Van der Schyf, C. J.; Youdim, M. B. Multifunctional drugs as neurotherapeutics. *Neurotherapeutics* **2009**, 6, 1-3.
3. Paéz J.A, Campillo, N.E, González P., Pérez C., Arán V.J., Martín M. I., Girón R., Sánchez E. M. Una nueva familia de éteres de 3-indazolilo con propiedades cannabinoides y/o colinérgicas. ESP. P200930775. **2009**. CSIC- Universidad Rey Juan Carlos.

POSTER COMMUNICATIONS – PC.066

A DIBROMOPYRIDO[2,3-D]PYRIMIDINE AS A GENERAL PLATFORM FOR THE DEVELOPMENT OF PROTEIN KINASE INHIBITORS

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Since the beginning of nineties, the research in the field of human signalling pathways has continuously provided hundreds of new therapeutic targets. Among those, protein kinases have proved to be highly relevant in cancer, immunological, nervous, endocrine and cardiac diseases, diabetes, Alzheimer¹, asthma², arteriosclerosis, leukemia³, arthritis⁴.

As a result of this growing knowledge about human transduction machinery, different inhibitory strategies have been proposed and studied: only few of them have succeeded. Some small molecules have been described as protein kinase inhibitors and potential therapeutic agents, some of the more promising candidates belonging to pyrido[2,3-*d*]pyrimidine family.

Traditionally, the heterocyclic moiety of this kind of compounds is synthesized in several steps, the required substituents being progressively introduced^{5,6}. Thus, starting from a pyrimidine ring bearing some of the final substitution pattern, the formation of the pyridine ring is achieved by condensation with reagents carrying another part of final substitution pattern. That is to say, diversity should be progressively introduced thus limiting the number of accessible products. In addition, some chemical incompatibilities could also limit the accessible chemical diversity.

As part of our research in the field of potential tyrosine kinase inhibitors, we have disclosed a series of straightforward methodologies for the synthesis of pyrido[2,3-*d*]pyrimidines which are based on a Michael addition between α,β -unsaturated esters and active methylene compounds, followed by cyclization with guanidines and amidines^{7,8}.

Herein we describe a new three-step protocol for the synthesis of a dibromo substituted 2-arylaminopyrido[2,3-*d*] pyrimidine scaffold in which the formation of pyridopyrimidine skeleton is achieved in one step from an α,β -unsaturated ester, malononitrile and an arylsubstituted guanidine. The subsequent regioselective bromination afforded, in two steps and in high yield the

aforementioned dibromosubstituted pyrido[2,3-*d*]pyrimidine. The two bromine atoms are easily and selectively replaced by carbon, nitrogen and oxygen substituents by standard Suzuki and Ullmann protocols, thus achieving new and already described biologically active pyrido[2,3-*d*]pyrimidines substituted in positions C6, C4 and in the phenylamino ring present in C2.

REFERENCES

1. Ho, G. J. *et al.*, *Current Drug Targets: Inflammation & Allergy*, **2005**, 4(2), 247
2. Wong, W. F., *Current Opinion in Pharmacology*, **2005**, 5(3), 264
3. Noriko, U., *Cancer & chemotherapy*, **2005**, 32(3), 297
4. Collett, M. S. *et al.*, *Proc. Natl Acad. Sci. USA*, **1978**, 75, 2021
5. Klutchko, S. R. *et al.*, *Journal of Medicinal Chemistry*, **1998**, 41(17), 3276
6. Schroeder, M. C. *et al.*, *Journal of Medicinal Chemistry*, **2001**, 44(12), 1915
7. Mont, N. *et al.*, *Molecular Diversity*, **2003**, 7, 153
8. Berzosa, X. *et al.*, *Journal of Organic Chemistry*, **2010**, 75, 487

POSTER COMMUNICATIONS – PC.067

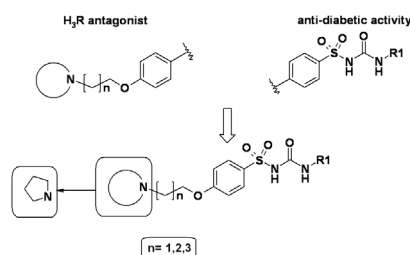
DUAL HISTAMINE H₃ ANTAGONISTS/ANTI-DIABETIC AGENTS: DESIGN, SYNTHESIS, BIOLOGICAL EVALUATION AND SAR

Silvia Galiano, Saioa Ancizu, Nerea Castrillo, Javier Ceras, Silvia Pérez-Silanes, Ignacio Aldana, Antonio Monge

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More than 400 million people suffer from obesity worldwide, making it one of today's global health issue. Hand in hand with the obesity rates is the rapid widespread of type 2 diabetes emerging in a new epidemic: the diabetes¹.

The combination of antagonism at histamine H₃ receptors² and the stimulation of insulin secretion through the sulfonylurea system³ has been proposed as an approach of new dual therapeutic agents for the treatment of obesity and type 2 diabetes mellitus.



Based on this strategy and as part of our ongoing programme to develop new anti-obesity drugs⁴, we have designed and synthesized a new series of non-imidazole derivatives, based on a basic pyrrolidine ring connected through an alkyl spacer of variable length to a phenoxysulfonylurea moiety. These compounds were initially evaluated for histamine H₃ receptor binding affinities suggesting that a propoxy chain linker between the amine and the core ring could be essential to the optimal binding affinity. The results confirmed these derivatives as a promising starting point for the design of new H₃-antagonists with anti-diabetic activity.

REFERENCES

1. Diabetes Prevention Program Research Group. *N. Engl. J. Med.* **2002**, 346, 393–403.
2. Malmlof, K.; Hastrup, S.; Wulff, B. S.; Hansen, B. C.; Peschke, B.; Jeppesen, C. B.; Hohlweg, R.; Rimvall, K. *Biochem. Pharmacol.* **2007**, 73, 1237.
3. Nissen, S.E.; Nicholls, S.J.; Wolsky, K.; Nesto, R.; Kupfer, S.; Pérez, A.; Jure, H.; Delarochellière, R.; Staniloae, C.S.; Mabromatis, K.; Saw, J.; Hu, B.; Lincoff, A.M.; Tuzcu, E.M. *JAMA* **2008**, 299(13), 1561-1573
4. Galiano, S.; Ceras, J.; Cirauqui, N.; Pérez, S.; Juanenea, L.; Rivera, G.; Aldana, I.; Monge, A. *Bioorg. Med.Chem.* **2007**, 15, 3896-3911

POSTER COMMUNICATIONS – **PC.068**

P-PIPERIDINYLLALKYL-PHENOXYLSULFONYLUREAS: NEW DERIVATIVES AS DUAL H₃ RECEPTOR ANTAGONISTS AND ANTI-DIABETIC ACTIVITY

Nerea Castrillo, Saioa Ancizu, Silvia Galiano, Javier Ceras, Silvia Pérez-Silanes, Ignacio Aldana and Antonio Monge

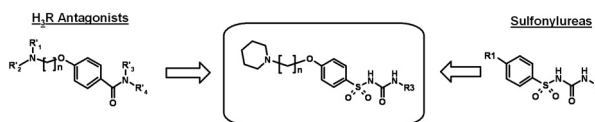
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A new threat has appeared in worldwide health: the diabetes epidemic. The World Health Organization estimated in 2005 that 1.1 million people died as a result of diabetes¹. Due to obesity is the major risk factor in type 2 diabetes, it's necessary to develop new treatments to the prevention in this epidemic.

It is known that histamine H₃ receptor antagonists are able to promote weight loss or prevent weight gain. Although most H₃ receptor antagonists had an imidazol moiety in their structure, recent studies have shown that N-containing non-aromatic heterocycles act as a potent anti-obesity agents². A-331440 is a novel non-imidazol-based antagonist of the histamine H₃ receptor which apparently reduces weight³.

The American Diabetes Association has suggested that sulfonylurea system is clinically significant due to its anti-diabetic properties. Glimepiride is the first third-generation sulfonylurea approved as oral anti-diabetic drug⁴.

Based on current findings, our research group proposed new compounds with dual activity as anti-obesity and anti-diabetic agents. Our aim was the design and synthesis of 22 new p-piperidinyllalkylphenoxysulfonylurea derivatives in order to evaluate the biological activity against H₃R and their affinity with hERG receptor.



ACKNOWLEDGMENTS

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REFERENCES

1. Nath, D., Heemels, M.T., Anson, L. *Nature* **(2006)**, 444 (7121), 839.
2. Meier, G.et al. *Eur. J. Pharm. Sci.* **(2001)**, 13, 249-259.
3. Hancock, A.A. *Curr. Opin. Investig.* **(2003)**, 4, 1190-1197.
4. Nissen, S.E.; Nicholls, S.J.; Wolsky, K.; Nesto, R.; Kupfer, S.; Pérez, A.; Jure, H.; Delarochellière, R.; Staniloae, C.S.; Mabromatis, K.; Saw, J.; Hu, B.; Lincoff, A.M.; Tuzcu, E.M. *JAMA* **(2008)**, 299(13), 1561-1573.

POSTER COMMUNICATIONS – **PC.069**

DOCKING OF SUBSTRATES AND INHIBITORS INTO THE SUBSTRATE BINDING SITE OF TWO SEROTONIN TRANSPORTER MODELS

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The serotonin transporter (SERT) belongs to the neurotransmitter:sodium symporter (NSS) family. Located in the membranes of presynaptic neurons, the transporter removes serotonin (5-HT) from the synaptic cleft, thus reducing the number of neurotransmitters available for interaction with postsynaptic receptors. The transporter is targeted by two of the main classes of antidepressant drugs - the selective serotonin reuptake inhibitors (SSRIs) and the tricyclic antidepressants (TCAs) - and is also the target for psychostimulants such as cocaine, MDMA (ecstasy), and d-amphetamine. The three-dimensional structure of SERT has not yet been determined. However, the first three-

dimensional structure of a NSS family member, the *Aquifex* aeolicus leucine transporter, LeuT, is available both in an occluded conformation (**1**) as well as in an outward-facing conformation (**2**). Based on a comprehensive alignment of NSS family members (**3**), two different homology models of SERT representing two different conformational states were generated using the ICM program package and the two LeuT crystal structures as templates and the models were used for docking of a set of known substrates and several inhibitors into the putative substrate binding site. Our results indicated that the bacterial leucine transporter crystal structures are reliable templates for generating SERT homology models for subsequent docking of substrates and inhibitors. The docking indicated that D98 is a key amino acid for binding both substrate and inhibitors. Transmembrane helices 1, 3, 6 and 8 were involved in interactions with substrates and inhibitors in both SERT models.

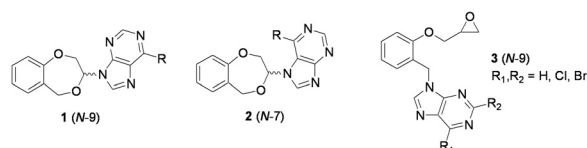
ACKNOWLEDGMENTS

This study was partly supported by a grant PNRF-103-AI-1/07 through the Norwegian Financial Mechanism and the Norwegian Research Foundation.

REFERENCES

1. Yamashita A., Singh S.K., Kawate T. et al. *Nature* 2005; 437:215-223.
2. Singh SK, Piscitelli CL, Yamashita A, Guaux E. *Science* 2008; 322(5908):1655-61.
3. Beuming T, Shi L, Javitch JA, Weinstein H. *Mol Pharmacol* 2006; 70:1630-42.

compounds with the advantage of selective obtaining the *N*-9' (**1**) or *N*-7' (**2**) regioisomers simplifying their isolation².



Figure

Trying to increase the anticancer activity of compounds **1** their structure was modified as followed: a) The salicyl alcohol fragment (present in the 2,3-dihydro-5H-1,4-benzodioxepin fragment) and the purine of **1** were maintained; b) The interaction of xenobiotics with DNA and the resulting adducts have been studied extensively, in order to understand how they exhibit their cytotoxic and carcinogenic properties³. It is within this philosophy that our group has focused on simple epoxides on which until now only little research has been performed. As a lead moiety the phenyl glycidyl ether was chosen (Figure).

We describe herein the synthesis and biological evaluation of a series of substituted 9-(2-oxiranylmethoxybenzyl)-9H-purines **3**.

REFERENCES

1. M.C. Núñez, M.G. Pavani, M. Díaz-Gavilán, et al. *Tetrahedron*, **2006**, 62, 11724-11733.
2. A. Conejo-García, M.C. Núñez, J.A. Marchal, et al. *Eur. J. Med. Chem.*, **2008**, 43, 1742-1748.
3. H. Bartsch. *Mutat. Res.*, 1996, 340, 67-79.

POSTER COMMUNICATIONS – PC.070

DI- AND TRI-SUBSTITUTED PURINES WITH THE PHENYL GLYCIDYL ETHER MOIETY: SYNTHESIS AND ANTICANCER ACTIVITIES

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Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge in the 21st century. Therefore, the development of new drugs against cancer belongs among the priorities of the development of science and fundamental research. Alkylating agents have been used for the treatment of cancer for over six decades and yet their repertoire continues to grow.

Studies on the synthesis and pharmacological evaluation of (RS)-6-substituted-7 or 9-(2,3-dihydro-5H-1,4-benzodioxepin-3-yl)-7H- or -9H-purines were published.¹ The microwave-assisted organic synthesis has provided faster access to the target

POSTER COMMUNICATIONS – PC.071

ENANTIOMERS OF 9-[1-(*p*-NITROBENZENESULFONYL)-1,2,3,5-TETRAHYDRO-4,1-BENZOXAZEPIN-3-YL]-2,6-DICHLORO-9H-PURINE, AS ANTICANCER AGENTS

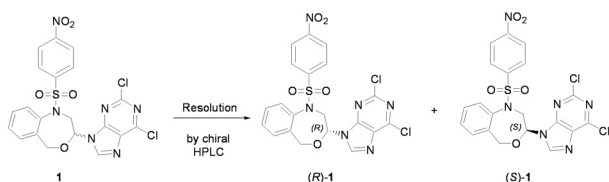
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Knowledge of the molecular signalling pathways that stimulate tumours has led to the identification of both Epidermal Growth Factor Receptor (EGFR) and Vascular Endothelial Growth Factor (VEGF) as key components implicated in the regulation of the tumour proliferation and angiogenesis, respectively. The dual inhibition of both molecular signalling pathways such as EGFR and VEGF may solve the multidrug resistance (MDR) problem and promote synergism¹. There are about ten scientific publications that deal with the dual EGFR and VEGF inhibition, and all of them concentrate in the period 2004-2010, which supports

the innovative character of the therapeutic approach. The drugs used are structures derived from 4-anilinoquinazolines, such as gefitinib and erlotinib, and monoclonal antibodies such as trastuzumab, used alone or in combination with other drugs. Nevertheless, only two homochiral drugs have been employed: a) (-)-gossypol, which is a polyphenol derived from the cotton plant (gender *Gossypium*, family *Malvaceae*)²; and b) AEE788, a synthetic product prepared in Novartis³. AEE788 presents the basic structure of 7H-pyrrolo[2,3-d]pyrimidine, that can be considered a bioisostere of the quinazoline moiety.

In this communication both enantiomers of a new tetracyclic prototype, such as 9-[1-(p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepin-3-yl]-2,6-dichloro-9H-purine, (R)-**1** and (S)-**1** are shown. Such homochiral drugs display a dual EGFR and VEGF inhibition, with production of an elevated level of apoptosis in tumour cells and revealing neither acute nor chronic toxicity.



Figure

REFERENCES

1. G.N. Naumov, M.B. Nilsson, T. Cascone, *et al.*, *Clin. Cancer Res.*, **2009**, *15*, 3484-3494.
2. R.M. Mohammad, S. Wang, S. Banerjee, *et al.*, *Pancreas*, **2005**, *31*, 317-324.
3. T. Sasaki, Y. Kitadai, T. Nakamura, *et al.*, *Neoplasia*, **2007**, *9*, 1066-1077.

POSTER COMMUNICATIONS – PC.072

INFLUENCE OF THE LINKER IN THE BIOLOGICAL ACTIVITY OF MONOCATIONIC INHIBITORS OF CHOK

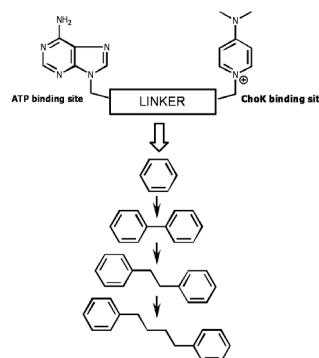
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Choline kinase (ChoK), the enzyme responsible for the generation of phosphorylcholine (PCho), is involved in cell proliferation. Studies using nuclear magnetic resonance (NMR) techniques have demonstrated elevated levels of PCho in human tumoral tissues with respect to normal ones, including breast, colon, lung, and prostate tumors [1]. Inhibition of ChoK has been

demonstrated to be a novel efficient antitumor strategy in onco-gene transformed cells [2].

Docking studies on the homology model of human ChoK based on the crystal structure of ChoK from *C. elegans* indicate that bis-cationic inhibitors can bind to the binding sites of ATP and choline [3]. Therefore we have design monocationic structures with a positive charge that could be stabilized into the choline binding site and a noncharged aromatic group that mimics the ATP adenine moiety connected by an appropriated linker. Here we report that, according to their inhibitory activities against human ChoK, the enzymatic inhibitory potency is closely related to the size of the linker being the 1,4-diphenylbutane the most suitable.



REFERENCES

1. Smith, T. A. D.; Bush, C.; Jameson, C. *et al.* *NMR Biomed.* **1993**, *6*, 318-323.
2. Hernández-Alcoceba, R.; Saniger, L.; Campos, J. *et al.* *Oncogene*, **1997**, *15*, 2289-2301.
3. Milanese, L.; Espinosa, A.; Campos, J. *et al.* *ChemMedChem*. **2006**, *1*, 1216-1228.

POSTER COMMUNICATIONS – PC.073

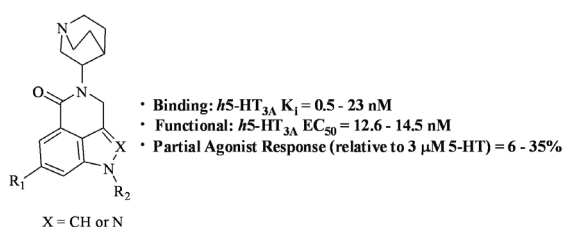
NOVEL 5-HT₃ RECEPTOR PARTIAL AGONISTS FOR THE POTENTIAL TREATMENT OF IRRITABLE BOWEL SYNDROME

David D. Manning¹, Christopher L. Cioffi¹, Kristen N. Ryan¹, Alexander Usyatinsky¹, Kevin Fitzpatrick¹, Carla Hassler¹, Svetlana Dobritsa¹, Cheng Guo¹, Liaqat Masih¹, Zhenjun Zhang¹, Sok Hui Choo¹, Jonathan D. Wierschke¹, William G. Early¹, Amy S. Butler³, Catherine A. Brady³, Nicholas M. Barnes³, Marlene L. Cohen², Peter R. Guzzo¹

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Partial activation of the 5-HT₃ receptor is an emerging strategy for the potential treatment of irritable bowel syndrome (IBS), a debilitating functional disorder of the bowel, which diminishes

quality of life for an estimated 7-12% of adults. The 5-HT₃ receptor is a pentameric, ligand-gated cation channel found in both the enteric and central nervous systems and has been a successful drug target for the treatment of chemotherapy-induced nausea and vomiting (CINV) and more recently for IBS. Initial drug development campaigns focused on the development of 5-HT₃ receptor antagonists as potential treatments for diarrhea predominant IBS (IBS-D). Although clinically successful, the first agent in this class to reach market (alosetron) has limited use due to severe constipation and rare instances of ischemic colitis (IC). Nevertheless, the 5-HT₃ receptor remains an attractive IBS drug target as shown by the subsequent approval in Japan of the 5-HT₃ receptor antagonist ramosetron (a repurposed CINV agent). 5-HT₃ receptor antagonists like ramosetron are believed effective in patients with IBS-D due to effects on visceral sensitivity, colonic transit and secretion, which lead to improvements in abdominal discomfort and stool consistency in patients. Targeting compounds that partially activate the 5-HT₃ receptor would minimize the potential for severe constipation or IC and may result in therapeutics that are applicable to a broader array of IBS patient populations (e.g. IBS-D, IBS-C (Constipation predominant) and IBS-M (Mixed; fluctuating between bouts of diarrhea and constipation)). Attenuation of the excessive serotonergic activity of the 5-HT₃ receptor without completely abolishing channel function is hypothesized to control or normalize gastroenteric function and to provide a novel, more clinically acceptable treatment of IBS. Two new chemical series bearing indazole and indole cores have been identified with nanomolar binding affinity for the h5-HT_{3A} receptor. A range of partial agonist activities in HEK cells expressing the h5-HT_{3A} receptor are reported. Excellent 5-HT₃ receptor selectivity, favorable eADME and good oral *in vivo* potency in the murine von Bezold-Jarisch reflex model is exemplified thereby indicating the series to have potential utility as improved IBS agents.



POSTER COMMUNICATIONS – PC.074

STUDY OF A NEW APPROACH FOR POTENT AND SELECTIVE MAO INHIBITORS

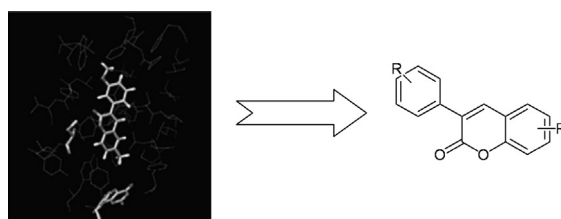
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Coumarins are a large family of compounds widely used in numerous applications¹. This is an important reason to study and develop different modifications on the coumarin skeleton. Phenylcoumarins are synthetic compounds in which an additional phenyl ring is present in any position of the pyrone or the benzenic ring of the coumarin's nucleus². For the last years, we have been studying deeply the 3-phenylcoumarin's scaffold. The inclusion of the resveratrol skeleton makes these molecules an interesting group to study. Recent studies pay special attention to their antioxidant, anticarcinogenic and enzymatic inhibition properties. In regard to the monoamine oxidase (MAO) inhibition, the recent findings revealed that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in the coumarin and 3-phenyl rings^{2,3}.

To a better understanding of the structure/activity relationships of MAO inhibitory activity and selectivity, and to improve them, in the present communication we report the design, synthesis and biological study of a new series of 3-phenylcoumarin derivatives.



The preliminary results of pharmacological study using recombinant human MAO-A and MAO-B isoenzymes indicated that some of the synthesized compounds are powerful and selective MAO-B inhibitors with IC₅₀ values in the low nanomolar-picomolar range. The present modifications, which we are studying more deeply, lead to improve coumarin's pharmacologic profile in the Parkinson's disease.

REFERENCES

- Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.*, **2005**, *12*, 887.
- Matos, M.J., Viña, D., Quezada, E., Picciau, C., Delogu, G., Orallo, F., Santana, L., Uriarte, E. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 3268.
- Matos, M.J., Viña, D., Picciau, C., Orallo, F., Santana, L., Uriarte, E. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 5053.

POSTER COMMUNICATIONS – PC.075

HOMOCHIRAL PURINE DERIVATIVES:
ANTICANCER ACTIVITIES

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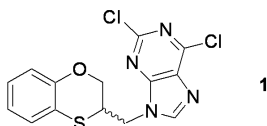
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The intrinsically chiral and non-racemic nature of the living world often results in its different interactions with the enantiomers of a given substance. If this substance is a drug, it might well be that only one of the two isomers is capable of exerting the desired therapeutic effect. The other may be inert (but must still be metabolized by the organism), harmful (causing even dramatic biological damages), or responsible for side effects that are difficult to predict.

Well aware of this fact and stimulated by the new policy statements issued by the regulatory agencies, the pharmaceutical industry has, over the past decade or so, systematically begun to develop chiral drugs in enantiometrically pure forms^{1,2}.

A series of eleven (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purines was obtained by applying the Mitsunobu protocol from (*RS*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol (**1**).

The racemic mixture (**1**) was resolved in its enantiomers: (*S*)-**1** (Retention time = 14.7 min) and (*R*)-**1** (Retention time = 17.5 min), using a CHIRALPAK® IA semipreparative column and a hexane/*i*PrOH mixture as eluent. The absolute configurations of the stereocentres were determined by X-ray diffraction. (*R*)-**1** and (*S*)-**1** show different antiproliferative activities against the human breast cancer cell line MDA-MB-231.



Several drugs are now marketed or being developed as single enantiomers in place of a previous racemic mixture, a process known as "chiral switching". It is easier to understand "pure" as opposed to "mixture" pharmacology but whether the promise of chiral (and metabolite) switches will translate into real clinical advances remains to be seen.

REFERENCES

1. M.C. Núñez; M.E. García-Rubiño; A. Conejo-García; O. Cruz-López; M. Kimatrai; M.A. Gallo; A. Espinosa; J.M. Campos. *Curr. Med. Chem.*, **2009**, 16, 2064-2074.

2. M.C. Núñez; M. Díaz-Gavilán; A. Conejo-García; O. Cruz-López; M.A. Gallo; A. Espinosa; J.M. Campos. *Curr. Med. Chem.*, **2008**, 15, 2614-2631.

POSTER COMMUNICATIONS – PC.076

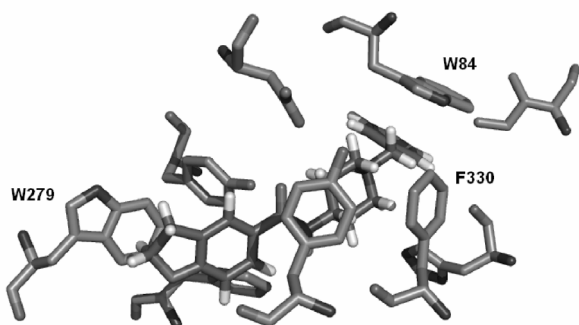
STRUCTURE-BASED SEARCH FOR NEW
INHIBITORS OF CHOLINESTERASES

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Alzheimer's disease (AD) is an irreversible, progressive, neurodegenerative disorder with memory and thinking disturbances. Due to the cholinergic hypothesis explaining the cognitive deficits, inhibitors of acetylcholinesterase (AChE) and also butyrylcholinesterase (BuChE) were introduced for the symptomatic treatment of mild and moderate AD [1]. Donepezil, rivastigmine and galantamine can increase cholinergic neurotransmission and lead to learning and memory improvement. They can also affect different mechanisms such as β -amyloid aggregation, regional cerebral blood flow, tau phosphorylation and oxidative stress which lead to the development of the disease [2,3]. Therefore, the search for new cholinesterase inhibitors is undertaken by many laboratories [4].

The aim of our study was to search for potential new inhibitors of cholinesterases with structure-based computational techniques. Based on properties of known inhibitors and requirements for blood-brain barrier penetration, the ZINC database was filtered for compounds fulfilling the following conditions: $1 \leq \log P \leq 3$, $1 \leq \text{charge} \leq 3$, rotatable bonds ≤ 8 , $1 \leq \text{H-bond donors} \leq 5$, $1 \leq \text{H-bond acceptors} \leq 10$, $100 \leq \text{MW} \leq 500$, $\text{PSA} \leq 90$. Almost 65 000 compounds passed these filters. Pharmacophore models were created for reference ligands such as donepezil, decamethonium and tacrine and used for virtual screening of the selected compounds. This resulted in a limited amount of well-fitting molecules. These compounds and some derivatives of compounds synthesized previously in our lab were used in a second screening which was based on a docking and scoring procedure optimized on the basis of known cholinesterase complexes. The top-ranked results were visually inspected to check for possible interactions, to assess the quality of the fitting and to suggest further chemical modifications. This enabled us to select potential hits which will be synthesized and tested for activity against AChE and BuChE *in vitro* with the spectrometric method of Ellman [5] and with electrophoretically mediated microanalysis [6].



One of the selected potential hits docked into AChE: Illustration of the suggested binding mode at the active site.

REFERENCES

1. Giacobini, E. *Neurochem. Res.* 2003, 28, 515.
2. Ballard, C. G. et al. *Curr. Alzheimer Res.* 2005, 2, 307.
3. Munoz, F. J.; Inestrosa, N. C. *FEBS Lett.* 1999, 450, 205.
4. Musiat, A.; Bajda, M.; Malawska, B. *Curr. Med. Chem.* 2007, 14, 2654.
5. Ellman, G. L.; Courtney, K.D.; Andres, V.; Feather-Stone, R. M. *Biochem. Pharmacol.* 1961, 7, 88.
6. Bajda, M.; Więckowska A.; Malawska B. *Acta Pol. Pharm.* 2009, 66, 357.

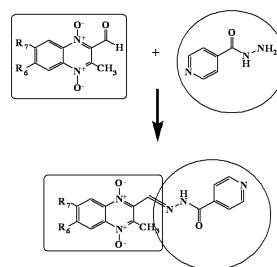
POSTER COMMUNICATIONS – PC.077

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW ISONICOTINIC HYDRAZIDE QUINOXALINE 1,4-DIOXIDE DERIVATIVES AS ANTITUBERCULAR AGENTS

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Tuberculosis (TB) is one of the most common infectious diseases known by the mankind. About 32% of the world's population is infected by *Mycobacterium tuberculosis*, with about 8 million new cases per year. The re-emergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB-cases. In this sense, the discovery of new and promising compounds with antimycobacterial activity remains essential for the control and prevention of TB (1).



As a continuation of our research in quinoxalines 1,4-di-N-oxide and with the aim of identifying new antitubercular drug candidates a new series of compounds have been prepared. Structure-based design has been used to obtain new lead molecules through the incorporation of isoniazid, one of the most effective first line anti-TB drugs, in a quinoxaline-1,4-di-N-oxide derivative (2).

Nine new quinoxalines 1,4-di-N-oxide derivatives containing isoniazid pharmacophore were prepared by a microwave assisted method optimized by our group in order to reduce the reaction times and increase the reaction yield. It has been necessary to optimize a new flash chromatography method due to the unexpected results obtained by the traditional chromatography purification methods.

In order to study the structure-activity relationship (SAR) of these compounds we use the Osiris program for calculating the fragment-based druglikeness of the compounds also using isoniazide and pirazinamide as reference compounds due to its structural closeness with our derivatives. With the aim of determining the antitubercular activity of the quinoxaline derivatives, the synthesized compounds have been sent to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF).

Taking into account the promising profile and the biological values of these compounds, it can be said that they should be considered for further experimental research.

ACKNOWLEDGMENTS

We wish to express our gratitude to the PIUNA project from the University of Navarra and the Antimicrobial Acquisition and Coordinating Facility for carrying out the biological assays through research and development contracts. Enrique Torres is indebted to the Navarra Government for a grant.

REFERENCES

1. WHO/HTM/TB/2008.393. WHO Report 2008
2. R. Villar, E. Vicente et al. *In vitro and in vivo* antimycobacterial activities of ketone and amide derivatives of quinoxaline 1,4-di-N-oxide. *J. Antimicrob. Chemother.* **2008**, 62, 547-554.

POSTER COMMUNICATIONS – PC.078

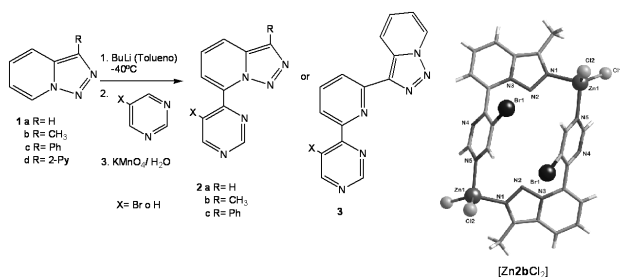
NEW PYRIMIDINYLTRIAZOLOPYRIDINES.
INTERACTION WITH NUCLEIC ACIDS

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Over the last four decades, intense research has focused on the effects of small organic compounds that noncovalently bind to nucleic acids, either by intercalation or groove binding. Classical intercalators typically have fused polyaromatic¹, while unfused polyaromatic compounds have been considered in literature as nonclassical intercalators².

In this work we have synthesized a series of compounds with the structure 7-(pyrimidin-4-yl)-[1,2,3]triazolo[1,5-a]pyridine **2** and 3-[6-(pyrimidin-4-yl)-pyridin-2-yl]-[1,2,3]triazolo[1,5-a]pyridine **3** both with fused and unfused aromatic rings. The synthesis of these compounds has been achieved by regioselective lithiation of the 7 position of [1,2,3]triazolo[1,5-a]pyridines **1**, and further reaction with the corresponding pyrimidine (Scheme 1). The reaction of 7-lithium-[1,2,3]triazolo[1,5-a]pyridine with electrophiles has been deeply studied,³ however it is the first time in which this reaction gives arylation products. All the obtained products have fluorescent properties, with λ emission between 474 and 500 nm.



Scheme 1

Figure 1

It has been reported that several metal complexes having polyaromatic ligands, such as metalloporphyrins⁴, bind to DNA. In this work we have prepared Zn^{II} complexes with ligands **2a** (X= Br) and **2b** (X= Br) can interact with DNA, while thermal denaturation assays show that complex **4** can destabilize the double helix of DNA.

Preliminary assays of DNA binding, by ethidium bromide quenching assay, show that compounds **2a** (X= Br) and **2b** (X= Br) can interact with DNA, while thermal denaturation assays show that complex **4** can destabilize the double helix of DNA.

REFERENCES

1. W.D. Wilson, Reversible interactions of small molecules with nucleic acids, in: M. Gait, M. Blackburn (Eds.), The Chemistry and Biology of Nucleic Acids, Oxford University Press, Oxford, England, **1990** (chapter 8).
2. L. Strekowski, B. Wilson, *Mutation Research*, **623**, **2007**, 3.
3. For example: a) G. Jones, D. R. Sliskovic, *Tetrahedron Lett* **1980**, 21, 4529. b) B. Abarca, R. Ballesteros, R. Ballesteros-Garrido, F. Colobert, F. R. Leroux, *Tetrahedron* **2007**, **63**, 10479.
4. M. Asadi, E. Safaei, B. Ranjbar, L. Hasani, *New J. Chem.*, **2004**, 1227.

POSTER COMMUNICATIONS – PC.079

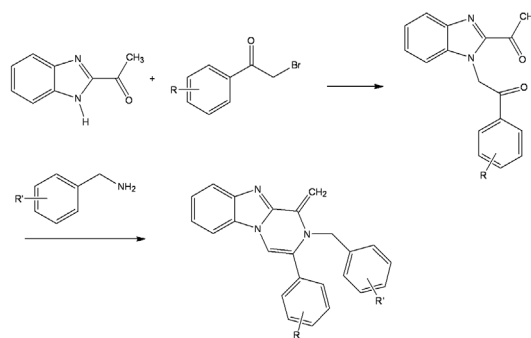
THE SYNTHESIS AND ANTICANCER ACTIVITY
OF SOME 1,2,3-TRISUBSTITUTED PYRAZINO-
BENZIMIDAZOLE DERIVATIVES

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The synthesis of some new pyrazino[1,2-a]benzimidazole derivatives and investigation of their anticancer activities were aimed in this work. Thus, 2-acetylbenzimidazole was reacted with 2-bromoacetophenone derivatives to give 1-(2-phenyl-2-oxoethyl)-2-aryloyl-4,5-diarylbenzimidazoles. These diketone compounds were reacted with appropriate benzylamine in acetic acid to obtain 1-methylene-2-benzyl-3-aryl-1,2-dihydropyrazino[1,2-a]benzimidazole derivatives. The structure of the compounds obtained were elucidated by using IR and ¹H-NMR spectral data and elemental analysis results.

All of the compounds were offered to American National Cancer Institute (NCI). Anticancer activities of the selected compounds were evaluated *in vitro* against approximately sixty human cell lines derived from nine neoplastic diseases and the results were given as growth percentage values. It was observed that the diketones were remarkable active compounds with low growth percentage. The most active compound was 1-[2-(3-chlorophenyl-2-oxoethyl)]-2-acetylbenzimidazole with the growth percentage of 24.53.



REFERENCES

1. Demirayak, Ş., Abu Mohsen, U., Karaburun, A.Ç., Synthesis and anti-cancer and anti-HIV testing of some pyrazino[1,2-a]benzimidazole derivatives, *Eur. J. Med. Chem.*, 37, 255-260 (2002).
2. Demirayak, Ş., Kayagil, I., Yurttas, L., Synthesis of Some 1,3-diarylpyrazino[1,2-a]benzimidazole derivatives by using either Rrflux or microwave irradiation method and investigation their Anticancer activities, *Eur. J. Med. Chem.*, Unpublished work.

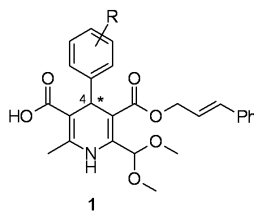
POSTER COMMUNICATIONS – PC.080

SYNTHESIS AND BIOLOGICAL EVALUATIONS OF OPTICALLY PURE 1,4-DIHYDROPYRIDINE-3-CARBOXYLIC ACID DERIVATIVES AS INHIBITORS FOR N-TYPE CALCIUM CHANNELS

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The N-type calcium channel (Ca_v2.2) is a well-established and characterized subtype of the voltage-dependent calcium channels (VDCCs), and distributes on the endings of central and peripheral nerves to control calcium influx into cells in response to changes in membrane potential. The activation of this channel triggers various physiological events such as neuronal excitability and secretion of neurotransmitters (ex. glutamate, substance P or CGRP) in strong association with the pathological process of neuropathic pain. Thus, blockade of the N-type VDCC has been considered as a promising therapeutic target for the pathological states, and many efforts have been made to discover potent small-molecule N-type VDCC blockers as candidates of next generation analgesics.¹ To serve this purpose, we have performed structure–activity relationship (SAR) studies for a unique 1,4-dihydropyridine-3-carboxylate compounds to find an effective and selective N-type calcium channel inhibitor with the least influence on cardiovascular system.² The latest SAR study on the 4-position of 1,4-dihydropyridine structure (**1**) will be disclosed with synthetic procedure and biological evaluations of two enantiomers.



REFERENCES

1. (a) Yamamoto, T.; Takahara, A. *Curr. Top. Med. Chem.* **2009**, 9, 377.; (b) Belardetti, F.; Zamponi, G. W. *Curr. Opin. Investig. Drugs* **2008**, 9, 707.
2. (a) Yamamoto, T.; Niwa, S.; Ohno, S.; Onishi, T.; Matsueda, H.; Koganei, H.; Uneyama, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.*, **2006**, 16, 798. (b) Yamamoto, T.; Niwa, S.; Iwayama, S.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Yamamoto, H.; Shoji, M. *Bioorg. Med. Chem.* **2006**, 14, 5333; (c) Yamamoto, T.; Niwa, S.; Ohno, S.; Tokumasu, M.; Masuzawa, Y.; Nakanishi, C.; Nakajo, A.; Onishi, T.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.* **2008**, 18, 4813.

POSTER COMMUNICATIONS – PC.081

A NOVEL CLASS OF DISUBSTITUTED ADAMANTYL DERIVATIVES AS POTENT MULTIDRUG RESISTANCE REVERSAL AGENTS

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A series of novel disubstituted adamantyl derivatives was conceived and synthesized as potent Multidrug resistance (MDR) reversal agents. MDR, which is a potentially surmountable obstacle to effective chemotherapy of cancer, is associated with excessively high expression of P-glycoprotein (P-gp) in cancer cells. P-gp, a plasma membrane transporter, which extrudes chemotherapeutic agents out of cells, can be considered as a therapeutic target. Here the design of target molecules was based on rational small molecules screening from in-house chemical library using an image-based high-throughput screening assay in a P-gp overexpressing MDR sarcoma cell line. The SAR studies on P-gp reversal activity indicated some adamantyl derivatives were more than 10-fold more potent than verapamil without considerable intrinsic cytotoxicity.

REFERENCES

1. Michael M. Gottesman, Tito Fojo and Susan E. Bates, *Nature Reviews Cancer*, **2002**, 2, 48-58.
2. Tripta Bansal, Naseem Akhtar, Manu Jaggi, Roop K. Khar and Sushama Talegaonkar, *Drug Discovery Today*, **2009**, 14 (21-22), 1067-1074.
3. Kyung Hoon Min, Yan Xia, Eun Kyung Kim, Yinglan Jin, Navneet Kaur, Eun Seon Kim, Dae Kyong Kim, Hwa Young Jung, Yongseok Choi, Mi-Kyung Park, Yong Ki Min, Kiho Lee, Kyeong Lee, *Bioorganic & Medicinal Chemistry Letters*, **2009**, 19, 5376–5379.

POSTER COMMUNICATIONS – PC.083

NEW HISTAPRODIFEN-DERIVED H₁-RECEPTOR AGONISTS

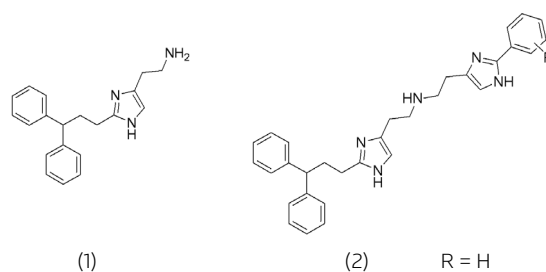
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In the histamine-receptor family (H₁, H₂, H₃, and H₄, respectively), the H₁-receptor subtype plays an essential role in the physiology of allergic reactions (e. g., rhinitis, flush) and in the control of sleep and waking state. Although this receptor is known for a long time and specific H₁-receptor antagonists have been introduced in the therapeutic market thirty years ago, the precise molecular binding mode of ligands and the binding pocket still remain to be elucidated. In our approach, we develop specific, highly potent histamine H₁-receptor agonists as pharmacological tools in order to get a deeper insight into the binding mode and to deduce further structure-activity relationships.

Histaprodiven (**1**)-type H₁-receptor agonists¹ offer an interesting starting point for these molecular investigations. Especially *suprahistaprodiven*, a “dimer” of histaprodiven and histamine which displays 36-fold higher activity than histamine, is an important milestone within this new class of H₁-receptor agonists². The idea to synthesize a hybrid-molecule of histaprodiven and the H₁-receptor agonist 2-phenylhistamine led to a new potent and selective H₁-agonistic compound showing 42-fold higher potency than histamine³. This agonist is also referred to as *phenoprodiven* (**2**). Searching for further structure-activity relationships, 2-phenylhistamines with different monosubstitution patterns (e. g., R = -CH₃, -OCH₃, -Hal) in the phenyl moiety – mostly H₁-receptor agonists by themselves – have been coupled with the conventional histaprodiven moiety in order to create a small library of *phenoprodiven*-derivatives. In the presented project the influence of different regioisomeric substituents in the 2-phenylimidazole moiety on H₁-receptor agonist potency and intrinsic activity is examined, and the results are compared to structure-activity relationships in the 2-phenylhistamine series. The *meta*-fluoro derivative of **2** was 47-fold more potent than histamine.

The syntheses included a reductive amination of an aldehyde containing the histaprodiven moiety with the appropriate 2-phenylhistamine as well as a microwave assisted *Suzuki* coupling, delivering the required 2-phenylhistamines⁴. Functional pharmacological characterisation was performed by means of the classical guinea-pig ileum H₁-receptor assay¹.



REFERENCES

1. Elz, S. et al., *J. Med.Chem.* **2000**, 43, 1071.
2. Menghin, S. et al., *J. Med. Chem.* **2003**, 46, 5458.
3. Striegl, B., *PhD thesis, University of Regensburg*, **2006**.
4. Skoumbourdis, A. et al., *Tetrahedron Lett.* **2007**, 48, 9140.

POSTER COMMUNICATIONS – PC.084

EXPLORING THE RIGID DIAMINE CHEMICAL SPACE: THEORY AND PRACTICE

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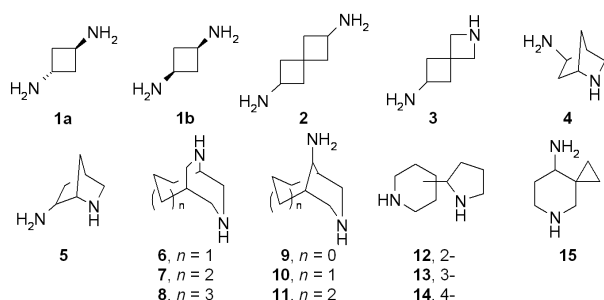
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Molecular rigidity has been repeatedly outlined as a distinguishing feature of approved drugs and natural products. Conformationally rigid aliphatic diamines exemplify privileged structures among other small molecules of importance to drug discovery. These building blocks have been widely used in drug discovery; in particular, more than 15 marketed drugs comprise residues of bicyclic diamines.

In this work, the theoretically available part of chemical space covered by rigid diamines was explored. It has been found that the known compounds comprise only a small part of all theoretically possible diamines, although even in case of known compounds, the diversity is quite large. MDL DDR data were analyzed for rigid diamines described in open or patent literature according to several parameters characterizing topology and spatial structure of the scaffolds.

Conformationally rigid diamines **1 – 15** either not described in the literature or recognized as hardly accessible by chemical synthesis using previously reported procedures were selected by us from the theoretically available chemical space. The practical approaches to the synthesis of these diamines as selectively monoprotected derivatives were developed. In the case of diamines **1 – 3**, spatial structure was also evaluated by X-Ray diffraction studies.



REFERENCES

- Shivanyuk, A. N.; Volochnyuk, D. M.; Komarov, I. V.; Nazarenko, K. G.; Radchenko, D. S.; Kostyuk, A.; Tolmachev, A. A. *Chem. Today* **2007**, 25, 12–13.
- Mityuk, A. P.; Denisenko, A. V.; Dacenko, O. P.; Grygorenko, O. O.; Mykhailiuk, P. K.; Volochnyuk, D. M.; Shishkin, O. V.; Tolmachev, A. A. *Synthesis* **2010**, 493–497.
- Mityuk, A. P.; Denisenko, A. V.; Dacenko, O. P.; Grygorenko, O. O.; Mykhailiuk, P. K.; Volochnyuk, D. M.; Tolmachev, A. A. *Tetrahedron Lett.* **2010**, 51, 1790–1792.

POSTER COMMUNICATIONS – PC.085

INHIBITION OF PEROXIDASE GENE EXPRESSION AND ENZYMATIC ACTIVITY BY RESVERATROL-COUMARIN HYBRID DERIVATIVES

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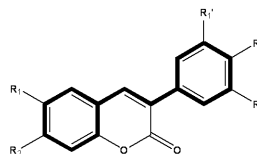
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Heme peroxidases are widespread in eukaryotes and prokaryotes and have a pivotal role in biology. [1] Both mammalian and plant peroxidases have been reported to produce aromatic oxyl radicals from several aromatic substrates and reactive oxygen species as part of a defense mechanism against pathogens. [2]

However, such reactive species generated by peroxidase-catalyzed reactions can also contribute to progressive human tissue damage in chronic inflammatory diseases as atherosclerosis, asthma and rheumatoid arthritis, which makes peroxidases interesting pharmacological targets. [3]

Considering the antioxidant and anti-inflammatory activities reported for *t*-resveratrol [4] and some coumarins [5], a series of resveratrol-coumarin hybrid derivatives have been evaluated as

inhibitors of enzymatic activity of peroxidases (horseradish peroxidase and peroxidases of HL-60 cells) and myeloperoxidase (MPO) gene expression.



T-resveratrol and some of the studied compounds caused significant inhibition of both peroxidase activity (HRP and peroxidase of HL-60 lysates). Not big differences could be found between inhibition of HRP or peroxidases of HL-60 lysates. Additionally, these compounds downregulated HL-60 MPO mRNA levels and at 100 μ M, some of them completely suppressed MPO gene expression.

Results obtained in this study may be helpful to understand better the interactions between resveratrol-coumarin hybrids and peroxidase catalytic site.

REFERENCES

- Everse, J.; Everse, K. E.; Grisham, M. B. In *Peroxidases in Chemistry and Biology*; CRC Press: Boca Raton, FL, 1991.
- Takahama, U.; Yoshitama, K. *J. Plant Res.* **1998**, 111, 97.
- Obinger, C. *Arch. Biochem. Biophys.* **2006**, 445, 197.
- Castro, R.; Lamas, J.; Morais, P.; Sanmartín, M.L.; Orallo, F.; Leiro, J. *Veterinary Immunology and Immunopathology* **2008**, 126, 9.
- Kabeya L.M.; de Marchi, A. A.; Kanashiro, A.; Lopes, N. P.; da Silva, C. H. T. P.; Pupo, M. T.; Lucisano-Valim, Y. M. *Bioorg. & Med. Chem. Lett.* **2007**, 15, 1516.

POSTER COMMUNICATIONS – PC.086

EFFECTS OF COUMARIN-RESVERATROL HYBRID DERIVATIVES ON LEUKOCYTE RESPIRATORY BURST RESPONSE.

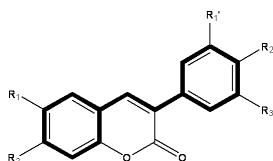
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Resveratrol [3,4',5-trihydroxystilbene] is a phytoalexin of natural origin presents in spermatophytes species such as vines, in response to damage. Resveratrol was already studied as cardioprotective, anticancer, enzymatic inhibitor, antioxidant and anti-inflammatory proving to be very efficient in a large group of in vitro, ex-vivo and/or in vivo experiments. [1]

On the other hand, coumarins consisting of fused benzene and α -pyrone rings, represent a vast family of compounds naturally found in plants that have been reported to possess a wide range of biological activities, including antioxidant and anti-inflammatory abilities without evidence of toxicity [2].

We have synthesized some coumarin/resveratrol hybrid derivatives in which the position of the hydroxy groups has been modified or the hydroxyl groups have been protected, fixing 3,4-double bond of the coumarin nucleus the trans disposition of the *t*-RESV. [3]



Effects of these compounds on the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during the respiratory burst and on the expression of inflammatory genes such as inducible nitric oxide synthase (NOS)-2 have been studied and compared with those obtained for *t*-resveratrol.

Obtained results indicate that some of these coumarin/resveratrol hybrid derivatives at micromolar concentrations have a significant antioxidant activity on the leukocyte response to proinflammatory stimuli and thus suggests that these compounds may have potential applications in the treatment of diseases involving leukocyte hyperresponsiveness.

REFERENCES

1. Leiro, J.; Alvarez, E.; García, D.; Orallo, F. *International Immunopharmacology* **2002**, 2, 767.
2. Bissonnette, E. Y.; Tremblay, G. M.; Turmel, V.; Piroette, B.; Reboud-Ravaux, M. *International Immunopharmacology*, **2009**, 9, 49.
3. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yáñez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. & Med. Chem. Lett.*, **2006**, 16, 257.

POSTER COMMUNICATIONS – PC.087

DESIGN AND SYNTHESIS OF HETERO ANALOGS OF THE SUBTYPE SELECTIVE EAAT1 INHIBITOR, UCPH-101

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Excitatory amino acid transporters (EAAT) play a pivotal role in regulating the synaptic concentration of glutamate in the central nervous system (CNS). Up-to-date five different subtypes

have been identified, named EAAT1-5, in humans. In rodents their naming is for historic reasons different: GLAST, GLT-1, EAAC1, EAAT4, and EAAT5, respectively. Recently, we have published the first subtype selective inhibitor of EAAT1 (GLAST), UCPH-101. As a part of our medicinal chemistry studies of this novel EAAT1 inhibitor, we have designed and synthesized 6-heteroatom analogs for improved solubility and investigation of the structure-activity-relation (SAR). In details, two new analogs were prepared 6-amino-UCPH-101 and 6-oxy-UCPH-101 by means of two distinct synthetic routes. However both analogs failed to inhibit the EAAT1 subtype as well as EAAT2 and EAAT3 ($EC_{50} > 300 \mu M$). Although this result was surprising to us it contributes to the SAR of UCPH-101 and will serve as important information in our future research work within this field.

POSTER COMMUNICATIONS – PC.088

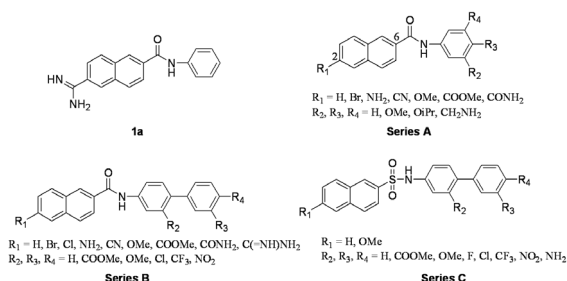
NON-BASIC UROKINASE PLASMINOGEN ACTIVATOR INHIBITORS AS POTENTIAL LEAD COMPOUNDS FOR AN ANTI-METASTATIC THERAPY

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Urokinase-type plasminogen activator (uPA) plays a key role in extracellular proteolytic events associated with tumor cell growth, migration, angiogenesis and metastasis.¹ Multiple independent groups have shown that uPA is a potent prognostic factor in breast cancer. Recently, this prognostic impact was validated in both a prospective randomized trial and a pooled analysis using LOE-1 evidence studies. As a result, uPA is an attractive target for the development of small molecule active site inhibitors. Recent drug development programs have focussed at nonpeptidic inhibitors that target at uPA have amidine or guanidine functional groups attached to aromatic or heterocyclic scaffolds.² There is a general problem of poor oral bioavailability of these charged inhibitors. We report the synthesis and evaluation of a series of uPA inhibitors having non-basic groups attached at 2-position of 6-substituted naphthamide or 6-substituted naphthalene sulfonamide. As a starting point we replaced amidine group of **1a**² by an amide (**Series A**, $R_1 = \text{CONH}_2$, $R_2 = R_3 = R_4 = \text{H}$) that resulted in same range of K_i value. With the help of molecular modeling a series of compounds (**Series B** and **C**) were synthesized for uPA inhibitory activity. The selective compounds were screened against a panel of other physiologically important trypsin-like serine proteases such as tissue plasminogen activator (tPA), plasmin and thrombin. It was noticed that four compounds have an enzymatic inhibition profile comparable to clinical uPA inhibitor **WX-UK1**. We propose that 2,6-disub-

stituted naphthamide template could be used further to design and optimize good clinical candidates.



REFERENCES

1. Duffy, M. J. The urokinase plasminogen activator system: Role in malignancy. *Curr. Pharm. Des.* **2004**, 10, 39–49.
2. Wendt M, Rockway T, Geyer A, McClellan W, Weitzberg M, Zhao X, Mantei R, Nienaber V, Stewart K, Klinghofer V.; Giranda, V. L. Identification of novel binding interactions in the development of potent, selective 2-naphthamide inhibitors of urokinase. Synthesis, structural analysis, and SAR of N-phenyl amide 6-substitution. *J. Med. Chem.* **2004**, 47, 303–324.

POSTER COMMUNICATIONS – PC.089

NOVEL CDC25 PHOSPHATASES SMALL COUMARIN-BASED INHIBITORS: DESIGN, SYNTHESIS AND BIOLOGICAL VALIDATION

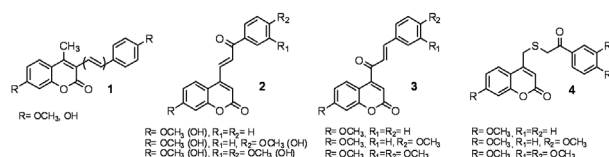
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Cell division cycle 25 (Cdc25) phosphatases are key actors in eukaryotic cell cycle control. The three human Cdc25 phosphatases (Cdc25A, Cdc25B, and Cdc25C) are responsible for dephosphorylating Cdk/cyclins on pThr14 and/or pTyr15 residues, this dephosphorylation triggers the final activation of Cdk/cyclin activity during normal cell cycle progression¹. Cdc25 phosphatases are also central targets and regulators of the G2/M checkpoint mechanisms activated in response to DNA injury. Cdc25A controls both the G1-to-S and G2-to-M transitions, whereas Cdc25B and Cdc25C are regulators of the G2-to-M transition². So far, several lines of experimental evidence have been provided for the involvement of Cdc25 phosphatases in oncogenic transformations and various human cancers³. Altered expression of these phosphatases is associated with checkpoint bypass and genetic instability. Accordingly, increased expression of Cdc25A and Cdc25B is found in many high-grade tumors and is correlated with poor prognosis in human cancers⁴. To date several Cdc25A and Cdc25B class of inhibitors are reviewed in

literature⁵ like potential agents for cancer therapy, mostly such as quinone-based compounds reported active *in vivo*⁶, nevertheless recently a new class of Cdc25B inhibitors were described displayed cytotoxic properties against HeLa cells and against prostatic LNCaP cells⁷. Moreover several Sylibin derivatives showed a strong cell cycle arrest in PC3 cells and differential effects of these compounds on the levels of some cell cycle regulators-cyclins, among these Cdc25A, Cdc25B and Cdc25C⁸, and several flavones were described able to inhibit Cdc-25.1 Phosphatase activity in *C. elegans*⁹. These findings prompted us to project the design, synthesis and biological validation of novel Coumarin-based (like flavon isomer) derivatives (**1-4**). Tested against the three Cdc25 isoform some of such molecules showed inhibiting activity at micromolar concentrations in highly repeatable fluorescence screening assays. The reversibility of fixation of those molecules on Cdc25 isoforms is currently assessed by fluorimetry. These molecules are also tested on commonly used breast cancer cell lines (MCF7 sensitive and MCF-7 resistant to chemotherapeutic drugs), in order to show a correlation between Cdc25 inhibition and a cytostatic potential (dephosphorylation of CDK and cell cycle blocking).



REFERENCES

1. Boutros, R.; Dozier, C., and Ducommun, B. *Curr. Opin. Cell Biol.* **2006**, 18, 185-191.
2. Rudolph, J. *Biochemistry* **2007**, 46, 3595.
3. Wang, P.; Zou, F.; Zhang, X.; Li, H.; Dulak, A.; Tomko, R.J.; Jr, Lazo J.S.; Wang, Z.; Zhang, L.; Yu, J. *Cancer Res.* **2009**, 69, 8157.
4. Aressy, B.; Ducommun, B. *Anticancer Agents Med Chem.* **2008**, 8, 818.
5. Lavecchia, A.; Di Giovanni, C.; Novellino, E.; *Curr Med Chem.* **2009**, 16, 1831.
6. Boutros, R.; Lobjois, V. and Ducommun, B. *Nature Reviews Cancer*, **2007**, 7, 495-507.
7. Kolb, S. ; Mondésert, O.; Goddard, M.L. Jullien, D.; Villoutreix, B.O.; Ducommun, B.; Garbay, C.; Braud, E. *Chem Med Chem.* **2009**, 4, 633.
8. Deep, G.; Oberlies, N.H.; Kroll, D.J.; Agarwal, R. *Int J Cancer.* **2008**, 123, 41.
9. Kim, K.S.; Kawasaki, I.; Chong, Y. and Shim, Y.H. *Mol. Cells* **2009**, 27, 345-350.

POSTER COMMUNICATIONS – PC.090

ANTI-*LEISHMANIA INFANTUM* ACTIVITY OF IMIDAZOQUINES

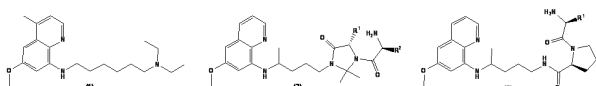
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Leishmanias are tropical and subtropical diseases caused by protozoal parasites of the genus *Leishmania*. The clinical expressions found in humans depend on the *Leishmania* species involved: cutaneous, mucosal and visceral leishmaniasis. This infection is endemic in 88 countries in the Horn of Africa, South Asia, and Latin America.¹

Sitamaquine (**1**), an 8-aminoquinoline (8-AQ), is a new anti-leishmanial oral drug, currently in Phase IIb clinical trials by GlaxoSmithKline. It has been well-tolerated in the most recent Phase II studies, but there are still some unanswered questions concerning its potential toxicities.^{2,3}

Our group developed imidazoquinones with general structure **2**, as peptidomimetic imidazolidin-4-one derivatives of the 8-aminoquinoline anti-malarial primaquine (PQ). Imidazoquinones **2** are stable compounds that preserve the overall bioactivity pattern of the parent drug and are not vulnerable to either oxidative deamination or proteolytic degradation by action of amino- or endopeptidases.⁴ Given their 8-aminoquinolinic nature and anti-protozoal activity, imidazoquinones were seen as possibly useful leishmanicidal agents. Their activity against *Leishmania infantum* was tested using the resazurin dye to measure parasite viability. This study also included dipeptide derivatives of PQ with general structures PQ-Proline-AA (**3**) that are mimetized by the imidazoquinones. We wish to report the results obtained in this study, i.e., on the leishmanicidal activities of compounds **2** and **3** against *L. infantum*.



REFERENCES

- Bern, C.; Maguire, J. H.; Alvar, J. Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS Neglected Trop. Dis.* 2008, 2, No. e313.
- Jha, T. K.; Sundar, S.; Thakur, C. P.; Felton, J. M.; Sabin, A. J.; Horton, J. A phase II dose-ranging study of sitamaquine for the treatment of

visceral leishmaniasis in India. *Am. J. Trop. Med. Hyg.* 2005, 73, 1005-1011.

- Wasunna, M. K.; Rashid, J. R.; Mbui, J.; Kirigi, G.; Kinoti, D.; Lodenyo, H.; Felton, J. M.; Sabin, A. J.; Albert, M. J.; Horton, J. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. *Am. J. Trop. Med. Hyg.* 2005, 73, 871-876.
- Vale, N.; Prudêncio, M.; Marques, C.A.; Collins, M.S.; Gut, J.; Nogueira, F.; Matos, J.; Rosenthal, P.; Cushion, M.T.; Rosário, V.E.; Mota, M.M.; Moreira, R.; Gomes, P. Imidazoquinones as antimalarial and antipneumocystis agents. *J. Med. Chem.* 2009, 52, 7800-7807.

POSTER COMMUNICATIONS – PC.091

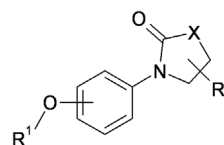
DISCOVERY OF LACTAMS AS A NOVEL CLASS OF HIGHLY POTENT H₃R INVERSE AGONISTS FOR THE TREATMENT OF COGNITIVE DISORDERS

Anne Valade, Sylvain Célanire, Bernard Christophe, Philippe Collart, Sabine Defays, Christel Delaunoy, Marie-Laure Delporte, Frédéric Denonne, Véronique Durieu, Michel Gillard, Delphine Hubert, Bénédicte Lallemant, Yves Lamberty, Geneviève Lorent, Jean-Marie Nicolas, Luc Quéré, Alain Vanbellinghen, Nathalie Vanhoutvin, Valérie Verbois and Laurent Provins

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Since the discovery and cloning of the human histamine H₃ receptor and the identification of the numerous potential therapeutic indications in which it may play a key role, pharmaceutical companies have put tremendous efforts in the identification of potent and selective H₃ ligands. Several of them successfully came up with drug candidates, currently in clinical trials for the treatment of cognitive disorders in Alzheimer's disease, ADHD or schizophrenia but also targeting sleep disorders such as narcolepsy.

In this poster, we would like to report the identification of highly potent H₃R inverse agonists bearing a lactam moiety. Lead generation and optimization efforts around those scaffolds with high ligand efficiency and low lipophilicity will be presented and will include the description of *in vitro* pharmacology, physico-chemical and pharmacokinetic properties as well as the evaluation of selected lead candidates in animal models of memory deficits.



The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5650)

POSTER COMMUNICATIONS – PC.092

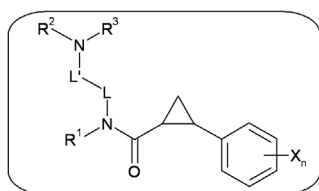
DISCOVERY OF NOVEL SELECTIVE SIGMA-1 LIGANDS AS COGNITIVE ENHANCERS

Anne Valade¹, Sophie Binet², Claire Brown², Eric Detrait¹, Doina Ene², Michel Gillard¹, Michel Guyaux¹, Yves Lamberty¹, Martin Maguire², Nivedita Namdev², Laurent Provins¹, Eric Schwartz², Céline Vermeiren¹

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The sigma receptor is a non-opioid transmembrane receptor which does not share any homology with other mammalian proteins. Since the 1970s, pharmaceutical companies have put tremendous efforts in the identification of potent Sigma-1 ligands. Several of them came up with non-selective pre-clinical candidates (e.g. (+)SKF-10,047). These compounds showed some improvement of cognitive impairment induced by Scopolamine, age or β -amyloid peptide in rodents. Despite promising preclinical data in several therapeutic areas, no clinical proof of concept has been reported to date.

In this poster, we would like to report the identification of phenyl-cyclopropylcarboxamide compounds as novel, selective and potent Sigma 1 ligands. Description of *in vitro* pharmacology, physicochemical and pharmacokinetic properties as well as the evaluation of selected compounds in animal models of memory deficit will be presented.



POSTER COMMUNICATIONS – PC.093

SYNTHESIS AND OPTIMIZATION OF PHARMACOLOGICAL PROFILE OF HEXAHYDRO- AND OCTAHYDRO-AZEPINO[4,3-b]INDOLES

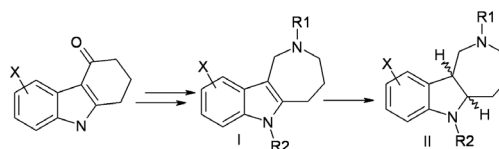
Sergey Tkachenko, Volodymir Kysil, Eugene Frolov, Alex Khvat, Ilya Okun, Oleg Mitkin, Alexander Ivachtchenko

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Possibly due to their unique intricacy, therapeutics for CNS disorders have not benefited from rational single targeted drug design. For major feature of many effective CNS drugs is their affinity for a large number of various biological targets involved

in the multifaceted pathogenesis. Design of multi-target-directed CNS-ligands has to be based on the concept of “magic shotgun”, where several key signalling elements are targeted simultaneously, which is opposite to the classic concept of single-target “magic bullet”.

We report the synthesis, biological evaluation and optimization of new CNS drug-like series of hexahydro- and octahydro-azepino[4,3-b]indoles (series I and II respectively).



X = H, Me, MeO, Hal, etc. R1 = Alkyl; R2 = Ar, Het, substituted Alkyl, Aralkyl, Alkynyl, etc.

Compounds of the series I were synthesized in a few steps starting from known tetrahydrocarbazol-4-ones. Series II was obtained by selective reduction of hexahydro-derivatives and included E and Z-isomers.

We have performed a molecular pharmacology profiling of a few representatives of series I and II on a panel of 70 therapeutic targets (GPCRs, ion channels, transporters, and enzymes) to characterize a spectrum of their activities. Selected compounds exhibited high affinity to constellations of histaminergic, serotonergic, alpha-adrenergic, dopaminergic receptors, Ca channels, transporters and cholinesterases. The pharmacological profiles of the compounds tested were sensitive to the nature of substituents X and R2. The decrease of compound lipophilicity by variation of X and R2 could result in diminishing of unwanted affinity toward hERG channel and H₁ receptors. The predominant activity toward 5-HT_{2A} receptors was shown for compounds I and II with R2 = Aryl. With R2 as Aralkyls, the compounds showed very high activity toward 5-HT_{2C}, 5-HT₆ and 5-HT₇ receptors.

Directed optimization of “pharmacological fingerprints” has led to the discovery of new series of chemotypes, which could be a reliable source of advanced drug candidates for the treatment of mood and cognition disorders.

POSTER COMMUNICATIONS – PC.094

IDENTIFICATION OF NOVEL ARYLOXYPROPANOLAMINES AS LIGANDS OF β -ADRENOCEPTORS: A vHTS APPROACH

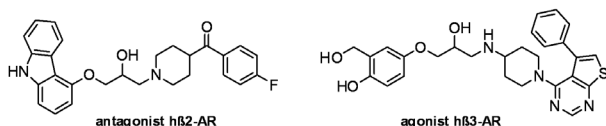
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Based on a distribution of the human β 3-adrenergic receptor (h β 3-AR) in different tissues like white and brown adipocytes, urinary bladder detrusor, gastrointestinal tract, near-term myometrium, brain and the human heart, several indications

emerged for a potential treatment with h β 3-AR ligands, especially agonists: obesity, type II diabetes, overactive bladder, irritable bowel syndrome (IBS), preterm labor, anxiety and major depression disorder.¹ Antagonists have recently been under evaluation for the treatment of chronic heart failure.² The inhalation of h β 2-adrenoceptor selective agonists has long been established as therapy of asthma and other bronchospastic conditions.³ Furthermore, systemic h β 2-agonists are used as tocolytics in preterm labor,³ and antagonists have just been described to have a beneficial effect in wound healing.⁴

Aiming for an *in silico* approach for the identification of new β -adrenoceptor ligands (with an initial focus on h β 3-AR), a direct docking procedure using a homology model for h β 3-AR was not selected as method of choice, as – at the given point in time – only the crystal structure of the bovine rhodopsin receptor was available as a representative of the target class of GPCRs, with only around 40% sequence homology to h β 3-AR. For an alignment approach, five literature-known h β 3-AR agonists were selected as templates based on their advancement in clinical development, their activity on β 3-receptors and/or for structural reasons. Each of these templates was used for a vHTS run of a virtual library comprising 4.6 Mio commercially available compounds using the 4Scan[®] technology,⁵ resulting in the identification of two hit classes of the aryloxypropanolamine type. Optimization programs for both of them resulted in the generation of two potent lead compounds, one as a selective antagonist at h β 2-AR,⁶ the other as a selective agonist at the h β 3-AR.⁷ Details of the hit-to-lead optimization will be presented, including tissue data for the latter (relaxation of pre-contracted human urinary bladder detrusor strips).



REFERENCES

1. Ursino, M. G.; Vasina, V.; Raschi, E.; Crema, F.; De Ponti, F. *Pharmacol. Res.* **2009**, 59, 221.
2. Lee, S.; Schwinger, R. H. G.; Brixius, K. *Pflugers Arch. Eur. J. Physiol.* **2008**, 455, 767.
3. Kolb, P.; Rosenbaum, D. M.; Irwin, J. J.; Fung, J. J.; Kobilka, B. K.; Shoichet, B. K. *Proc. Natl. Acad. Sci. USA* **2009**, 106, 6843.
4. Sivamani, R. K.; Pullar, C. E.; Manabat-Hidalgo, C. G.; Rocke, D. M.; Carlsen, R. C.; Greenhalgh, D. G.; Isseroff, R. R. *PLoS Med.* **2009**, 6, 105.
5. Seifert, M. H. J.; Wolf, K.; Vitt, D. *Biosilico* **2003**, 1, 143.
6. Tasler, S.; Baumgartner, R.; Aschenbrenner, A.; Ammendola, A.; Wolf, K.; Wieber, T.; Schachtner, J.; Blisse, M.; Quotschalla, U.; Ney, P. *Bioorg. Med. Chem. Lett.*, submitted.
7. Tasler, S.; Baumgartner, R.; Ammendola, A.; Schachtner, J.; Wieber, T.; Blisse, M.; Rath, S.; Zaja, M.; Klahn, P.; Quotschalla, U.; Ney, P. *Bioorg. Med. Chem. Lett.*, in preparation.

POSTER COMMUNICATIONS – PC.095

LABELLING STRATEGIES TO STUDY LARGE PROTEIN-LIGAND INTERACTIONS BY NMR

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The application of Nuclear magnetic Resonance (NMR) spectroscopy to large proteins is still a challenge. In spite of technical improvements in recent years, signal overlap continues to be a serious handicap when working with high molecular weight proteins. Therefore, simplification of the spectra of these macromolecules by selective labeling is a useful technique to facilitate their study. In this presentation, we report on two cost-effective labeling strategies for the NMR study of prolyl oligopeptidase (POP). In the first approach, a ¹⁵N-label is selectively incorporated into the tryptophan side chains of the protein and high quality simplified [1H15N]-TROSY HSQC spectra are obtained without the need for deuteration. The assignment of the indole resonances by site-directed mutagenesis and the interaction with a classical covalent POP inhibitor, Z-prolyl-prolinal, is described. In the second strategy, a ¹³C-label is selectively incorporated into the methyl groups of Methionine side chains and high resolution [1H13C]-HMQC spectra are recorded rapidly in a straightforward manner. After assignment of the methyl-¹³C-Met resonances, structural information about POP interaction with Z-prolyl-prolinal, as well as with new POP inhibitors, is obtained. The main advantage of these two methodologies is that they allow the study of POP-ligand interactions and POP conformational changes in solution.

REFERENCES

1. Tarragó, T.; Claasen, B.; Kichik, N.; Rodriguez-Mias, R.A.; Gairí, M.; Giralt, E. A cost-effective labeling strategy for the NMR study of large proteins: selective ¹⁵N-labeling of the tryptophan side chains of prolyl oligopeptidase. *Chembiochem.* 2009, 10, 2736-9.
2. Tarragó, T.; Martín-Benito, J.; Sabidó, E.; Claasen, B.; Madurga, S.; Gairí, M.; Valpuesta, J.M.; Giralt, E. A new side opening on prolyl oligopeptidase revealed by electron microscopy. *FEBS Lett.* 2009, 583, 3344-8.

POSTER COMMUNICATIONS – PC.096

INVESTIGATION OF AGENTS TARGETING NUCLEAR RECEPTOR CO-REPRESSOR PROTEIN

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Acute promyelocytic leukemia (APL), resulting from the fusion of promyelocytic leukemia (PML) protein and retinoic acid receptor (RAR), is a subtype of acute myelocytic leukemic. APL displays resistance to programme cell death and is characterized by an accumulation of blast cells in the bone marrow and peripheral blood. Previous reports by Ng *et al* illustrated the significant role of Nuclear receptor Co-Repressor (NCoR) protein in the resistance to apoptosis in APL cells. Accumulation of misfolded NCoR protein as insoluble aggregates in APL cells was shown to induce endoplasmic reticulum (ER) stress and to activate unfolded protein response (UPR) –induced apoptosis, ultimately contributing to differentiation arrest of APL cells. This establishes the misfolded NCoR protein as an attractive molecular target in APL. Curcumin, an active component in turmeric was shown to selectively promote apoptosis in APL cells containing elevated levels of misfolded NCoR protein over non-APL cells. While curcumin has been known to rescue the native conformation of misfolded proteins such as the amyloid β , its mechanism of action on APL cells appears to involve the inhibition of proteasome mediated degradation of misfolded NCoR protein. Accumulation of misfolded NCoR therefore aggravates ER stress and results in the selective sensitization of APL cells to UPR-induced apoptosis. This finding illustrates the potential of curcumin in targeting misfolded NCoR and thus APL. We aim to develop more potent curcumin analogues with high fidelity for NCoR protein in APL cells. A series of 32 analogues of curcumin have been designed and synthesized with the objective of interrogating structure-activity relationships and providing greater insight into the role of misfolded NCoR as a drug target in APL. Activity and selectivity profiles have been established on the APL cell line NB4-R1 (retinoic acid resistant) and non-APL cell lines HL60 and K562. Herein we report several scaffolds with up to 18-fold improved potency on NB4-R1 while maintaining at least 3-fold selectivity between APL and non-APL cell lines. In particular, one compound exhibited over 200-fold selectivity for NB4-R1 with a 3-fold improved potency over curcumin. With analogues of improved potency and selectivity to NCoR protein, we aim to study the influence of the analogue on the conformational status of NCoR in APL and non-APL cells and its implication in the response of cells to UPR-induced apoptosis.

REFERENCES

1. Ng APP; Fong J.H.; Nin D.S.; Hirpara J.L.; Asou N.; Chen C-S.; Pervaiz S.; Khan M., *Cancer Res.*, **2006**, 66, 9903-9912
2. Ng A.P-P; Khan M., Submitted
3. Hafner-Bratkovic I.; Gaspersic J.; Smid L., *J Neurochem.*, **2008**, 104, 1553-1564

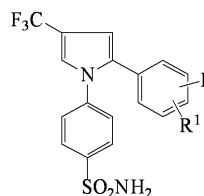
POSTER COMMUNICATIONS – PC.097

ANTITUMOR PROPERTIES OF DERIVATIVES 1,2-DIPHENYLPYRROLE AND THEIR PREPARATION PROCESS

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The development of antitumoral therapies of general applicability is one of the main goals in medicinal chemistry¹. Cancer is a heterogeneous disease characterized by the accumulation of tumor cells, which can cause the death of both animals and humans. Among the strategies envisaged for developing new antitumoral treatments, the promotion of cell death, in particular the induction of apoptosis by the inhibition of signaling through focal adhesions, a mechanism barely explored so far, is especially attractive². The reason for this interest lies in the anti-tumor and antimetastatic activity induced by the blockade of the constitutive signaling through focal adhesions. Herein is described the synthesis and bio-evaluation of 1,2-diphenylpyrrole derivatives as potential drug candidates for treatment or prevention of tumours³.



The synthetic procedure is based in the heterocyclic ring construction, followed by the introduction of arylsubstituted nucleus on position two of pyrrole by a Pd(0) catalyzed cross-coupling reaction.

Discussion of structure-activity relationships will be done in the presentation.

REFERENCES

1. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57-70.
Ashkenazi, A. *Nature Reviews Cancer* **2002**, *2*, 420-430.
2. Schimmer, A. D. *Cancer Research* **2004**, *64*, 7183-7190.
Schimmer, A. D.; Welsh, K.; Pinilla, C.; Wang, Z.; Krajewska, M.; Bonneau, M.-J.; Pedersen, I. M.; Kitada, S.; Scott, F. L.; Bailly-Maitre, B.; Glinsky, G.; Scudiero, D.; Sausville, E.; Salvesen, G.; Nefzi, A.; Ostresh, J. M.; Houghten, R. A.; Reed, J. C. *Cancer Cell* **2004**, *5*, 25-35.
3. Mangues, R.; Casanova, I.; Albericio, F.; Álvarez, M.; Savina, S. EP Patent 09166668.

POSTER COMMUNICATIONS – PC.098

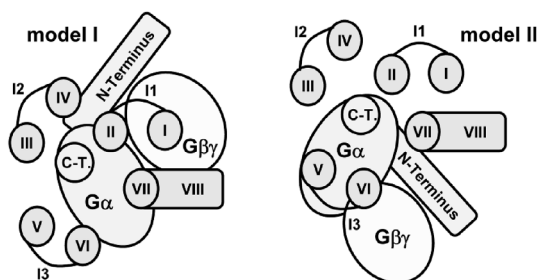
TWO MODELS FOR DISTINCT INTERACTIONS BETWEEN THE HUMAN ADRENERGIC β_2 RECEPTOR AND $G\alpha_s$ – AN IN SILICO STUDY

Straßer, A., Wittmann, H.-J.

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G protein-coupled receptors (GPCR), important targets for several drugs, couple intracellular to heterotrimeric G proteins. These G proteins are switches on molecular level in order to turn on intracellular signal cascades, induced by activation of the corresponding GPCR. Some new crystallographic studies [1] give information about the interaction between receptor and a part of the C-terminus of the $G\alpha$ -subunit of the G protein. But still, there is no crystal structure about a complete GPCR G-protein complex.

The aim of this study was an in silico analysis of the interaction of the human adrenergic β_2 receptor ($h\beta_2R$) with the $G\alpha_s$ subunit. A systematic potential energy surface scan between $h\beta_2R$ and $G\alpha_s$ revealed two energetically preferred $h\beta_2R$ - $G\alpha_s$ complexes. These two complexes were embedded into a lipid bilayer and surrounded by intra- and extracellular water. Subsequently, molecular dynamic simulations were performed in order to get knowledge about distinct hydrogen bond interactions between $h\beta_2R$ and $G\alpha_s$. Based on the modelling results, two stable $h\beta_2R$ - $G\alpha_s$ complexes with completely different orientation between $h\beta_2R$ and $G\alpha_s$ were obtained.



In general, these two models are in good accordance to experimental results, available in literature. However, all experimental results found for several GPCRs, can not be explained by one distinct orientation between receptor and $G\alpha$ subunit at one time. Thus, based on the results of this in silico study, it may be hypothesized, that there exist two different interaction states between receptor and G protein, in dependence of the length of I3-loop or C-terminus. Further experimental mutagenesis studies have to be performed to proof this hypothesis. However, this in silico study gives distinct suggestions for interaction between GPCR and G proteins, which is important for a better understanding on molecular level [2].

REFERENCES

1. Scheerer P, et al., *Nature*, 455:497-503
2. Strasser A, Wittmann HJ, *J Mol Model*, DOI:10.1007/s00894-010-0646-3

POSTER COMMUNICATIONS – PC.099

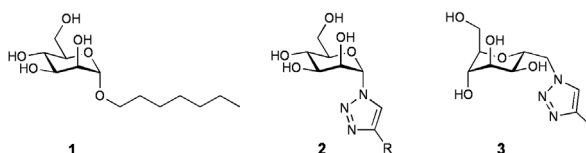
BIOLOGICAL EVALUATION OF α -D-MANNOSYL TRIAZOLES AS LIGANDS FOR FimH

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Uropathogenic strains of *Escherichia coli* (UPEC) are accounting for more than 70% of all urinary tract infections (UTIs).¹ These infections are initiated by the adhesion of the mannose-binding lectin FimH, located at the tips of bacterial type 1 pili, to uroplakin receptors in the uroepithelium. Since bacterial resistance to antibiotics is the major problem of recurrent infections, blocking of bacterial adhesion with mannose derivatives like *n*-heptyl α -D-mannoside **1**² is a promising therapeutic approach to prevent infection.

In our search for metabolically stable and pharmacokinetically improved FimH antagonists, a library of mannosyl triazoles of types **2** and **3** was synthesized and tested in target-based and cell-based binding assays. Compared to the reference compound **1**, the affinity of the triazoles **2** and **3** was decreased about 2- to 10-fold. A thorough analysis by NMR spectroscopy (NOESY, COSY, HSQC) revealed that the C-mannosides **3** adopt an unusual ¹C₄ conformation.



REFERENCES

1. T. M. Hooton, W. E. Stamm, *Infect Dis. Clin. North. Am.* **1997**, *11*, 551.
2. J. Bouckaert, J. Berglund, M. Schembri, E. De Genst, L. Cools, M. Wuhrer, C.-S. Hung, J. Pinkner, R. Slättegård, A. Zavialov, D. Choudhury, S. Langermann, S. J. Hultgren, L. Wyns, P. Klemm, S. Oscarson, S. D. Knight, H. De Greve, *Mol. Microbiol.* **2005**, *55*, 441.

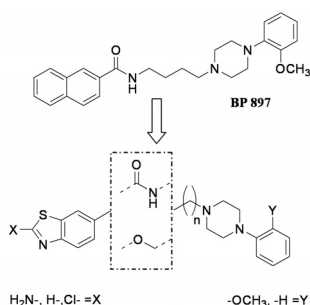
POSTER COMMUNICATIONS – PC.100

POTENT AND SELECTIVE DOPAMINE D_{2/3} LIGANDS WITH BENZTHIAZOLE ELEMENTS

Schübler, M., Schwed, S., Weizel, L., Kottke, T., Stark, H.

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Dopamine D₂ and D₃ receptor subtypes belong to the dopamine D₂-like receptor family, a group of class A G-protein coupled receptors (GPCRs). They have become highly interesting drug targets of intensive research over the past decades. The both receptor subtypes offer valuable points of action for the development of medications against neuropsychiatric disorders like Parkinson's disease, schizophrenia and drug addiction.¹ A former clinical candidate BP 897, (K_i hD₂: 67 nM, hD₃: 0,92 nM, ratio hD₂/hD₃: 66) with an in vitro partial agonist functionality on the hD₃ receptor, has been used as prominent point of beginning in the development of potent dopamine D₂/D₃ receptor compounds.²



In our bioisosteric approach we have exchanged the naphth-2-yl aromatic substructure of BP 897 by benz[d]thiazol-6-yl moieties with different connecting groups. Substituents for variations in X and Y as well as different alkyl spacer lengths n on the benz[d]thiazol-6-ylcarboxamides and on the corresponding ether derivatives have been evaluated concerning their influence on hD₂ and hD₃ receptor subtype affinities (cf. Scheme). The compounds showed moderate to high affinities in the nanomolar concentration ranges in which slight changes of the structural parameters led to a change in the receptor profile.

Synthesis, screening assays, pharmacological affinity data, and structure-activity-relationships on these two new classes of compounds will be described.

REFERENCES

1. A.E. Hackling, H.Stark, *ChemBioChem* **2002**, *3*, 946.
2. M. Pilla et al., *Nature* **1999**, *400*, 371.

POSTER COMMUNICATIONS – PC.101

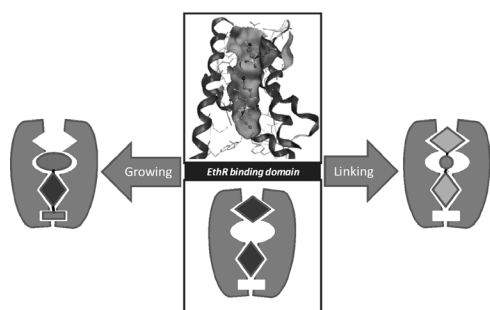
FRAGMENT LINKING AND FRAGMENT GROWING STRATEGIES FOR THE DESIGN AND DISCOVERY OF EthR INHIBITORS AS POTENT ETHIONAMIDE BOOSTERS: A NEW STRATEGY TO FIGHT MDR-TUBERCULOSIS.

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Tuberculosis remains a major cause of mortality killing each year more than two million people. Current tuberculosis therapies include a large number of prodrugs that are activated inside of the mycobacteria. The protein EthA is responsible for the activation of Ethionamide, a second-line antibiotic. The negative regulation of *ethA*, exerted by the bacterial transcriptional repressor EthR, limits the effectiveness of the prodrug. [1] Thus, EthR contributes to the innate resistance of mycobacteria to this antibiotic. We recently reported that drug-like EthR inhibitors are able to boost the antimycobacterial efficacy of Ethionamide both *in vitro* and *in vivo*. [2] In the course of identification of new chemical entities, BIAcore assay and X-ray crystallography were used to identify a fragment binder featuring arylsulfonamide framework. Based on this scaffold we developed parallel fragment-based strategies to improve activities. Analysis of the binding mode, design, screening of virtual libraries and hit expansion allowed us to rapidly establish improved molecules. Further elaboration using structure-based drug design resulted in drastic potency improvement while maintaining good physicochemical and pharmacokinetic properties.

This presentation will discuss the design, synthesis and preliminary *in vitro* and *ex vivo* biological evaluation of fragment-based EthR inhibitors.



REFERENCES

1. Engohang-Ndong, J. et al. Mol Microbiol 2004, 51, (1), 175.
2. Willand, N. et al. Nat Med 2009, 15, (5), 537.

POSTER COMMUNICATIONS – PC.102

DISCOVERY AND SAR OF NOVEL FURAN-FREE IMIDAZOPYRIDINE LIGANDS AS SELECTIVE ADENOSINE A_{2A}-ANTAGONISTS FOR PARKINSON DISEASE

Schann Stephan; Mayer Stanislas; Lydie Cardona; Sophie Gardan; Nadia Hubert ; Anne Haberkorn ; Edith Steinberg ; Luc Baron; Franchet Christel; Neuville Pascal

Domain Therapeutics, BioParc, Illkirch-Strasbourg, France

A_{2A}-receptor antagonism is a novel therapeutic strategy for the treatment of Parkinson's Disease, a neurodegenerative disorder resulting from a gradual loss of dopaminergic neurons. A_{2A}-receptor antagonists have shown efficacy in animal models and promising results in clinical evaluations. Whilst a number of different ligands were described as A_{2A}-receptor antagonists, they can be broadly divided into two families: the xanthine and the non-xanthine, the majority of the latter containing a furan moiety that can be subject to an oxidative metabolism leading to reactive species.

At Domain Therapeutics, we have identified a novel series of imidazopyridine compounds as A_{2A}-receptor ligands. These compounds are completely distinct from the known non-xanthine A_{2A}-receptor antagonists. Their optimization led to compounds with low-nanomolar affinity and efficacy for the A_{2A}-receptor, good selectivity profile and no flag in early ADME-Tox. The best representatives were further evaluated in two rodent models of Parkinson's Disease and showed efficacies after oral administration.

POSTER COMMUNICATIONS – PC.103

A JOURNEY IN PHYSICO-CHEMICAL PROPERTY SPACE, FROM LEAD TO DRUG CANDIDATES: METRICS, CHALLENGES AND PROGRESS IN PEPTIDERGIC GPCRS MODULATORS: OPTIMIZATION OF A V1a ANTAGONIST

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The development of small-molecule ligands of peptidergic receptors (with the exception of opioid receptor ligands) is still considered a challenge by medicinal chemists. The average molecular weight, total polar surface area (TPSA) and cLogP of peptidergic ligands are significantly higher than those of ligands of other targets such as aminergic GPCRs, ion channels and transporters. Since high molecular weight, high TPSA and high cLogP are associated with poor drug-like properties, increased risk of off-target toxicity and poor CNS penetration, small-molecule peptidergic GPCR ligands have suffered from high attrition between the bench and the clinic. It is therefore not surprising that despite significant efforts from the pharmaceutical industry, a relatively small number of peptidergic GPCRs antagonists have been approved for marketing. We will present a range of tools to measure progress along lead optimization, as applied to the specific example of small-molecule, drug-like V1a antagonist.

The talk will include

- A brief outlook of the pharmacology of V1a
- The properties of peptidergic GPCR ligands (in terms of high cLogP, H-Bond donor/acceptor profile, MW) and the challenges associated with these properties, especially regarding absorption, polypharmacology and toxicity.
- The use of in-silico models beyond the "rule-of-five"
- The use of calculated parameters to assess progress within a series (LipE)
- Early results, and several rounds of optimization using library design software among other techniques
- The structure and properties of final compound
- Key learnings

POSTER COMMUNICATIONS – PC.104

NOVEL [1]BENZOTHIENO[3,2-*d*]PYRIMIDIN-4(3*H*)-ONES. LIGANDS FOR THE 5-HT₇ RECEPTOR

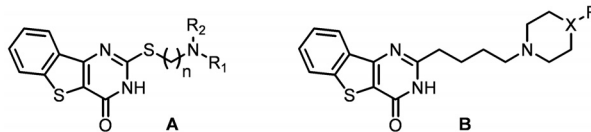
*Giuseppe Romeo*¹, *Valeria Pittalà*¹, *Maria N. Modica*¹,
*Loredana Salerno*¹, *Francesco Guerrera*¹, *Maria A. Siracusa*¹,
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The 5-HT₇ receptor (5-HT₇R) is the last added member of the serotonin (5-HT) receptors family. This receptor, characterized in 1993, belongs to the G protein-coupled receptor family and is positively coupled with adenylyl cyclase. Alternative splicing of the 5-HT₇R gene has been reported to generate different isoforms (5-HT_{7(a), (b), (c)} in rat and 5-HT_{7(a), (b), (d)} in human) which differ only in the length and amino acid composition of their carboxyl-terminal tail. In human, the most abundant isoform is the 5-HT_{7(a)}. These isoforms do not show significant differences in their pharmacological profile, signal transduction or tissue distribution.

Since their discovery, 5-HT₇R has been the subject of intense research due to their widespread distribution in the brain. It has been proposed to be involved in learning and memory, circadian rhythms, locomotor activity, thermoregulation, nociception, as well as in depression, anxiety, psychiatric disorders, epilepsy and some inflammatory processes [1].

In recent years our research group has been involved in the development of selective serotonin receptor ligands [2] and recently our studies have been focused on the design and synthesis of selective 5-HT₇R ligands. In particular, tricyclic [1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-one derivatives bearing an aminoalkylthio side chain in 2-position of the tricyclic system (general formula **A**) had shown affinity for the human cloned 5-HT₇R [3]. We now report the synthesis of a new series of [1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-ones of general formula **B**. These compounds bear in the 2-position of the heterocyclic system an aminobutyl side chain in which the basic moiety is represented by 4-phenyl or 4-benzyl piperazines or piperidines (**B**, X = N, CH). Title compounds were prepared following two different synthetic pathways (each consisting of four steps) starting from methyl 3-aminobenzo[*b*]thiophene-2-carboxylate. As a general trend, B compounds (having a connecting butyl chain between the tricyclic system and the basic moiety) showed better affinity values for the human cloned 5-HT₇R than those displayed by their corresponding analogues of the A series (with a propylthio chain). Moreover, some of them showed high selectivity over the serotonin 5-HT_{1A} receptor. Complete synthetic pathways to title compounds along with binding data will be reported at the symposium.



REFERENCES

1. Pittalà, V; Salerno, L; Modica, M; Siracusa, M A; Romeo, G. 5-HT₇ receptor ligands: recent developments and potential therapeutic applications. *Mini-Rev. Med. Chem.* 7, 945-960 (2007).
2. Siracusa, M A; Salerno, L; Modica, M; Pittalà, V; Romeo, G; Amato, M E; Nowak, M; Bojarski, A J; Mereghetti, I; Cagnotto, A; Mennini, T. Synthesis of new arylpiperazinylalkylthiobenzimidazole, benzothiazole, or benzoxazole derivatives as potent and selective 5-HT_{1A} serotonin receptor ligands. *J. Med. Chem.* 51, 4529-4538 (2008).
3. Romeo, G; Modica, M; Pittalà, V; Salerno, L; Siracusa, M A; Cagnotto, A; Mennini, T. New Thienopyrimidinone derivatives as potential 5-HT₇ receptor ligands. *XIX National Meeting on Medicinal Chemistry*, 14-18 September 2008, Verona (Italy).

POSTER COMMUNICATIONS – PC.105

DESIGN AND SYNTHESIS OF PROTEIN-PROTEIN INTERACTION MIMICS AS *PLASMODIUM FALCIPARUM* CYSTEINE PROTEASE FALCIPAIN-2 INHIBITORS

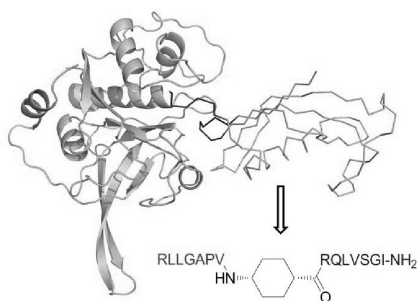
Sergio Romeo^{1,3}, *Luca Rizzi*¹, *Srividhya Sundararaman*²,
*Katarina Cendic*¹, *Nadia Vaiana*¹, *Reshma Korde*², *Dipto Sinha*², *Asif Mohammed*², *Pawan Malhotra*^{2,3}

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Cysteine proteases play a significant role in the growth and the development of several human parasites such as *Plasmodium*, *Leishmania*, and *Trypanosoma* which affect more than 3.5 billion people worldwide. Falcipain-2 (FP-2) is an important cysteine protease of the human malaria parasite, *P. falciparum* (Pf), and is one of the most promising target for the development of new Pf inhibitors.¹ FP-2 plays roles in haemoglobin degradation and merozoites egression at the asexual blood stages of *Plasmodium* development. Endogenous cysteine protease inhibitors have been described in a number of eukaryotic systems and they represent an important platform for new drug design. Cystatin and its homologues are natural inhibitors of Papain and others cysteine proteases, but the high molecular weight and the peptidic structure raise difficulties in their exploitation in the drug design process. In the present study, we designed a series of small peptides that mimic the protein-protein interaction between falcipain-2 and Chicken Egg White Cystatin and analyzed their effects on falcipain-2 activity.

These peptide mimics were designed keeping in mind the distance of about 4-5 Å between the carbonyl of Pro¹¹ or Val¹² of cystatin. Three different linkers, γ -aminobutyric acid (GABA), *cis*-4-aminocyclohexane carboxylic acid and a macrocycle formed by GABA and two cysteines joined by a disulfide bridge were used to link peptides. Some of these compounds showed considerable inhibition ($\geq 50\%$) at 5 μM (K_i 2-7.5 μM). When these peptide mimics were tested against the mature parasite, they also produced morphological abnormalities especially in the food vacuole, similar to what have been observed with others cysteine protease inhibitors. We believe this approach could be an excellent starting point for the development of new cysteine protease inhibitors and in general for the development of new drugs against *P. falciparum*.



REFERENCES

1. Sijwali, P. S.; Rosenthal, P. J. Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (13), 4384-4389.

POSTER COMMUNICATIONS – PC.106

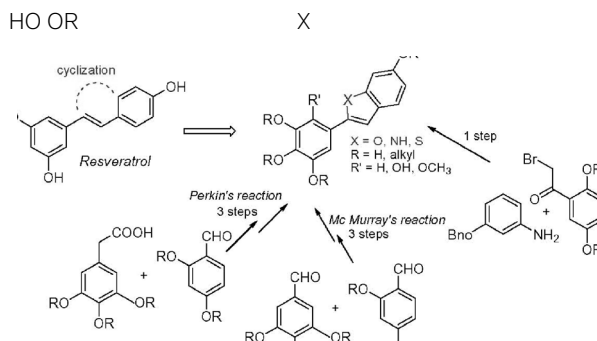
ANTITUMOR AGENTS. SYNTHESIS AND CANCER CELL GROWTH INHIBITION OF NEW RESVERATROL DERIVATIVES

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Pharmaceutical studies suggested that the polyphenol resveratrol (*trans*-3,4',5-trihydroxystilbene) is one of the main wine grape components that protect from vascular and neurodegenerative diseases and cancers. The ability of resveratrol to prevent the occurrence of carcinomas was related to the inhibition of tumor cell cycle and induction of tumor cell death.¹⁻² We focused on the synthesis of new structural resveratrol analogues obtained by cyclization of stilbene (Scheme 1). The double bond was replaced by an unsaturated ring. This modification should allow us to assess the importance of the double bond of natural product.

Scheme 1 OR



OR Three different synthetic pathways for the preparation of the resveratrol analogues have been proposed. A series of these analogues was synthesized via McMurray reaction between two substituted aldehydes or by condensation of the substituted arylacetic acid with the corresponding aldehyde under Perkin's conditions. An analog containing a nitrogen in the aromatic ring was synthesized in one step from the substituted aniline and the corresponding 2-bromo-1-(2,5dimethoxyphenyl) ethanone via Bischler reaction. The dimethoxy compounds were deprotected using BBr₃ giving the desired compounds in high overall yields. The synthesis and biological data will be reported.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Ministry of Science and Technology (CTQ2007-60614/BQU) for the financial support.

REFERENCES

1. G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J. M. Schmidt, F. Hogan, *J. Med. Chem.* **1995**, *38*, 1666-1672.
2. G. R. Pettit, A. Thornhill, N. Melody, J. C. Knight. *J. Nat. Prod.* **2009**, *72*, 380-388.

POSTER COMMUNICATIONS – PC.107

MUTUALLY COMPLEMENTARY LIGAND AND STRUCTURE-BASED COMPUTATIONAL STUDIES ON A NOVEL SERIES OF POTENT AND SELECTIVE A₃ ADENOSINE RECEPTOR ANTAGONISTS

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Adenosine receptors (ARs) are a family of proteins belonging to the superfamily of G-Protein Coupled Receptors (GPCRs), which is the main pharmacological target nowadays. ARs are divided into 4 subtypes (A_1 , A_{2A} , A_{2B} and A_3), and are considered potential drug targets in disorders such as schizophrenia, Parkinson, asthma or cardiovascular diseases. [1] We have recently reported the synthesis of a diarylpyrimidine library as selective A_3 AR antagonists. [2] In a further step, we have grown and experimentally tested against the four human AR subtypes a collection of 150 members of these chemical libraries. [3]

In the present work we determine the binding mode of the family of diarylpyrimidine compounds, and establish structure-activity relationships, all of which will be used as a guide the design of the new libraries herein described. On the basis of the generated model, we also pursue to obtain structural information of the ARs that may account for both for potency and selectivity of AR antagonists.

A homology model of A_3 AR receptor was built with MODELLER using the recently crystallized A_{2A} structure as template. We then carried out an automated docking exploration with the set of 63 compounds for which an experimental value of K_i was obtained on the A_3 AR, with the software GOLD v3.2. This protocol was based on our experience on the GPCR Dock 2008 competition. [4] The molecular alignment generated on the docking step was used as input for a 3D-QSAR study, by means of the second generation of Grid-INdependent Descriptors (GRIND-2), [5] with the software PENTACLE v10.4.

Our systematic docking exploration consistently identifies a conserved binding mode for the series of 2 and 4-amidopyrimidines. The main anchoring point is a double hydrogen bond between the ligand and residue Asn6.55, together with a π -stacking interaction with Phe5.29. This binding mode is consistent with that experimentally observed for the A_{2A} antagonist ZM241385. Regarding the 3D-QSAR study, we obtained a model using the ligands aligned by their docking poses as input, yielding statistical values of good quality ($r^2=0.88$, $q^2=0.70$). With this model we can relate the size and disposition of the different substituents of the ligands, with their location and interactions within receptor sub-sites. Importantly, the conservation of the binding site residues, and their role in affinity and selectivity of antagonists binding is addressed in this work.

REFERENCES

1. Jacobson K.A. et al. Nat. Rev. Drug Discov. 2006; 5(3):247-64
2. Yaziji V. et al. J. Comb. Chem. 2009; 11:519-522
3. Manuscript in preparation.
4. Michino M. et al. Nat. Rev. Drug Discov. 2009; 8(6):455-63
5. Durán A. et al. J Chem. Inf. Mod. 2009; 49: 2129-38

POSTER COMMUNICATIONS – PC.108

POSITIVE ALLOSTERIC MODULATION OF THE mGluR5 RECEPTOR: SAR, SPR AND IN VIVO ACTIVITY OF ADX47273 DERIVATIVES

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Glutamate is the major excitatory neurotransmitter in the brain. It mediates its actions via the activation of both ligand gated ion channels (iGluRs) and a family of class C G-Protein Coupled Receptors known as metabotropic Glutamate Receptors (mGluRs). mGluRs play important roles in various neurological disorders.

The allosteric binding sites are topographically distinct and are less conserved within the 8 mGluR subtypes compared to the glutamate orthosteric binding site. Consequently, allosteric ligands of mGluR₁₋₈ have higher pharmacophore diversity than orthosteric ligands. Also, allosteric modulators of mGluR₁₋₈ have been shown to offer a valid strategy to develop non amino-acid like therapeutics that can be administered orally and that readily cross the blood-brain barrier. In addition, positive allosteric modulators (PAMs) are unlikely to induce a rapid desensitization such as the currently available agonists.

mGluR₅ receptors are located mainly on post-synaptic elements in the brain (cortex, thalamus, hippocampus and cerebellum), from where they regulate the activity of NMDA & AMPA, as well as the excitability of the post-synaptic neurons.

The aryl-oxadiazole ADX47273 has been the topic of intense interest for the pharmacological evaluation of the mGluR₅ PAM approach; this compound demonstrated promising antipsychotic activity in preclinical rodent models for positive symptoms of schizophrenia and it was also active in cognition models. ADX47273 has opened an avenue to a novel class of drug-like molecules in this field. We will present the multiparametric lead optimization program which led to the identification of several chemical series of highly selective mGluR₅ receptor PAM. Compared to ADX47273 which suffered of a poor bioavailability; the new molecules are systematically active in rodent behavioral models.

POSTER COMMUNICATIONS – PC.109

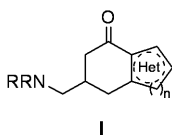
SYNTHESIS, BINDING AFFINITY AND SAR OF NEW HETEROCYCLIC ANALOGUES OF HALOPERIDOL AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

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The newer generation of treatments for schizophrenia, referred to as atypical antipsychotics, add to the blockade of dopamine receptors, a potent activity at serotonin receptors. It is thought that 5-HT_{2A} antagonism together with relatively weaker dopamine antagonism are principal features that differentiate the side-effect profile of atypical antipsychotics, such as clozapine, from the first generation of treatments [1]. Although the novel atypical antipsychotics olanzapine, risperidone and quetiapine have brought about improvements in toleration and negative symptomatology, chronic treatment may lead to unwanted weight gain, blood dyscrasias, and motor dysfunctions, such as extra-pyramidal side effects (EPS) and tardive dyskinesia (TD). These side effects may be linked to drug-dependent affinity for other receptors. The search in our group continues for new atypical antipsychotics that would be more efficacious and would have fewer side effects than currently available treatments [2].

Since antidopaminergic therapies continue in the first line of schizophrenia's treatment, in this communication, we will describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D₂ antagonists for potential use as treatments for schizophrenia. In these compounds a typical bioisosteric replacement of the benzene in the aminobutyrophe none pharmacophore by a five- or six-membered heterocycle (I) containing at least one heteroatom has been applied.



ACKNOWLEDGMENTS

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REFERENCES

1. Meltzer, H. Y.; Matsubara, S.; Lee, J. J. *Pharm. Exp. Ther.* **1989**, 251, 238.
2. (a) Dezi, C.; Brea, J.; Alvarado, M.; Raviña, E.; Masaguer, C. F.; Loza, M. I.; Sanz, F.; Pastor, M. J. *Med. Chem.* **2007**, 50, 3242; (b) Aranda,

R.; Villalba, K.; Raviña, E.; Masaguer, C. F. Brea, J.; Areias, F.; Dominguez, E.; Selent, J.; Lopez, L.; Sanz, F.; Pastor, M.; Loza, M. I. *J. Med. Chem.* **2008**, 51, 6085; (c) Carro, L.; Raviña, E.; Domínguez, E.; Brea, J.; Loza, M.I.; Masaguer, C.F. *Bioorg. Med. Chem. Lett.* **2009**, 19, 6059

POSTER COMMUNICATIONS – PC.111

NATURAL PRODUCT FRAGMENTS AS A NOVEL DIVERSITY SET FOR FRAGMENT-BASED SCREENING

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Our objective is to provide a number of interconnecting approaches to allow natural products to be a competitive source of new leads in modern drug discovery.

We use these new approaches to address the timelines for mining natural products for pharmacologically interesting compounds. In recent years we have -

1. published our work in developing a drug-like natural product library with properties that comply with Lipinski rules for orally active drugs. This demonstrates that many natural products display the physicochemical properties required in orally acting pharmaceuticals.¹
2. demonstrated, using X-ray crystal structure data, that a similarity exists between the recognition of compounds to three biosynthetic enzymes in the flavonoid biosynthetic pathway and the recognition of protein kinases. This demonstrates natural products retain an imprint of biology space and explains why many natural products recognise the ligand binding domain in many therapeutically important proteins.^{2,3}
3. demonstrated that it is possible to take a complex natural product extract and identify those constituents that bind reversibly to proteins using a FTMS method. We have subsequently isolated the active constituent using mass directed fractionation, demonstrated that the compound has functional activity and obtained an X-ray structure of the complex. This mass spectrometry based approach can be used to discover naturally occurring chemicals that bind to native unmodified proteins.⁴

We now present a chemo-informatics study that shows we can capture the diversity of natural products by using naturally occurring chemical fragments. Results from fragment-based drug discovery based on natural product fragments will be presented.

In combination these technological approaches contribute to addressing druggable natural products within normal lead identification timelines.

REFERENCES

1. Quinn, R. J.; Carroll, A. R.; Pham, N. B.; Baron, P.; Palframan, M. E.; Suraweera, L.; Pierens, G. K.; Muresan, S. Developing a drug-like natural product library. *J. Nat. Prod.* **2008**, *71*, 464-468.
2. McArdle, B. M.; Campitelli, M. R.; Quinn, R. J. A Common Protein Fold Topology Shared by Flavonoid Biosynthetic Enzymes and Therapeutic Targets. *J. Nat. Prod.* **2006**, *69*, 14-17.
3. Maresca, A.; Temperini, C.; Vu, H.; Pham, N. B.; Poulsen, S.-A.; Scozzafava, A.; Quinn, R. J.; Supuran, C. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide Inhibitors. *J. Am. Chem. Soc.* **2009**, *131*, 3057-3062.
4. Vu, H.; Pham, N. B.; Quinn, R. J. Direct screening of natural product extracts using mass spectrometry. *J. Biomol. Screen.* **2008**, *13*, 265-275.

POSTER COMMUNICATIONS – PC.112

SYNTHESIS AND VASORELAXANT ACTIVITY OF PHTHALAZINONES

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Hypertension is one of the most common cardiovascular diseases that can cause coronary disease, myocardial infarction, stroke and sudden death and is the major contributor to cardiac failure and renal insufficiency. [1]

Within the drugs in the market, hydralazine, one of the first anti-hypertensive agent, has attracted much attention in the last decade and it is newly considered as a lead for developing new drugs, due to its direct vasodilator action. [2]

Structural modification of hydralazine led to the discovery of some pyridazinones and other phthalazine derivatives with broad spectra on the cardiovascular system, including antihypertensive effects and inhibition of platelet aggregation and of phosphodiesterases. [3][4]

A series of N2-substituted phthalazine-1,4-dione derivatives has been synthesized through condensation of substituted phthalic anhydride either with hydrazine or with substituted hydrazines or through N-alkylation of phthalazine-1,4-dione derivatives previously synthesized. [4]

The vasorelaxant activity of these compounds was evaluated on isolated rat aorta rings either with or without endothelium, previously stimulated with phenylephrine. [5] Some of the synthesized compounds have been characterized as agents with clear vasorelaxant effects and therefore with a promising future as antihypertensive agents.

REFERENCES

1. Oates, J. A. Antihypertensive Agents and the Drug Therapy of Hypertension. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th ed.; Pergamon Press, **2001**; Vol.780, Section V.33.
2. Florez, J.; Armijo, J. A.; Mediavilla A. *Farmacología Humana*, 3rd ed.; Masson S.a., Barcelona, 2000; Vol. 671.
3. Heinisch, G.; Kopelent-Frank, H. *Prog. Med. Chem.* **1992**, *29*, 141.
4. del Olmo, E.; Barboza, B.; Ybarra M. I.; López-Pérez, J. L.; Carrón, R.; Sevilla M. A.; Boselli, C.; San Feliciano, A. *Bioorg. & Med. Chem. Lett.* **2006**, 2786.
5. Quezada E., Delogu G., Picciau C., Santana L., Podda G., Borges F., García-Morales V., Viña D., Orallo F. *Molecules* **2010**, *15* (1), 270-9.

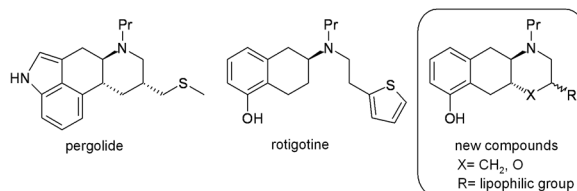
POSTER COMMUNICATIONS – PC.113

SYNTHESIS, X-RAY CRYSTALLOGRAPHY AND IN VITRO CHARACTERIZATION OF NOVEL DOPAMINE RECEPTOR AGONISTS

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Pergolide and rotigotine are used clinically for the treatment of Parkinson's disease. Rotigotine contains a structural alert in the form of the thiophene ring, while the ergot alkaloid-derived dopamine receptor agonists pergolide and cabergolide have been associated with severe multivalvular heart disease.¹ Computational analysis of the presumed binding conformations of rotigotine and pergolide suggested to us that the methyl thioether and thiophene ring of the two drugs occupy the same space in the dopamine receptor. Herein we report the synthesis and structure-activity relationships at dopamine D1 and D2 receptors of a 'hybrid' series in which conformationally restricted rotigotine scaffolds have been combined with R side chains, which were chosen in part based on previously reported pergolide analogs.² The compounds (R = lipophilic group) were prepared by a novel method using (2R, 3R)-8-Methoxy-3-propylamino-1,2,3,4-tetrahydro-naphthalen-2-ol and (R)-(-)-Epichlorohydrin or (S)-(+)-Epichlorohydrin, or (R = H) from an intermediate described by Nozulak *et al.*³ The absolute conformations of these novel compounds were elucidated by X-ray crystallography.



REFERENCES

- Horvath, J.; Fross, R.D.; Kleiner-Fisman, G.; Lerch, R.; Stalder, H.; Liaudat, S.; Raskoff, W.J.; Flachsbar, K.D.; Rakowski, H.; Pache, J.-P.; Burkhard, P.R.; Lang, A.E. *Movement Disorders* (2004), 19, 656-662. Pergolide was withdrawn from the US market in 2007 by due to the possibility of heart valve damage.
- Ohno, S.; Koumori, M.; Adachi, Y.; Mizukoshi, K.; Nagasaka, M.; Ichihara, K. *Chem. Pharm. Bull.* (1994), 42(10), 2042-2048.
- Nozulak, J.; Vigouret, J. M.; Jaton, A. L.; Hofmann, A.; Dravid, A. R.; Weber, H. P.; Kalkman, H. O.; Walkinshaw, M. D. *J. Med. Chem.* (1992) 35, 480-489.

POSTER COMMUNICATIONS – PC.115

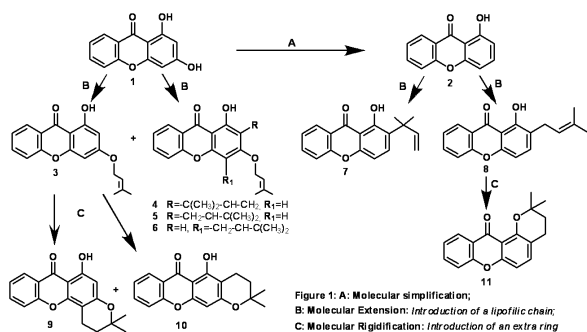
MOLECULAR MODIFICATIONS ON XANTHONIC SCAFFOLD: STRUCTURE-ACTIVITY RELATIONSHIP APPROACHES

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Xanthonic compounds show interesting biological activities associated with their tricyclic scaffold depending on the nature and/or position of the different substituents¹⁻². A relationship between activity and the presence of prenyl groups in key positions on the xanthone nucleus was associated with some biological activities, such as antibacterial, PKC modulation and antitumor¹.

Therefore, prenylated xanthenes could represent an excellent model for the development of new and more effective drugs, particularly in the improvement of antitumor activity, and for this reason the introduction of prenyl groups in "hit" compounds, has been one of the strategies used in our research group³⁻⁴. Based on previous results concerning the effect of several xanthonic derivatives on the *in vitro* growth of some human tumor cell lines³, we have selected 1,3-dihydroxyxanthone (**1**), as starting material to carry out some molecular modifications in order to improve its antitumor activity. The strategy used is showed in **Figure 1**:



Compounds **3-10** were evaluated for their effect on the *in vitro* growth of human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung) and SF-268 (central nervous system). Compound **11** is under study. Xanthenes **4** and **5** exhibited a selective and potent growth inhibitory effect against the MCF-7 cell line, comparing with respective building block **1**. On the other hand, it was observed for compounds **7** and **8**, the appearance of a moderate effect on the activity against the MCF-7 cell line, when compared with their synthetic precursor **2**. Considering prenylated xanthenes **4, 5, 7** and **8**, all with a prenyl group at C2 position of the xanthone scaffold, it was found that compounds **4** and **5** were significantly more active than **7** and **8**, which may indicate that the existence of an additional prenyloxy group at C3 in xanthenes **4** and **5**, could be an important feature for this activity.

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REFERENCES

- Pinto, M., Castanheiro, R., "Natural Prenylated Xanthenes: Chemistry and Biological Activities", Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, Índia, **2009**, Chap. 17, pp.520-676.
- Pinto, M., *et al. Curr. Med. Chem.* **2005**, 12, 2517.
- Castanheiro, R., *et al. Bioorg. Med. Chem.* **2007**, 15, 6080.
- Castanheiro, R., *et al. Pharmaceuticals* **2009**, 2(2), 33.

POSTER COMMUNICATIONS – PC.116

ANTIMITOTIC AGENTS BEARING PYRIDINE N-OXIDE MOIETIES

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Antimitotic agents act by binding to tubulin and thus altering its normal polymerisation / depolymerisation dynamics. This causes inhibition of mitotic spindle formation, which leads to cell cycle arrest and, subsequently, to cell death. Combretastatins and phenstatins are potent antimitotic agents that bind to tubulin at the colchicine site¹. Several combretastatins have entered phase II/III clinical trials as their prodrugs (Fig 1).

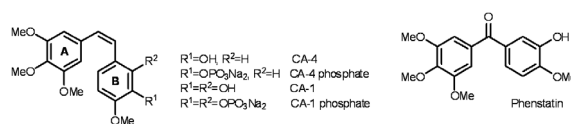


Fig 1. Structure of combretastatin A-4, combretastatin A-1, their prodrugs and phenstatin.

Several modifications have been carried out on the basic structure of combretastatins and phenstatins,² most of them addressed at the so-called B ring and at the bridge between both aromatic systems. Modifications of the A ring (3,4,5-trimethoxyphenyl) are much scarcer, and usually lead to compounds displaying decreased potency.

Recently, we proposed the replacement of ring A by 2,6-dimethoxy-4-pyridyl and its *N*-oxide,³ in order to investigate whether this system can effectively replace such A ring and thus produce potent antitubulin compounds (Fig 2). The *N*-oxide Oxygen could replace the 4-methoxy group of the model compounds and thus contribute to the binding to tubulin by hydrogen bonding, as it has been proposed as binding mode for these agents.

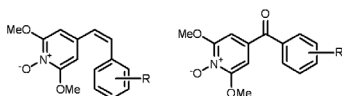


Fig 2. Structure pyridine *N*-oxide analogues of combretastatins and phenstatins.

Oxidation of pyridines to their *N*-oxides is usually described as an easy process, using for example MCPBA. However, for 2,5-disubstituted pyridines with electron-withdrawing groups such oxidation is more difficult to perform. A variety of reagents and conditions failed, although finally the desired pyridine *N*-oxides were obtained. Preliminary activity results indicate that these analogues are less potent than the parent compounds. Synthetic and biological results will be described in detail.

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REFERENCES

1. (a) Lin, C.M., Ho, H.H., Pettit, G.R., Hamel, E. *Biochemistry* **1989**, *28*, 6984. (b) Pettit, G.R., Toki, B., Herald, D.L., Verdier-Pinard, P. Boyd, M.R., Hamel, E., Pettit, R.K. *J. Med. Chem.* **1998**, *41*, 1688.
2. Tron, G.C., Pirali, T., Sorba, G., Pagliai, F., Busacca, S., Genazzani, A.A. *J. Med. Chem.* **2006**, *49*, 3033.
3. Lozano, M. Aramburu, L. Degree Dissertations. University of Salamanca, 2007, 2009.

According to the World Health Organization, about 1.5-2% of the world's population living with epilepsy or will experience it at some point in their life span¹. About (20-30%) of epilepsy patients are resistant to the currently available therapeutic agents. More or less 70% of patients with epilepsy are well controlled by monotherapy with currently available antiepileptic drugs, and another 5-10% of patients could be stabilized by add-on therapies. In the remaining 20% of patients seizures are not efficiently controlled². A series of novel schiff bases of 3-aminomethyl pyridine were synthesized by condensation reaction with substituted aryl aldehydes/ ketones and / cyclic ketones. These schiff bases were screened for anticonvulsant activity³⁻⁵. The chemical structures of synthesized compounds were confirmed by FT-IR, ¹H-NMR, spectra and elemental analysis. A number of compounds were observed to exhibit protection after intraperitoneal administration at the dose 30 & 100mg kg⁻¹ in various models employed. In MES screen five compounds (1_c) N-(2-chlorobenzylidene) (pyridin-3-yl) methanamine, (1_f) 2-methoxy-4-[(pyridine-3-ylmethylimino)methyl]phenol, (2_a) N-(3-phenylallylidene) (pyridin-3-yl) methanamine, (3_b) 3-[1-(pyridin-3-ylmethylimino) ethyl]benzenamine, and (4_a) N-(diphenyl-methylene) (pyridin-3-yl) methanamine, emerged out as most active compounds in series (ED₅₀) 11.70, 6.39, 11.70, 8.64 and 9.13mg kg⁻¹ with high protective index (PI) > 10. Whereas four compounds (1_e) 4-[(Pyridine-3-ylmethylimino) methyl]benzene-1,3-diol, (1_g) N-(3,4-dimethoxybenzylidene) (pyridin-3-yl)methanamine, (2_b) N-[(1H-indol-3-yl)methylene] (pyridin-3-yl) methanamine, and (5) N-cyclohexylidene (pyridine-3-yl) methanamine showed remarkable protection over clinically used drugs in Sc.-PTZ screen (ED₅₀) 6.44, 11.70, 6.47, and 14.16 with (PI > 10). Compound (4_b) showed good anticonvulsant activity (ED₅₀) 20.79 with higher neurotoxicity (PI = 0.57). Compound (1_e) was active in both Sc.-PTZ and in Sc.-STR seizures. Some selected compounds were subjected to oral MES screen (30mg kg⁻¹) in rats, most of the compounds showed peak activity after 0.5h of oral administration. The new series of schiff bases can significantly be used for future development of novel anticonvulsant agent.

REFERENCES

1. Brown TR, Holmes GL. Epilapsy *N. Engl. J. Med.*; 344:1145-1151, (2001).
2. Swann, A.C. *J. Clin. Psychiatry*, 62(14), 16-21, (2001).
3. Krall R. L., Penry J. K., White B. G., Kupferberg H. J., Swinyard E. A., *Epilepsia*, 19, 409-428, (1978).
4. [20] Kupferberg H. J., *Epilepsia*, 30, 51-56, (1989).
5. [21] Dunham M. S., Miya T. A., *Amer. J. Pharm. Ass. Sci.*, 46, 208, (1957).

POSTER COMMUNICATIONS – PC.118

SOME NOVEL HETEROCYCLIC SCHIFF BASES: AS POTENTIAL ANTICONVULSANT AGENTS

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POSTER COMMUNICATIONS – PC.120

NON-COVALENT MASS SPECTROMETRY :
A POWERFUL, INNOVATIVE TOOL FOR EARLY-STAGE DRUG DISCOVERY

Jean-Paul Renaud

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Non-covalent mass spectrometry is emerging as a powerful tool that can be used at different stages of early-stage drug discovery: (i) as an efficient secondary screening to remove false positives from a primary high-throughput screening or a virtual screening by assessing the physical binding of the primary hits to the target, and (ii) as a high-content technique for the detailed characterization of ligand-target interaction in terms of stoichiometry, reversibility, specificity, and affinity. Collision-induced dissociation experiments can serve to evaluate the enthalpic part of the binding energy, and thus to guide the hit-to-lead process.

This label-free technique consumes low protein amounts, it can be automated and it is suitable for fragment-based drug discovery. The use of non-covalent mass spectrometry in ligand screening and drug design will be illustrated by literature and internal examples.

REFERENCES

1. Vivat et al. (2010) "Native MS: an 'ESI' way to support structure- and fragment-based drug discovery" *Future Med. Chem.* 1 : 35-50
2. Renaud and Delsuc (2009) "Biophysical techniques for ligand screening and drug design" *Curr. Op. Pharmacol.* 9 : 622-628

POSTER COMMUNICATIONS – PC.121

5-BENZYLIDENERHODANINE AND
5-BENZYLIDENETHIAZOLIDINE-2,4-DIONE-
BASED INHIBITORS OF MurD LIGASE

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The escalating rate of bacterial resistance to currently available antibiotics is a well-known problem, and the need for novel anti-

bacterial drugs is increasingly important clinically. Peptidoglycan is an essential bacterial cell-wall polymer, unique to prokaryotic cells and therefore optimal for the selective targeting of microbial vital pathways. The formation of a cytoplasmic peptidoglycan precursor is catalysed by a group of enzymes, called Mur ligases (MurC-MurF), that catalyze a series of reactions, starting from UDP-*N*-acetylmuramic acid (UDP-MurNAc), to yield UDP-*N*-acetylmuramoyl-pentapeptide (UDP-MurNAc-pentapeptide) by sequentially adding L-Ala (MurC), D-Glu (MurD), meso-diaminopimelic acid or L-Lys (MurE), and the D-Ala-D-Ala dipeptide (MurF).^{1,2,3}

We synthesized and biologically evaluated a series of glutamic acid- and 5-benzylidenerhodanine- or 5-benzylidenethiazolidine-2,4-dione-based derivatives as inhibitors of MurD ligase. The series was designed based on the 2,4-diaminoquinazoline-containing highest ranked compound from virtual screening, performed with FlexX docking program using ZINC database.^{4,5} The best compounds were found to inhibit MurD from *E. coli* with IC₅₀ values in the range of 45 to 206 μM. Details of the binding mode of derivatives within the active site were established with three high-resolution cocrystal structures. In the MurD-inhibitor complex, the D-Glu moiety occupies the same site as the D-Glu residue of the product UMAG of the enzymatic reaction catalyzed by MurD. Rhodanine or thiazolidine-2,4-dione rings of the inhibitor occupy the uracyl-binding pocket. The ring nitrogen forms a hydrogen bond with the O^γ of Thr36, and the carbonyl oxygen at position 2 interacts with the Thr36 backbone NH group. NH Group on the *para*-position of benzylidene ring interacts with the enzyme through active site crystal water molecules. The details of the binding mode of compounds in the MurD active site will help to understand the structure-activity relationships and provide a good foundation for structure-based design of a novel generation of MurD inhibitors.

REFERENCES

1. Barreateau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* **2008**, 32, 168-207.
2. Kotnik, M.; Štefanič Anderluh, P.; Preželj, A. *Curr. Pharm. Design* **2007**, 13, 2283-2309.
3. Vollmer, W.; Blanot, D.; de Pedro, M. A. *FEMS Microbiol. Rev.*, **2008**, 32, 149-167.
4. Tomašić, T.; Zidar, N.; Rupnik, V.; Kovač, A.; Blanot, D.; Gobec, S.; Kikelj, D.; Peterlin Mašič, L. *Bioorg. Med. Chem. Lett.* **2009**, 19, 153-157.
5. Zidar, N.; Tomašić, T.; Šink, R.; Rupnik, V.; Kovač, A.; Turk, S.; Patin, D.; Blanot, D.; Contreras Martel, C.; Dessen, A.; Zega, A.; Gobec, S.; Peterlin Mašič, L.; Kikelj, D. *Submitted*

POSTER COMMUNICATIONS – PC.122

IMPROVING ENRICHMENT AND HIT RATE IN VIRTUAL SCREENING

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In the drug discovery process, accurate methods of computing the affinity of small molecules with a desired biological target are strongly needed. Even if, in the last years, the accuracy and efficiency of the available virtual screening algorithms have been improved, many drawbacks and limitations still exist. For example, docking methods lack a reliable simulation of both ligand and receptor flexibilities, as well as good scoring functions able to estimate ligand binding energies in reasonable agreement with experimental data. These limitations often lead to a high level of false positives or false negatives in the hit list. For that reason, it is generally agreed that docking results need to be post-processed with more accurate tools.

To this end, we developed Binding Estimation After Refinement (BEAR), a new and automated post-docking procedure for the conformational refinement of docking poses through molecular dynamics (MD) followed by accurate prediction of binding free energies using MM-PBSA and MM-GBSA¹ (Figure1).

The BEAR performance in virtual screening was evaluated on several macromolecular targets and related sets of known ligands, determining the enrichment factors and assessing the correlation between predicted and experimental binding affinities. These analyses suggested critical improvements with respect to standard docking softwares^{2,3}.

Moreover, when applied in virtual screening campaigns, BEAR was able to discover novel and potent inhibitors of Plasmodium falciparum plasmepsin II with an impressive hit rate⁴, and has been successful in identifying promising scaffolds for the design of irreversible protein kinase inhibitors⁵. Therefore, taken as a whole, the results obtained so far prospect that BEAR may become a prominent tool in the drug discovery pipeline.

The BEAR virtual screening procedure is reliable and strongly automated, and can be tailored to the needs of the end-user in terms of computational time and the desired accuracy of the results. BEAR is under constant development and validation on additional biological targets in order to further improve accuracy, automation and calculation speed.

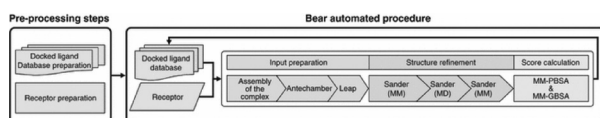


Figure 1 : The BEAR workflow

REFERENCES

1. Rastelli G, Degliesposti G, Del Rio A, Sgobba M (2009) *Chem Biol Drug Des* 73:283–28
2. Ferrari AM, Degliesposti G, Sgobba M, Rastelli G (2007) *Bioorg Med Chem* 15:7865–787
3. Rastelli G, Del Rio A, Degliesposti G, Sgobba M (2010) *J Comput Chem* 31:797–81
4. Degliesposti G, Kasam V, Da Costa A, Kang HK, Kim N, Kim DW, Breton V, Kim D, Rastelli G. (2009) *ChemMedChem* 7:1164–73.
5. Del Rio A, Sgobba M, Parenti MD, Degliesposti G, Forestiero R, Percivalle C, Conte PF, Freccero M, Rastelli G. (2010) *J Comput Aided Mol Des*. Doi: 10.1007/s10822-010-9324-x.

POSTER COMMUNICATIONS – PC.123

SYNTHESIS OF MYCOBACTERIUM TUBERCULOSIS DXR INHIBITORS BY PALLADIUM(II)-CATALYZED HECK ARYLATION OF ACROLEIN

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Tuberculosis remains one of the big infectious diseases around the world. Furthermore, the occurrence of multidrug resistant and extensively drug resistant *Mycobacterium tuberculosis* (*Mtb*) strains makes it even more urgent to develop new drugs to treat tuberculosis infected patients. The enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is involved in the second step of the non-mevalonate pathway for the synthesis of isoprenoids. This biochemical pathway is found in many bacteria and parasites, but in humans isoprenoids are synthesized by the mevalonate pathway. Thus, DXR is a very promising antibacterial drug target.

Fosmidomycin (3-(*N*-hydroxyfomamido)propylphosphonic acid) is known to inhibit *Mtb* DXR with an IC₅₀ of 80 nM. However, this compound is not active at the *Mtb* whole cell level, possibly due to the lack of uptake. In the search for more potent and cell wall permeable compounds for the inhibition of *Mtb* DXR, we have synthesized analogues of fosmidomycin by the introduction of an aromatic substituent in the 1-position of the propylphosphonic acid scaffold. The use of an oxidative Heck reaction facilitated the selective preparation of the essential terminally arylated starting materials directly from acrolein and a number of selected boronic acids. Scope and limitations of the reaction will be discussed as well as the biological activity and suggested binding mode of the target compounds.

POSTER COMMUNICATIONS – PC.124

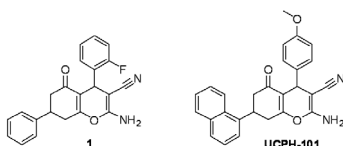
DISCOVERY OF UCPH-101 - STRUCTURE-ACTIVITY RELATIONSHIPS FOR NOVEL CLASS OF EAAT1 INHIBITORS

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L-Glutamate (Glu) is believed to play a key role in a range of neurodegenerative diseases, and psychiatric disorders. Therefore the glutamatergic neurotransmitter system is a highly attractive target from a therapeutic perspective. The excitatory amino acid transporters (EAATs) are transmembrane proteins responsible for the uptake of Glu from the synaptic cleft, thereby terminating the glutamatergic neurotransmitter signal. To date five human subtypes have been identified, termed EAAT1-EAAT5. We believe that valuable information about the physiological functions and thus therapeutic potentials of the specific EAAT subtypes may be addressed by studying the actions of selective ligands. (1,2)

A small commercially available compound library consisting of 3040 compounds was screened for inhibitory activity at EAAT1, EAAT2- and EAAT3-expressing HEK293 cell lines in a [^{3} H]-D-Asp uptake assay.(3) Interestingly, we found **1** to be a selective inhibitor at the EAAT1 (IC_{50} : 4.7 μ M). The discovery of **1** prompted us to commence building a SAR for this class of EAAT1 inhibitors. Analogues which bear one aromatic substituent in both the 4- and 7-position show differential inhibitory potencies at EAAT1 with **UCPH-101** being the most potent inhibitor (IC_{50} : 0.66 μ M). (2)



Furthermore analogues with varying substituent (-H, -COOH, -COOMe, -COOEt, -COO^tBu, -CONH₂) instead of the cyano group have been synthesized. We found that the cyano group is of great importance in retaining the inhibitory activity for this class of EAAT1 inhibitors as none of these analogues showed any inhibitory activity at EAAT1-3. Analogues with variations addressing the importance of the amino group have also been synthesized.

REFERENCES

1. Bunch L, Erichsen MN, Jensen AA, *Expert Opin. Ther. Targets* 2009, 13(6), 719-731
2. Jensen AA, Erichsen MN, Nielsen CW, Stensbøl TB, Kehler J, Bunch L, *J. Med. Chem.* 2009, 53, 912-915
3. Jensen AA, Bräuner-Osborne H, *Biochem. Pharmacol.* 2004, 67, 2115-2127

POSTER COMMUNICATIONS – PC.125

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL ANALOGUES OF OLANZAPINE

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Olanzapine is an atypical antipsychotic and currently one of the most effective treatments to date being able to reduce the symptoms of schizophrenia, whilst also having reduced propensity to cause adverse side effects. Its therapeutic effect arises from its mixed D₂/5-HT_{2A} receptor antagonism, which acts to stabilise the dopamine imbalance commonly observed in sufferers of schizophrenia.¹ Olanzapine, however, has strong binding affinity for a number of other receptors including muscarinic (M₁), histaminergic (H₁) and adrenergic (α_1), which contribute to its side effect profile. There still remains a need for new antipsychotic treatments with improved efficacy as well as reduced side effect potential.

Using olanzapine as a starting point, a series of analogues were synthesised based on a pharmacophore model of currently available antipsychotics (Figure 1).² The analogues comprise various aromatic functionalities linked to the olanzapine core by diverse aliphatic linkers. The pharmacological potential of the analogues were determined through *in vitro* receptor binding assays, with promising analogues further screened in *in vivo* animal models for schizophrenia.

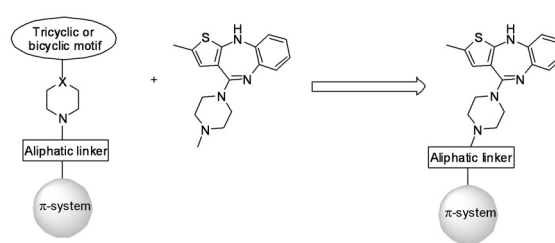


Figure 1. Olanzapine analogues based on a proposed antipsychotic pharmacophore model.

REFERENCES

1. Littrell, K. H.; Petty, R. G.; Wolf, N. M. Olanzapine: a 5-year perspective. *Expert Review of Neurotherapeutics* **2006**
2. Podloucka, A., Synthesis and Preliminary Pharmacological Evaluation of Novel Antipsychotics for the Treatment of Schizophrenia. *Honours Thesis, Monash University* **2002**

POSTER COMMUNICATIONS – PC.127

MEDICINAL PLANTS FROM ANGOLA: FROM CITOTOXICITY TO BACTERICIDE ACTIVITY

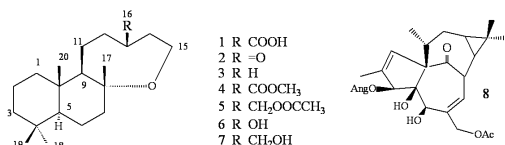
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The majority of the Angola population, as in other African countries, uses medicinal plants and goes to traditional healers in his search for medical care. However little has been done to study these medicinal plants. [1] As part of our ongoing study of the plants of Angola [2,3,4], we report here on the study of *Adenodolichos huillensis*, *Hymenodictyon floribundum*, *Parinari pumila*, *Peucedano angolense*, *Eragrostis viscosa*, *Euphorbia conspicua*, *Boscia microphylla* and *Tinnea antiscorbutica*.

Antiradicalar and antioxidant capacity of the extracts in different solvents were evaluated by DPPH and ABTS methods. The disk diffusion method was used to evaluate antimicrobial activity, while the broth microdilution method was used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). The Ames assay and the micronucleus assay were used to determine genotoxicity and evaluate the mutagenic activity of the isolated compounds. Cell viability was evaluated by MTT assay. Molluscicidal activity was determined for adults and egg masses of *Biomphalaria glabrata*.

From *Euphorbia conspicua* some molluscicide compounds with ingenane and ingol skeleton were isolated. *E. viscosa* presents a low antiradicalar capacity, no antifungal activity, inhibit Gram positive bacteria and present bacteriostatic activity against *Bacillus cereus* and *Staphylococcus aureus*. *E. viscosa* afforded citotoxic and bactericide compounds with a new 8 α ,15-epoxy-labdane skeleton.



ACKNOWLEDGMENTS

This work was partially funded by the project PTDC/QUI/66507/2006 of Fundação para a Ciência e Tecnologia (FCT) with FEDER funding and Textile and Paper Materials Center.

REFERENCES

1. Bossard, E.; *Medicine traditionnelle au Centre et à l'Ouest de l'Angola*, Vol. 1, Ministério da Ciência e Tecnologia, Instituto de Investigação Científica Tropical, Lisbon, 1996.

2. dos Santos, A.F.; Lopes, L.A.; Mata, R.C.S.; de Mendonça, D.I.M.D.; Sant'Ana, A.E.G. 2007. *Bioresour. Technol.* 98, 135-139.
3. Borges, C.M.P.; Diakanawma, C.; de Mendonça, D.I.M.D. *J. Braz. Chem. Soc.* advance online publication 22 March 2010.
4. Sebastião, N'S.N.; Cordeiro, I.J.S.; dos Santos, A.F.; Gaspar, J.F.; Martins, C.; Rueff, J.; Diakanawma, C.; Sant'Ana, A.E.G.; de Mendonça, D.I.M.D. *Phytochemistry*; doi: 10.1016/j.phytochem.2010.02.003.

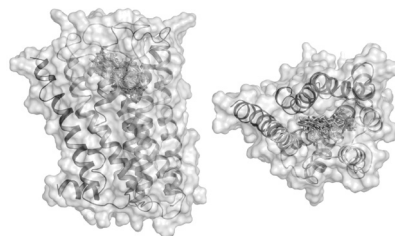
POSTER COMMUNICATIONS – PC.128

AMINERGIC G PROTEIN-COUPLED RECEPTORS; HOMOLOGY MODELING AND EVALUATION BY VIRTUAL SCREENING

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G protein-coupled receptors (GPCRs) are therapeutically significant proteins, targeted by a wide range of drugs. Until recently, the only GPCR crystal structure available was that of rhodopsin, which greatly limited structure-based drug design. The recent release of high-resolution crystal structures of several class A GPCRs has allowed the development of homology models, based on templates other than rhodopsin.



We report the development of homology models of nine therapeutically significant GPCRs (such as the serotonin 5HT_{2A} receptor, pictured).¹ The models were generated and refined in Prime, using the β_2 AR crystal structure (PDB: 2RH1) as the template. The orthosteric sites of the models were optimized using Induced Fit Docking, to allow for sidechain flexibility and the resulting models have been assessed using protein validation tools. Virtual screening was employed to evaluate the models by docking a database of active compounds and drug-like decoys, using Glide. Of the nine homology models developed, six showed moderate to good enrichment in virtual screening experiments (5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, D₂, D₃ and M₁). It is hoped that these models, based on the recent β_2 AR crystal structure, will provide a better starting point for structure-based drug design.

REFERENCES

1. McRobb, F. M. et al. *Journal of Chemical Information and Modeling* 2010. DOI: 10.1021/ci900444q

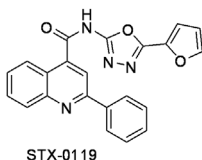
POSTER COMMUNICATIONS – PC.129

DISCOVERY OF NOVEL STAT3 DIMERIZATION INHIBITORS

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Signal Transducer and Activator of Transcription-3 (STAT3) is considered to be a good therapeutic target in oncology. Using virtual screening against the STAT3-SH2 domain and in vitro assays (luciferase reporter gene assay and fluorescence resonance energy transfer-based STAT3 dimerization assay in cells), we identified STX-0119 as a novel STAT3 dimerization inhibitor. STX-0119 abrogated both the subsequent DNA binding of STAT3 selectively and the expression of its target proteins in MDA-MB-468 cells. Structure activity relationships of STX-0119 analogues were investigated and reconciling this with the docking model of the STAT3-SH2 domain/STX-0119. Furthermore, STX-0119 induced selective apoptosis of a cancer cell line with constitutively active STAT3 and suppressed SCC-3 growth in the nude mouse xenograft model by oral administration. This is the first demonstration of in vivo efficacy following oral administration of a STAT3 dimerization inhibitor.



POSTER COMMUNICATIONS – PC.130

PRIMAQUINE-FERROCENE CONJUGATES: ON THE WAY TO SAFER AGENTS AGAINST PNEUMOCYSTIS PNEUMONIA

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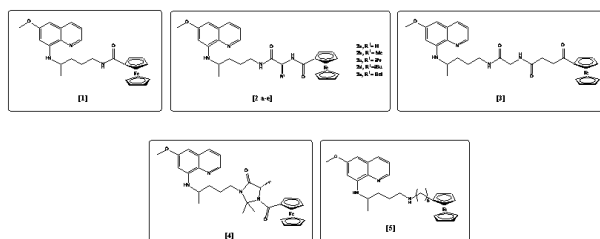
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Ferrocene (Fc) has been used in the preparation of bioactive organometallic structures, such as Fc-conjugates of: peptides and nucleotides¹, clinically relevant drugs as dopamine receptor ligands² and organometallic anti-malarials derived from chloroquine (Ferroquine).³ This chloroquine isostere with Fc-based side chain was 22 times more potent than parental drug and is now under clinical trials.^{3,4}

Our research group has been focused on imidazoquinones, a new class of imidazolidin-4-one peptidomimetic derivatives of Primaquine (PQ) that display anti-malarial and anti-pneumocystis activity while being resistant to premature metabolic inactivation by oxidative deamination or proteolysis.⁵⁻⁸ We hypothesized that conjugation of ferrocene with PQ-based structures (**1-4**) or with PQ's heteroaromatic core 8-amino-6-methoxyquinoline (**5**), could enhance both their anti-plasmodial and anti-pneumocystis activities.⁹



Interestingly, while organometallic conjugates were inactive against *Plasmodium*, they turned out to be moderately to markedly active against *Pneumocystis carinii*, while devoid of cytotoxicity. Compound **2e** was the best of the set, with higher activity than the parent drug (IC₅₀ of 1.1 μM against 1.8 μM for PQ) and dramatically reduced cytotoxicity against A549 human lung fibroblast carcinoma (IC₅₀ >100 μg/mL against 22 μg/mL for PQ). This is an unparalleled finding on PQ-based anti-*Pneumocystis* agents.

REFERENCES

1. H.-B. Kraatz, J. Inorg. Organometal. Polym. Mat., 2005, **15**, 83.
2. D. Huber et al., J. Med. Chem., 2009, **52**, 6860.
3. M. A. L. Blackie et al., J. Organometal. Chem., 2003, **688**, 144.
4. W. Daher et al., Malaria J., 2006, **5**, 11, and references therein.
5. P. Gomes et al., Tetrahedron, 2004, **60**, 5551.
6. M. J. Araújo et al., J. Med. Chem., 2005, **48**, 888.
7. N. Vale et al., Bioorg. Med. Chem. Lett., 2008, **18**, 485.
8. N. Vale et al., J. Med. Chem., 2009, **52**, 7800.
9. J. Matos et al., MedChemComm, 2010, submitted for publication.

POSTER COMMUNICATIONS – PC.131

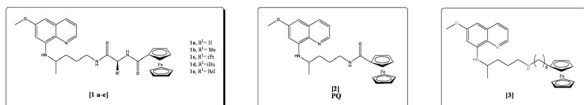
ANTI-PLASMODIUM ACTIVITY OF PRIMACENES

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Primacenes (**1a-e**), our first generation of ferrocene-primaquine conjugates, were recently prepared by us through coupling ferrocenecarboxylic acid to amino groups present in primaquine (PQ, **2**) amino acid derivatives, and tested for their *in vitro* blood-schizontocidal activity against *P. falciparum* and *in vivo* gametocytocidal activity against *P. berghei*.

Ferrocene (Fc) has been used in the preparation of bioactive organometallic structures of clinically relevant drugs.² Insertion of the ferrocene core in well known anti-malarial drugs as Chloroquine (CQ) showed an improvement in the anti-malarial activity. Ferroquine (FQ), a chloroquine isostere with an Fc-based side chain is 22 times more potent than CQ itself.^{3,4} Given the excellent results reported for FQ, we hypothesized that acylation of PQ itself or of its amino acid derivatives might enhance their blood-schizontocidal activity. However, primacenes **1a-e** were practically devoid of any type of anti-malarial activity. Believing that the deleterious effect of ferrocenylation on PQ anti-malarial activity was due to the consequent abolition of a basic aliphatic amine group, earlier proposed as a key feature for activity,⁵ we prepared and tested compounds **1f** and **3**, both possessing such a group. Full recovery of activity was observed, confirming beyond any doubt that PQ derivatives must possess an aliphatic amine in order to display anti-malarial activity of any kind.



REFERENCES

1. J. Matos et al., *MedChemComm*, 2010, submitted for publication.
2. H.-B. Kraatz, *J. Inorg. Organometal. Polym. Mat.*, **2005**, 15, 83.
3. W. Daher et al., *Malaria J.*, **2006**, 5, 11, and references therein.
4. F. Dubar et al., *Molecules*, **2008**, 13, 2900, and references therein.
5. M. J. Portela et al., *Pharm. Res.*, **1999**, 16, 949.

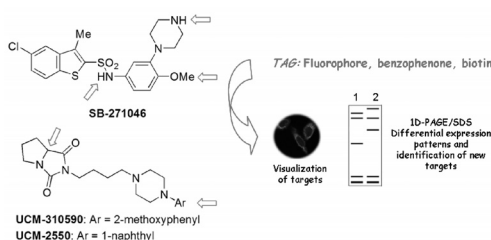
POSTER COMMUNICATIONS – PC.132

SYNTHESIS OF CHEMICAL PROBES FOR THE STUDY OF SEROTONIN 5-HT_{1A} AND 5-HT₆ RECEPTORS

Henar Vázquez-Villa,¹ Mar Martín-Fontecha¹, Dulce Alonso¹, Ana M. Gamo¹, Juan A. González-Vera¹, María F. Martínez-Espesón³, Mariola Tortosa³, Alma Viso³, Roberto Fernández de la Pradilla³, Elena Junquera², Emilio Aicart², Bellinda Benhamú¹, María L. López-Rodríguez¹ and Silvia Ortega-Gutiérrez¹

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Development of compounds that enable direct observation of sub-fractions of the proteome has become of paramount importance in the quest of understanding biological processes at the systems level. In this context, activity-based protein profiling (ABPP) can be considered as one of the major contributions. This approach has been successfully applied to the study of different enzyme families.¹ However, the superfamily of G protein-coupled receptors (GPCRs), which account for more than the 50% of the druggable genome,² remains to be addressed. Among the different families of GPCRs, we have been focused on the serotonin receptors, particularly 5-HT_{1A} and 5-HT₆ subtypes, due to their therapeutic potential for the treatment of neuropsychiatric disorders. Herein we present our results concerning the synthesis and evaluation of novel labeled ligands for the study of both 5-HT_{1A} and 5-HT₆ receptors. Our strategy encompasses the selection and tagging of known potent ligands of these receptors with a fluorescent, photoactivatable and/or affinity tags. Fluorescent ligands were synthesized by attachment of the corresponding fluorophore (dansyl, lissamine or NBD) to different positions of known 5-HT_{1A} (UCM-310590 and UCM-2550)³ and 5-HT₆ (SB-271046)⁴ ligands (Figure below). Synthesized probes have shown both good fluorescent properties and high binding affinities, which enable direct observation of the h5-HT_{1A} and h5-HT₆ receptors in cells.^{5,6} These results have provided the basis for the introduction of benzophenone and biotin affording a new series of high affinity probes for covalent binding and affinity pull-down of receptors. These strategies should contribute to optimize the therapeutic exploitation of known or new members of the GPCR superfamily by providing valuable information about their location or level of expression.



REFERENCES

1. Cravatt, B.F. *et al. Annu. Rev. Biochem.* **2008**, *77*, 383.
2. Lagerström, M.C. *et al. Nat. Rev. Drug Discov.* **2008**, *7*, 339.
3. López-Rodríguez, M.L. *et al. J. Med. Chem.* **1996**, *39*, 4439; *J. Med. Chem.* **2005**, *48*, 2548.
4. Bromidge, S.M. *et al. J. Med. Chem.* **1999**, *42*, 202.
5. Alonso, D. *et al. ACS Med. Chem. Lett.* Submitted. 6) Vázquez-Villa, H. *et al. Chem. Eur. J.* Submitted.

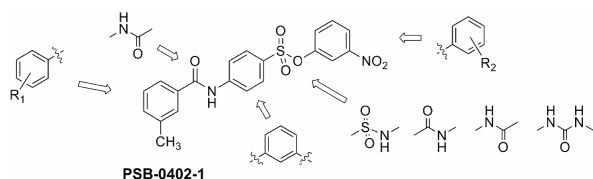
POSTER COMMUNICATIONS – PC.133

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A NEW CLASS OF P2Y₂ RECEPTOR ANTAGONISTS

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P2Y₂ receptors, which are activated by UTP, ATP, and dinucleotides, belong to the family of G protein-coupled nucleotide (P2) receptors.¹ Antagonists for this P2Y receptor subtype have been proposed as anti-inflammatory, neuroprotective and anti-hypertensive agents. However, potent and selective P2Y₂ antagonists are currently not available.² In a screening campaign, we identified 3-nitrophenyl 4-(3-methylbenzamido)benzenesulfonate (PSB-0402-1) as a relatively potent antagonist at human P2Y₂ receptors with an IC₅₀ value of 3.64 µM. In the present study we investigated the properties and structure-activity relationships of this new class of P2Y₂ receptor antagonists.



The stability of PSB-0402-1 was assessed by LCMS. A series of phenyl 3- and 4-benzamidobenzenesulfonates and -sulfonamides was synthesized. Furthermore, the sulfonic acid ester was replaced by amide and urea groups. The compounds were investigated in calcium assays at the G_q-coupled human P2Y₂, P2Y₄ and rat P2Y₆ receptors stably expressed in 1321N1 astrocytoma cells.

The ester function of the lead structure was shown to be highly stable at different pH values. Depending on the substitution of the benzamido moiety, the synthesized phenyl sulfonates showed antagonistic activity at P2Y₂ receptors mostly in the very low micromolar range and selectivity versus P2Y₄ and P2Y₆ receptors. In contrast to the sulfonates the corresponding sulfonamides were inactive. Further optimized compounds, includ-

ing carbamides and diamides, are currently investigated and preliminary test results look promising.

In conclusion, a novel class of relatively potent and selective P2Y₂ receptor antagonists has been identified. They may be useful as pharmacological tools to elucidate the (patho)-physiological roles of the P2Y₂ receptor and have potential as lead structures for drug development.

REFERENCES

1. Abbraccio, M. P.; Burnstock, G. *et al. Pharmacol. Rev.* **2006**, *58*, 281-341
2. (a) Brunschweiler, A.; Müller, C. E. *Curr. Med. Chem.* **2006**, *13*, 289; (b) Müller, C. E. *Curr. Pharm. Des.* **2002**, *8*, 99-110

POSTER COMMUNICATIONS – PC.134

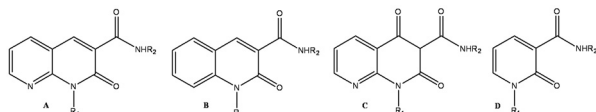
CB2 SELECTIVE CANNABINOID RECEPTOR LIGANDS: PHARMACOMODULATION AROUND THE 1,8-NAPHTHYRIDIN-2(1H)-ON-3-CARBOXAMIDES

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The endocannabinoid signaling system is composed of cannabinoid (CB) receptors, their endogenous ligands and the enzymes that produce and inactivate these ligands. To date, two distinct cannabinoid receptors, CB1 and CB2, have been identified in mammalian tissues and cloned. The psychotropic effects associated with nonselective cannabinoid agonists are thought to be mediated through the CB1 receptor, which is present in the central and peripheral nervous system as well as the periphery. While there is some evidence of the CB2 receptor in the central nervous system, the CB2 receptor is found primarily in the immune system. Over the last few years, the interest for CB2 selective agonists has been growing with proposed indications in the control of both neuropathic and inflammatory pain and more recently in liver pathophysiology, osteoporosis, growth of malignant gliomas, tumors of immune origin, and immunological disorders such as multiple sclerosis. Furthermore, given the presence of the CB₂ receptors in the brain microglial cells, it has recently been shown that CB₂ agonists might provide neuroprotection by blockade of microglial activation. On the basis of docking studies developed using three-dimensional models of

the two CB receptors [1,2], recently we described the synthesis, pharmacological characterization, and molecular modeling studies of a novel series of 1,8-naphthyridin-2(1*H*)-on-3-carboxamide (**A**) acting as selective and potent CB2 receptor agonists and further studies revealed that one of these compounds induced a concentration-dependent decrease of cell viability in Jurkat cells [3].



In order to specify structure-affinity relationships, the derivatives of general structure **B**, **C** and **D** were synthesized using compounds **A** as a template. The binding results indicate that most of these compounds exhibited a remarkable CB2 affinity, with a K_i value in the nanomolar range. This affinity was accompanied by a high selectivity with respect to CB1 receptor. The newly synthesized CB₂ receptor ligands, showing the better binding profiles in the series, were evaluated *in vitro* for their antiproliferative activity.

REFERENCES

1. Manera, C. *et al.*, J. Med. Chem., **2006**, 49, 5947-5957.
2. Tuccinardi, T *et al.*, J. Med. Chem., **2006**, 49, 984-994
3. Manera, C. *et al.*, J. Med. Chem., **2009**, 52, 3644-3651

POSTER COMMUNICATIONS – PC.135

N-ALKYLATED DIKETOPIPERAZINES: EASILY AVAILABLE CSA FOR DETERMINATION OF ENANTIOMERIC PURITY

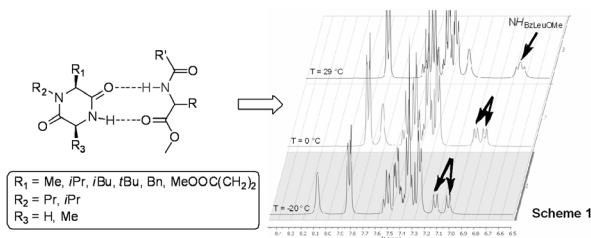
Črt Malavašič, Jernej Waggoner, Uroš Grošelj, Anton Meden, Branko Stanovnik, Jurij Svete

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Diketopiperazines, the smallest cyclic peptides known, show wide spectrum of biological activities.¹ These compounds display some interesting chemical properties regarding medicinal chemistry, among them substituent group stereochemistry, conformational rigidity and donor and acceptor groups for hydrogen bonding.¹

A series of novel chiral nonracemic *N*-alkylated piperazine-2,5-diones were prepared and their properties were investigated by X-ray analysis and NMR spectroscopy. Chiral solvating properties of *N*-alkylated diketopiperazines arise from their ability of hydrogen bonding with *N*-acylated amino acid esters in CDCl₃ solution (Scheme 1).²⁻⁴ Recently, we have shown that diketopiperazines are easily available chiral solvating agents (CSA) for racemic *N*-acylated α -amino acid esters. They were applied for fast and easy determination of enantiomer composition of

fully protected α -amino acid ester derivatives by ¹H NMR devoiding peak broadening which is otherwise a typical disadvantage of chiral lanthanide shift reagents.^{3,4} Besides, these CSA can be used for determination of absolute configuration as well. In summary, these CSA might find use in determination of optical purity of pharmaceutically interesting compounds.



REFERENCES

1. Martins, M. B.; Carvalho, I. *Tetrahedron* **2007**, 63, 9923-9932.
2. Malavašič, Č.; Waggoner, J.; Stanovnik, B.; Svete, J. *Tetrahedron: Asymmetry* **2008**, 19, 1557-1567.
3. Waggoner, J.; Golič Grdadolnik, S.; Grošelj, U.; Meden, A.; Stanovnik, B.; Svete, J. *Tetrahedron: Asymmetry* **2007**, 18, 464-475.
4. Waggoner, J.; Svete, J.; Stanovnik, B. *Synthesis* **2008**, 1436-1442.

POSTER COMMUNICATIONS – PC.136

DISCOVERY OF SELECTIVE INHIBITORS OF UBIQUITIN SPECIFIC PROTEASES

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Regulated protein degradation is primarily controlled by the ubiquitin-proteasome system. This system was established as a valid oncology target since the approval of the proteasome inhibitor Velcade®. Targeting upstream on the ubiquitin conjugation/deconjugation system opens the way to anticancer agents with increased specificity and selectivity.

Ubiquitin-specific proteases (USP) are involved in the deubiquitination of specific target substrates regulating their stability, subcellular localization and/or activation status. Those USP represent druggable targets due to their cysteine protease activity amenable to pharmacological inhibition by small molecules. Genome-wide RNAi screening of the catalytically active human USPs in cancer-relevant cellular models and phenotypic assays allowed us to identify USP7/HAUSP and USP8/UBPY as promising cancer targets. After successful assay development, HTS performed on our chemically diverse library resulted in the discovery of novel USP inhibitors. From these series, hit to lead and

lead optimization programs were performed in order to improve potency, selectivity, cellular activity and pharmacokinetic profile.

Our progress made from the first generation series towards the specificity issue which culminated with the discovery of the first USP8- and USP7-specific series, will be presented.

POSTER COMMUNICATIONS – PC.137

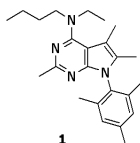
SYNTHESES AND BIOLOGICAL EVALUATION OF A NEW NAPHTHYRIDIN DERIVATIVES AS POTENTIAL CRF₁ ANTAGONIST

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Depression and anxiety are a complex psychiatric disorder that affects an enormous number of people in society, and constitute a major health concern worldwide. Despite the number of treatments available, significant unmet needs still remain in terms of efficacy, onset of effect and tolerability. Corticotropin releasing factor (CRF) is a 41-amino acid peptide that plays a central role in mediating the behavioral, neuroendocrine, and autonomic responses to stress. CRF is synthesized in the hypothalamus and liberated into the hypophyseal-portal system triggering the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. Clinical data indicate that patients with depression and post-traumatic stress disorder show significantly elevated concentrations of CRF in cerebrospinal fluid when compared to normal controls. The CRF receptor has two well-characterized subtypes CRF₁ and CRF₂, which are widely distributed throughout the central nervous system (CNS) and peripheral tissues. A selective CRF₁ antagonist may represent a novel class of compounds for the treatment of anxiety, depression and stress-related diseases.

Several companies have disclosed potent CRF₁ receptor antagonists based on monocyclic and bicyclic cores. The extensively researched antalarmin **1** (1) (2) falls within this broad structural class, and has proven to be functional antagonists, both in vitro and in vivo with exquisite selectivity for CRF₁ receptor, but it has been suggested that the clinical development of this early CRF₁ antagonist was limited (3).



Our efforts have been focused on the modifications in the pyrrolo-pyrimidine core template and peripheral side chains. A series of new [1,8]naphthyridin with amine functionality have been designed and synthesized as possible antagonist CRF₁.

Quite a few compounds have been synthesized and evaluated. CRF receptor binding affinities have been determined by displacement of [¹²⁵I].Tyr⁰-CRF from rat frontal cortex. The results of these studies will be reported and discussed.

This work was co-supported by the Secretariat General for Science and Technology Policy of the Ministry of Science and Innovation and by the INNOTEK Program of the Department of Industry, Commerce and Tourism of the Basque Government.

REFERENCES

1. Webster, E. L.; Lewis, D. B; Torpy, D. J.; Zachman, E. K.; Rice, K. C.; Chrousos, G. P. *Endocrinology* **1996**, 137, 5747-5750.
2. Chen, Y. WO 9413676.
3. Hsin, L. W.; Tian, X. : Webster, E. L.; Coop, A.; Caldwell, T. M. *Bioorg. Med. Chem.*, **2002**, 10, 175-183.

POSTER COMMUNICATIONS – PC.138

DESIGN SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION TOWARDS AROMATASE OF C-4 DERIVATIVES OF FORMESTANE AND 5 α -ANDROST-3-EN-17-ONE

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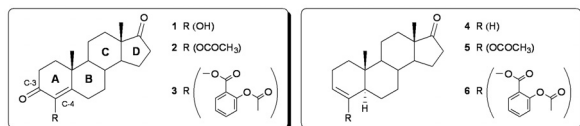
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Hormone-dependent breast tumors require estrogens for their growth.¹ One main approach to block estrogen action targets aromatase, the cytochrome P-450 enzyme responsible for estrogen biosynthesis. In breast cancer, intratumoral aromatase is the source for local estrogen production and its inhibition by aromatase inhibitors (AIs) is an important approach for reducing tumor growth.

Recently, the active site of aromatase has been elucidated, and the molecular basis for enzyme-substrate (androstenedione) interaction was established.² It was found that the volume of the binding pocket is relatively short (< 400 Å) allowing to enter into the cleft only molecules with appropriate dimensions such as androstenedione derivatives with small substituents.

Numerous recent studies have associated cyclooxygenase-2 (COX-2) with breast cancer pathology. Inhibition of COX-2 activity is described to be protective against tumorigenesis in animal models of breast cancer and epidemiologic studies suggest that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid, protects against breast cancer development.³

Formestane **1** is the C-4 hydroxy analogue of the enzyme substrate androstenedione and was the first steroidal AI clinically used. Its C-4 acetoxy derivative **2**, also revealed to have strong anti-aromatase activity.⁴ The 3-deoxy steroid **5 α** -androst-3-en-17-one **4** is also a potent AI recently prepared and evaluated by our team.⁵



In this work, we present a structure-activity study, based on the synthesis and aromatase inhibitory activity evaluation of C-4 acetoxy- and acetylsalicyloxy-derivatives of formestane (**2** and **3**) and of **5 α** -androst-3-en-17-one (**5** and **6**). The results obtained so far suggest that the presence of bulky substituents in C-4 diminishes the aromatase inhibitory activity which is consistent with the short volume of the binding pocket of aromatase.

REFERENCES

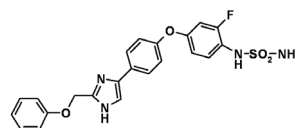
- Chen, S. *Frontiers of Bioscience* **1998**, 3(9), 22.
- Glosh, D.; Griswold, J.; Erman, M.; Pangborn, W. *Nature* **2009**, 457, 219.
- Takkouche, B.; Regueira-Méndez, C.; Etminan, M. *J Natl Cancer Inst* **2008**, 100, 1439.
- Marsh, D.A.; Brodie, H.J.; Garrett, W.; Tsai-Morris, C.; Brodie, A.M.H. *J Med Chem* **1985**, 28, 788.
- a) Cepa, M.D.S.; Tavares-da-Silva, E.J.; Correia-da-Silva, G.; Roleira, F.M.F.; Teixeira, N.A.A. *J Med Chem* **2005**, 48, 6379. b) Cepa, M.; Correia-da-Silva, G.; Tavares-da-Silva, E.J.; Roleira, F.M.F.; Teixeira, N. *Steroids*, **2008**, 73, 1409.

POSTER COMMUNICATIONS – PC.139

IRC-083927: A NOVEL TUBULIN POLYMERIZATION INHIBITOR ACTIVE AGAINST CELL LINES RESISTANT TO STANDARD TUBULIN BINDING AGENTS

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IRC-083927

Microtubules are a validated target for antitumor drugs. However, the effectiveness of these microtubule-interacting agents is limited by the fact that they are substrates for drug efflux pumps (PgP) or/and by the acquisition of point mutations in tubulin residues important for drug-tubulin binding.

In order to bypass these resistance systems, we have identified and characterized a novel synthetic imidazole derivative IRC-083927 which prevents polymerization of purified tubulin in vitro by binding to the colchicine site. IRC-083927 inhibits in vitro cell growth of human cancer cell lines displaying resistance to microtubule interacting agents due to the presence of efflux pumps (NCI-H69/LX4 resistant to Navelbine and Paclitaxel) and or due to the presence of mutations on beta tubulin (A549EpoB40 resistant to Epothilone B or Paclitaxel) and on alpha- and beta tubulin (A549EpoB480 resistant to Epothilone B or Paclitaxel). As expected for numerous tubulin binding agents, IRC-083927 also displayed antiangiogenic properties by inhibiting the endothelial cell proliferation and in vitro vessel formation in the low nanomolar range. The high potency and efficacy of IRC-083927 has been shown using chronic oral treatment (2.5 or 5 mg/kg during 28 day period) in all tested models. In addition, IRC-083927 is more efficient than: 1) Velcade against the human myeloma RPMI-8226, 2) ABT-751 against androgen independent prostate cancer PC3 and 3) Combretastin CA4P or ABT-751 against human hormone-independent breast cancer MDA-MB-231. Finally, no signs of toxicity have been observed in mice with this regimen. Chronic exposure of rats to IRC83927 at the maximum tolerated dose induced reduction of the gastric emptying and a slight decrease of white blood cells.

Together, the biological effects induced by IRC-083927 on tubulin interacting agent resistant tumor models and its synthetic nature support further investigations to fully evaluate its significant potential for the treatment of advanced cancers resistant to current clinically available drugs.

REFERENCES

- WO2007/125197
- Molecular Cancer Therapeutics* (2008), 7(8), 2426-2434
Erratum *Molecular Cancer Therapeutics* January 1, 2009 8:274

POSTER COMMUNICATIONS – PC.140

FRAGMENT-BASED APPROACHES TO GPCRS: EFFICIENT DEVELOPMENT OF HISTAMINE H₄ RECEPTOR LIGANDS WITH ANTI-INFLAMMATORY PROPERTIES

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The human histamine H₄ Receptor (hH₄R) is considered a promising therapeutic target for inflammation and pruritis. Fragment screening resulted in the identification of various fragment hits, including quinazolines, quinoxalines and aminopyrimidines. Guided by a pharmacophore model, and supported by homology models, the fragment hits were efficiently optimized into potent hH₄R ligands with nanomolar affinities. In vivo studies in the rat reveal that some of these ligands show excellent anti-inflammatory properties in the carrageenan induced paw edema model.

tent with antagonism of the histamine H₃ receptor. The synthesis and *in vitro* characterization of compound **4** with respect to affinity, functional antagonism and pharmaceutical properties will be discussed along with pre-clinical pharmacokinetic data in mouse, rat and canine. These data will show that compound **4** is a high affinity histamine H₃ antagonist that is orally bioavailable in pre-clinical species. Data demonstrating robust histamine H₃ receptor occupancy in rat (i.e. Figure 1), significant increases in histamine levels in the frontal cortex of rats and the effects of compound **4** on total wake time in rats will also be presented. Based on this pre-clinical data compound **4** was chosen as a clinical candidate.

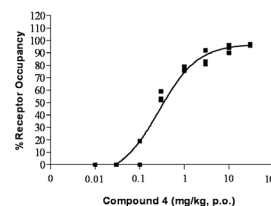
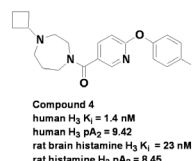


Figure 1. Receptor Occupancy vs Dose in a Rat.

POSTER COMMUNICATIONS – PC.142

COMPUTATIONAL PERSPECTIVE ON THE OPTIMIZATION OF CYP450 2D6 LIABILITIES IN DRUG DESIGN: SELECTIVE ALPHA-2C AGONISTS CASE STUDIES

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Human cytochrome P450 2D6 (CYP2D6) is a heme containing enzyme which plays a critical role in the metabolism of many xenobiotic compounds. CYP2D6 liabilities in drug discovery can result in poor pharmacokinetic properties and a potential for drug-drug interactions associated with severe side effects.

To address CYP2D6 liabilities in lead generation and optimization stages of projects, we use a 3D comparative model structure of the enzyme for which we developed docking and post-processing protocols. CYP2D6 inhibition issues are addressed using 2D-QSAR & 3D-QSAR methods in order to develop predictive models which help medicinal chemists in the decision making process of the projects.

In this poster we will describe the development of these methodologies and show how the models were used to support the design of selective alpha-2C agonists.

The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5601)

POSTER COMMUNICATIONS – PC.141

THE DISCOVERY OF (4-CYCLOBUTYL-[1,4]DIAZEPAN-1-YL)-[6-(4-FLUOROPHENOXY)-PYRIDIN-3-YL]-METHANONE: A NOVEL HISTAMINE H₃ ANTAGONIST SUITABLE FOR CLINICAL DEVELOPMENT

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The discovery and pre-clinical characterization of (4-Cyclobutyl-[1,4]diazepan-1-yl)-[6-(4-fluoro-phenoxy)-pyridin-3-yl]-methanone (**4**) will be discussed. Compound **4** is a high affinity, selective histamine H₃ antagonist that penetrates the central nervous system and shows efficacy in pre-clinical models consist-

POSTER COMMUNICATIONS – PC.143

DESIGN OF MORE POTENT SQUALENE SYNTHASE INHIBITORS COMBINING ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Atherosclerosis is a multifactorial disease with several mechanisms participating in its initial manifestation and progression. These mainly involve hyperlipidemia, oxidative stress and inflammation. Squalene Synthase (SQS), a key enzyme in the cholesterol biosynthetic pathway, occupies the first solely committed step towards the biosynthesis of the sterol nucleus of cholesterol. Because of its unique and strategic location in this pathway, it is an attractive target for the development of novel and improved antidyslipidaemic agents. These agents are considered to offer a better side-effect profile than inhibitors of (upstream) HMG-CoA reductase (i.e. statins) which are presently extensively used in the management of atherosclerosis.^{1,2} We have previously synthesized and investigated 2-aromatically substituted morpholine derivatives that were shown to reduce cholesterol and triglyceride levels, acting as squalene synthase inhibitors, as well as to combine antioxidant and anti-inflammatory activity^{3,4}.

We hereby evaluated the relationship between the *in vitro* SQS inhibitory action of several of these derivatives and the theoretically derived molecular interactions obtained by docking studies based on the available crystal structure of human SQS and use of *Autodock 4*. Thus, by visualizing preferred binding modes and comparing best fit energy values (ΔG) with experimental binding data of several derivatives, we designed potentially more potent SQS ligands. This design took into consideration structural features that potentially maintained or increased antioxidant and anti-inflammatory activity.

The synthesis of the new derivatives was performed by modification of existing methodology while the new structures were further evaluated *in vitro* (inhibition of SQS and antioxidant activity) and *in vivo* (antidyslipidemic and anti-inflammatory effect).

The new derivatives were significantly more active than our formerly developed SQS inhibitors, as predicted by our structure-theoretical-activity relationships. Simple regression calculations between $\text{Log}(1/IC_{50})$ (SQS inhibition) and ΔG of derivatives revealed a significant correlation between ΔG and experimental activity ($R^2 = 0.8$, $F = 35$, $p = 0.0002$). Furthermore, the new derivatives had an improved antioxidant profile.

In conclusion, application of findings from docking studies led to the design and development of more potent SQS inhibitors. To

our knowledge, this is the first time that an *in silico* docking study has been performed on the crystal structure of human SQS. Combination of the above approaches may lead to further optimization and development of multi-pharmacophore antiatherosclerosis agents.

REFERENCES

1. Elsayed R et al., Emerging lipid-lowering drugs: squalene synthase inhibitors. *Expert Opin Emerg Drugs*. **2008** 13, 309.
2. Meng CQ, Atherosclerosis is an inflammatory disorder after all, *Cur Top Med Chem*. **2006**, 6, 93.
3. Kourounakis et al., Lipid-lowering (hetero)aromatic tetrahydro-1,4-oxazine derivatives with antioxidant and squalene synthase inhibitory activity. *J. Med. Chem.* **2008** 51 5861.
4. Matralis A. Kourounakis A. Multi-site-directed molecules as a therapeutic approach for atherosclerosis. *20th International Symposium on Medicinal Chemistry*, Vienna, Sept. 2008.

POSTER COMMUNICATIONS – PC.144

EXHAUSTIVE SAMPLING OF DOCKING POSES REVEALS POSSIBLE BINDING MODES OF PROPAFENONES IN P-GLYCOPROTEIN

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The failure of chemotherapies can often be traced back to the acquisition of multidrug resistance (MDR), which can be directly associated with the over expression of P-glycoprotein (P-gp, ABCB1, MDR1). As an ABC-exporter it actively transports a high number of chemically diverse substances out of the cell. This drug translocation process is involved in a catalytic cycle where P-gp undergoes several conformational changes in connection with the binding of substrate and nucleotide as well as ATP hydrolyzation (1).

Propafenones are known for their ABCB1 modulating activity wherefore they have been in focus of several ligand based analyses. In this study the binding of five propafenone derivatives to the human ABCB1 transporter was investigated. Since no X-ray structure of the human protein is available, homology models were built on the basis of the mouse ABCB1 exporter (PDB code: 3G5U, resolution: 3.80 Å, apo) (2) and the bacterial homolog Sav1866 (PDB code: 2HYD, resolution: 3.00 Å, ADP-bound) (3). As these templates represented two different states of the catalytic cycle the docking results were expected to explain the affinity differences caused by ATP binding. As the conformational change of the transporter may lead to a shift in the hydrophobic environment all ligands were docked in a neutral and a protonated form.

As the high poly-specificity of P-gp is connected with its flexibility, we docked into homology models of two different catalytic

states treating selected side chains flexible. To fully explore the pose space, we allowed 500 poses for each docking run, which were subsequently rescored using the external scoring function XScore. With the assumption that propafenone derivatives bind in a similar manner to P-gp the poses were clustered according to the RMSD of the common scaffold. In addition, the protein ligand interaction fingerprint, which is implemented in the MOE software package, also served as clustering principle. Prioritisation of different clusters was further guided by information from structure-activity relationships of the five selected compounds.

With this exhaustive approach it was possible to explore all potential poses inside the large poly-specific binding pocket of P-gp. However, a high number of poses generated could be sorted out by the hypothesis of a common binding mode of all compounds used. The results suggest that interactions with transmembrane helices 5, 6, 7, 8 are present in both states of the catalytic cycle, whereas interactions with helix 12 get lost with the conformational change of the protein.

We acknowledge financial support provided by the Austrian Science Fund, grant F03502.

REFERENCES

1. Seeger MA, van Veen HW. Molecular basis of multidrug transport by ABC transporters. *Biochim Biophys Acta*. 2009;1794(5):725-37
2. Aller SG et al. Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding. *Science*. 2009;323(5922):1718-22
3. Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature*. 2006;443(7108):180-5

POSTER COMMUNICATIONS – PC.145

NOVEL ESTRONE MIMETICS WITH HIGH 17 β -HSD1 INHIBITORY ACTIVITY

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17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) catalyzes the reduction of estrone into estradiol, which is the most potent estrogen in humans. Lowering intracellular estradiol concentration by inhibition of this enzyme is a promising new option for the treatment of estrogen dependent diseases like breast cancer and endometriosis. Besides the already described (hydroxyphenyl)naphthols¹ and bis(hydroxyphenyl)heterocycles², we identified heterocyclic substituted biphenyls and their azanalogues as new class of 17 β -HSD1 inhibitors³. The latter resulted from the combination of ligand- and structure-based design, which was based on mimicking estrone, especially focusing on

the imitation of the D-ring keto group with (substituted) heterocycles. Molecular docking provided insights into plausible protein-ligand interactions for this class of compounds. The most promising compound **12** showed an inhibitory activity in the high nanomolar range and very low affinity for the estrogen receptors α and β . Thus, compound **12** is a novel tool for the elucidation of the pharmacological relevance of 17 β -HSD1 and might be a lead for the treatment of estrogen dependent diseases.

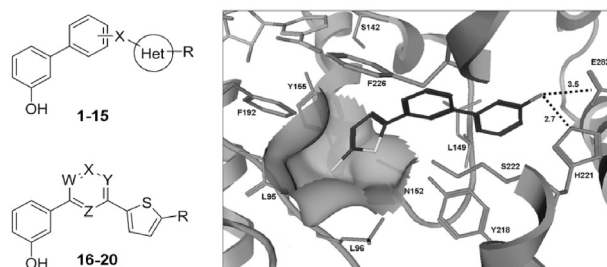


Figure 1: General structures of synthesized compounds are shown on the left. Docking complex between 17 β -HSD1 (PDB-ID: 1EQU) and compound 12 (violet) is depicted on the right.

REFERENCES

1. Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. *J. Med. Chem.* **2009**, 52, 6724.
2. Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. *Mol Cell Endocrinol.* **2009**, 301, 205-211.
3. Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. *Bioorg. Med. Chem.* **2010**, doi: 10.1016/j.bmc.2010.03.065

POSTER COMMUNICATIONS – PC.146

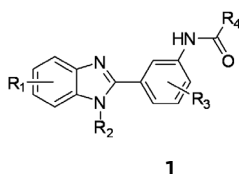
SYNTHESIS AND ANTICHLAMYDIAL ACTIVITY OF 2-ARYLBENZIMIDAZOLE DERIVATIVES

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Chlamydia pneumoniae is a common intracellular bacterium causing mild acute respiratory infections and community acquired pneumonia. However, pathogen can turn chronic, and these infections are associated with atherosclerosis¹, asthma², and Alzheimer's disease³. Due to the biphasic life cycle of *C. pneumoniae* there is an obvious demand to continue developing new type of antibiotics and effective new antichlamydial compounds.

In our previous study certain 2-arylbenzimidazole derivatives were found to have activity against *C. pneumoniae*.⁴ In this present study a new set of 2-arylbenzimidazole derivatives was synthesized, and their inhibitory effect against *C. pneumoniae* was evaluated. Thirty-one 2-arylbenzimidazole derivatives based on the general structure **1**, and two 2-arylimidazoles, were synthesized. Structural modifications of the compounds were designed based on the activity results. The best compounds inhibit the growth of the bacterium at micromolar concentration. Synthesis of the library and the structure-activity relationships will be described.



REFERENCES

1. Watson C, Alp NJ. *Clin. Science* **2008**, 114: 509-531.
2. Hahn DL, Peeling RW, Dillon E, McDonald R, Saikku P. *Ann. Allergy Asthma Immunol.* **2000**, 84: 227-233.
3. Gérard HC, Dreses-Werringloer U, Wildt KS, Deka S, Oszust C, Balin BJ, Frey II WH, Bordayo EZ, Whittum-Hudson JA, Hudson AP. *FEMS Immun. & Med. Microb.* **2006**, 48: 355-366.
4. Alvesalo JKO, Siiskonen A, Vainio MJ, Tammela PSM, Vuorela PM. *J. Med. Chem.* **2006**, 49: 2353-2356.

POSTER COMMUNICATIONS – PC.147

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL COMT INHIBITORS

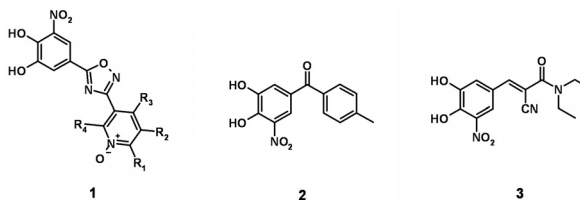
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BIAL - Portela & C^ª, S.A. À Av. da Siderurgia Nacional, 4745-457 S. Mamede do Coronado, Trofa, Portugal Psoares.silva@bial.com

Catechol-O-methyltransferase (COMT) is a magnesium-dependent enzyme that catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the phenolic group of substrates, inactivating endogenous catechol neurotransmitters and xenobiotic catechols such as L-DOPA. In mammals, COMT is distributed throughout various organs such as liver, kidney, lung, blood cells and skeletal muscles. Inhibitors of COMT are used in the treatment of central and peripheral nervous system disorders such as Parkinson's disease [1] (PD). In the presence of a decarboxylase inhibitor COMT becomes the major metabolizing enzyme for levodopa, catalyzing the metabolism to 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD) both in the brain and periphery.

We have recently discovered a new class of peripherally selective, long acting COMT inhibitors [2] of general formula **1** that

may possess therapeutic advantages over established COMT inhibitors such as tolcapone **2** and entacapone **3**.



Synthesis and biological evaluation will be presented.

REFERENCES

1. (a) Axelrod, J.; Senoh, S.; Witkop, B. *J. Biol. Chem.* **1958**, 233, 697. (b) Guldberg, H.; Marsden, C. *Pharmacol. Rev.* **1975**, 27, 135.
2. Learmonth, D.; Kiss, L.; Leal Palma, P.; Dos Santos Ferreira, H.; Araújo Soares-Da-Silva, P. WO2007013830A1, **2007**.

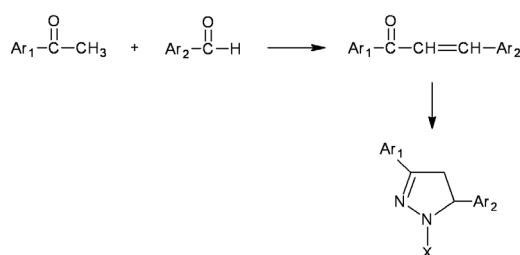
POSTER COMMUNICATIONS – PC.148

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NEW 2-PYRAZOLINE DERIVATIVES

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Chalcones, 1,3-diphenyl-2-propene-1-ones, which are member of the flavonoid family, have been shown to display a various array of pharmacological activities such as antimicrobial, antiinflammatory. Besides their important pharmacological activities, chalcones are also well known intermediates for synthesizing various heterocyclic compounds like flavonoids, flavanone, pyrazoles, oxazoles, pyrimidines and 1,5-benzothiazepines. Among these compounds, 2-pyrazolines are attracting interest of many researchers in medicinal chemistry because of their bioactivity such as antimicrobial [1], antiinflammatory [2], antidepressant and anticonvulsant [3] activities. In an effort to develop new 2-pyrazoline derivatives as anticonvulsant agents, a novel series of chalcones were prepared by Claisen Schmidt condensation. Thus, 1,3,5-trisubstituted-2-pyrazolines were synthesized by the reaction of chalcones with thiosemicarbazide, substituted thiosemicarbazides or substituted semicarbazides. Structures of the compounds have been confirmed by IR, ¹H-NMR spectral data and elemental analysis. 1,3,5-trisubstituted-2-pyrazoline derivatives have been testing for the anticonvulsant activity by pentylenetetrazole induced seizure and maximal electroshock seizure tests.



X: CSNH₂, CONHAr₃, CSNHAr₄

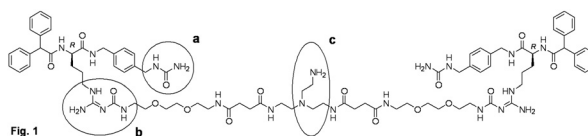
ACKNOWLEDGMENT

The authors wish to thank Marmara University Scientific Research Projects Commission (BAPKO, Project number, SAGA-040609-0140, 2009) to financial support for this study.

REFERENCES

- Özdemir A, Turan-Zitouni G, Kaplancıklı ZA, Revial G, Güven K. Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. *Eur J Med Chem.* 42(3):403-409, 2007.
- Shoman ME, Abdel-Aziz M, Aly OM, Farag HH, Morsy MA. Synthesis and investigation of anti-inflammatory activity and gastric ulcerogenicity of novel nitric oxide-donating pyrazoline derivatives. *Eur J Med Chem.* 44(7):3068-3076, 2009.
- Özdemir Z, Kandilci HB, Gümüşel B, Çaliş Ü, Bilgin AA. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. *Eur J Med Chem.* 42(3):373-379, 2007.

assay [4]: IC₅₀ = 0.71 µM; flow cytometric Y₄R binding assay⁴: K_i = 0.30 µM).



In contrast to the Y₁R, which prefers (R)-configured argininamides, stereochemical discrimination by the Y₄R was not detected. Highest Y₄R antagonistic activity resided in compounds having an H-donor group **a** (urea or hydroxyl) as well as an additional basic moiety **c** at a certain distance from the crucial acylguanidine **b**. This suggested the synthesis of monovalent argininamides combining the putative pharmacophoric groups **b** and **c** by spacers of different lengths.

In summary, the presented class of compounds is considered a promising starting point in the search of more potent and selective Y₄R antagonists, superior to recently identified acylguanidines derived from histamine H₂ receptor ligands [4, 5].

REFERENCES

- Kamiji, M. M. et al., *Curr. Topics Med. Chem.*, **2007**, 7, 1734-1742,
- Balasubramaniam et al., *J. Med. Chem.*, **2006**, 49(8), 2661-2665,
- Nagaaki, S. et al., *Expert Opin. Ther. Patents*, **2009**, 19(10), 1401-1415,
- Ziemek, R. et al., *J. Recept. Signal Transduct.*, **2007**, 27, 217-233.
- Kaske, M. et al., *Frontiers in Medicinal Chemistry*, Heidelberg, March **2009**, Poster No. HMC 24

POSTER COMMUNICATIONS – PC.149

MONOVALENT AND BIVALENT ARGININAMIDE-TYPE ANTAGONISTS OF THE HUMAN NPY Y₄ RECEPTOR

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The neuropeptide Y (NPY) system has emerged as a complex network of peptides and receptors. Among the NPY receptors (Y₁R, Y₂R, Y₄R, Y₅R) the Y₄R is unique due to preferential binding of pancreatic polypeptide (PP) instead of NPY. The biological role of the Y₄R is far from being understood. PP-based peptides are described to inhibit food intake, suggesting the Y₄R to be a potential target for the treatment of obesity. With respect to more detailed investigations, potent *non-peptidic* Y₄R ligands are required as pharmacological tools. However, such compounds are not reported in the literature so far [1, 2, 3].

Surprisingly, the investigation of a library of argininamides, initially designed as Y₁R antagonists, revealed that certain bivalent compounds are Y₄R antagonists with activities in the low micromolar or sub-micromolar range (example cf. **Fig. 1**; Y₄R Ca²⁺

POSTER COMMUNICATIONS – PC.150

MODULATION OF PHYSICO-CHEMICAL PROPERTIES OF PHENOL DERIVATIVES OF 1,3-DIMETHYL-PYRIMIDO[2,1-f]PURINEDIONES – ADENOSINE A_{2A} RECEPTOR LIGANDS.

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The adenosine receptor (AR) family consists of four G protein-coupled receptor subtypes: A₁, A_{2A}, A_{2B} and A₃. Adenosine A₁ or A_{2A} receptor antagonists may be useful for the treatment of acute and chronic neurodegenerative disorders including cerebral ischemia, Parkinson's and Huntington's diseases, cognitive deficits and Alzheimer's disease as well as for the treatment of peripheral diseases, such as cardiac failure [1, 2].

In our efforts to develop novel adenosine receptor ligands a series of N⁹-phenyl-substituted 1,3-dimethylpyrimido[2,1-

tional interfaces (particularly that of TM1-TM2) besides TM4-TM5 (4). On the basis of these results, we have further explored the oligomerization interface of GPCRs with Adaptive Poisson-Boltzmann solver (APBS) (5).

APBS is a software package for modeling biomolecular solvation through solution of the Poisson-Boltzmann equation (PBE) and is one of the most popular continuum models for describing electrostatic interactions between molecular solutes in a given medium. Using VMD and APBS we built the system to simulate receptor molecules in a membrane placed between water layers. The obtained patterns of electrostatic interactions allowed to rationalize our earlier results obtained with iterative protein-protein docking and to point out key regions of transmembrane bundles and intracellular loops of GPCRs engaged in the oligomerization process.

REFERENCES

1. Filizola, M. *Life Sci.* **2010**, in press.
2. Salom, D.; Lodowski, D.T.; Stenkamp, R.E.; Le Trong, I.; Golczak, M.; Jastrzebska, B.; Harris, T.; Ballesteros, J.A.; Palczewski, K. *Proc. Natl. Acad. Sci.*, **2006**, *103*, 16123.
3. Fotiadis, D.; Liang, Y.; Filipek, S.; Saperstein, D.A.; Engel, A.; Palczewski, K. *FEBS Lett.* **2004**, *564*, 281.
4. Kaczor, A.; Selent, J.; Pastor, M. unpublished results.
5. Baker, N.A.; Sept, D.; Joseph, S.; Holst, M.J.; McCammon, J.A. *Proc. Natl. Acad. Sci.* **2001**, *98*, 10037.

POSTER COMMUNICATIONS – PC.153

ITERATIVE PROTEIN-PROTEIN DOCKING AS A METHOD FOR MODELING G-PROTEIN COUPLED RECEPTORS DIMERS

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Until very recently, structure-based drug design methods using G-protein coupled receptors (GPCRs) as targets focused almost exclusively on the inhibition of a single receptor at the orthosteric binding pocket. However, more and more experimental evidence from AFM, FRET, BRET, cysteine cross-linking and receptor chimeras studies indicate the existence of physiologically relevant GPCR dimers/oligomers. Targeting these complexes selectively or designing small molecules that inhibit receptor-receptor interactions might provide new opportunities for novel drug discovery (1). In order to study the mechanisms and dynamics which rule GPCRs oligomerization, it is essential to understand the dynamic process of receptor-receptor association, and to identify regions that are suitable for selective drug binding. Since nearly no X-ray structure of any GPCRs dimer is currently

available, various computational techniques have been applied for modeling of GPCRs dimers, based on the sequence analysis, protein-protein docking algorithms, or molecular dynamics techniques. As it has recently been indicated in our study (2), commonly used protein-protein docking approach may prove unsuitable for membrane proteins. This drawback lies in the fact that most protein-protein docking algorithms were built considering only globular and water-soluble proteins.

In this scenario, the aim of this work was the elaboration of an efficient protocol for enhancing results of protein-protein docking for modeling GPCRs dimers. Two dimers were studied: rhodopsin-rhodopsin homodimer and the recently published mGluR2-5HT_{2A} receptor heterodimer (3). The method involved the application of Rosetta software (4) to obtain populations of dimers with all the possible interfaces followed by scoring according to (i) surface of dimer interface, (ii) the ratio of hydrophobic versus polar surface and (iii) the surface of selected amino acids in dimer outface. The best models were minimized and the whole cycle was iteratively repeated until the results converged to a consistent dimer formation. In the case of the rhodopsin dimer, a consistent percentage in the highest-scored population of dimers was obtained immediately after the first cycle for dimers which direct their transmembrane helix (TM) TM4 and TM5 into the dimer interface. For the mGluR2-5HT_{2A} receptor dimer, several runs led to TM45 interface for 5-HT_{2A} receptor and TM56 for mGluR2. The proposed protocol might be used as an efficient approach for the modelling of GPCRs dimers.

REFERENCES

1. Filizola, M. *Life Sci.* **2010**, in press.
2. Kaczor, A.; Selent, J.; Pastor, M. unpublished results.
3. Bruno, A.; Guadix, A.E.; Costantino, G. *J. Chem. Inf. Model.* **2009**, *49*, 1602.
4. Daily, M.D.; Masica, D.; Sivasubramanian, A.; Somarouthu, S.; Gray, J.J. *Proteins.* **2005**, *60*, 181.

POSTER COMMUNICATIONS – PC.154

MORACIN O AND MORACIN P AS HIF-1 α INHIBITORS

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Moracins O and P were isolated from the oriental medicine Mori Cortex Radicis. The moracin O (IC₅₀ = 0.14 nM) and P (IC₅₀ = 0.65 nM) exhibited excellent inhibitory activity against hypoxia inducible factor (HIF-1). HIF-1, a key mediator for adaptation process of cancer cells to tumor hypoxia, has emerged as an important molecular target for anticancer drug discovery. Herein we report the synthetic methodology for moracin O and P. We initially have accomplished syntheses of the moracin O and P via 2-prenylated phenol as a common intermediate. The key steps

during the process of synthesis are regiospecific intramolecular cyclization. Using 2-prenylated phenol, benzohydrofuran moiety in moracin O was proceed through epoxidation and subsequent intramolecular cyclization with base and benzohydropyran moiety in moracin P was formed by *in situ* epoxidation following intramolecular cyclization with acid. This synthetic methodology was further utilized for derivatization of moracin O and asymmetric synthesis.

REFERENCES

1. Harris AL. *Nat. Rev.*, **2002**, 2, 38–47.
2. Navneet Kaur, Yan Xia, Yinglan Jin, Nguyen Tien Dat, Kondaji Gajulapati, Yongseok Choi, Young-Soo Hong, Jung Joon Lee and Kyeong Lee, *Chem. Commun.*, **2009**, (14), 1879 – 1881.
3. Amato Giaccia, Bronwyn G. Siim and Randall S. Johnson, *Nature Reviews Drug Discovery*, 2003, 2, 1-9.

POSTER COMMUNICATIONS – PC.155

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 1,4-DITHIOSUBSTITUTED PHTHALAZINES AND 1,2-BIS-(4-(THIOSUBSTITUTED)-PHTHALAZIN-1-YL)-DISULFIDES

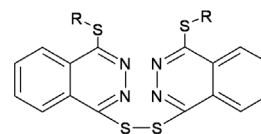
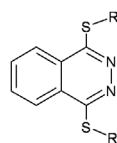
losu Jiménez^{1*}, *Elena Ibáñez*¹, *Iranzu Lamberto*¹, *Daniel Plano*¹, *María Font*², *Ignacio Encío*³, *Juan Antonio Palop*^{1*}, *Carmen Sanmartín*¹.

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A serie of 1,4-dithiosubstituted phtlazines have been designed in order to combine the biological effects attributed to the heterocycle ring and sulfur derivatives separately. Cytotoxicity, VEGFR-2 inhibition, and DNA bisintercalation are the main therapeutic activities^{1,2} observed for phtlazinic derivatives; while organic and inorganic sulfur compounds have been used in medicine due to their antioxidant, apoptosis induction and chemopreventive properties³⁻⁵.

The synthesis of thioderivatives was performed by aliphatic nucleophilic substitution of different benzyl and alkyl halides with 1,4-dithiophthalazine, or by aromatic electrophilic substitution of 1,4-di-chlorophthalazine with the corresponding benzylthiol.

In order to study the relationship between molecular symmetry and biological activity⁶ a serie of 1,2-bis-(4-(thiosubstituted)-phtlazin-1-yl)-disulfides have been prepared by aliphatic nucleophilic substitution of different benzyl and alkyl halides with 1,2-bis-(4-(thiol)-phtlazin-1-yl)-disulfide.



R= H, CH₃, benzyl, 4-chlorobenzyl, 4-substitutedbenzyl.

All products are being tested as cytotoxic agents against five human cancer cells: CCRF-CEM (leukemia), HT-29 (colon adenocarcinoma), HTB-54 (lung carcinoma), MCF-7 (breast adenocarcinoma) and PC-3 (prostatic adenocarcinoma); and a non-tumoral cell line 184B5 (breast epithelium). The biological activity results will allow us to compare 1,4 symmetrical substitution and dimeric symmetry.

Currently, we are working on a new synthetic derivatives based on the sulfur atoms substitution by selenium in order to study if this modification increases the biological activity of our compounds.

REFERENCES

1. Xin Zhai; Juan Li; Lei He; Su Zheng; Yao Bin Zhang; Ping Gong. *Chinese Chemical Letters*. 19 (2008)29-32.
2. Evgueni L. Piatnitski, Matthew A. J. Duncanson, et al. *Bioorganic & Medicinal Chemistry* 16 (2006) 1579 -1581.
3. Erin, E. Battin; Julia ; *Journal of Inorganic Biochemistry* 102 (2008) 2036–2042.
4. Juli Bai; Ziqiang Meng. *Regulatory Toxicology and Pharmacology* 43 (2005) 272–279
5. Rosa M. Pascale; Maria M. Simile; Maria R. De Miglio; Francesco Feo. *Alcohol* 27 (2002) 193–198
6. Sanmartín, C.; Font, M.; Palop, J.A. *Mini-Reviews in Medicinal Chemistry*, 2006, 6, 639-650.

POSTER COMMUNICATIONS – PC.156

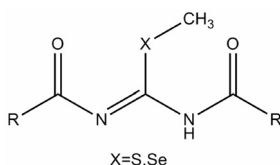
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW IMIDOSELENOCARBAMATES AS POTENTIAL ANTIPROLIFERATIVE AGENTS IN CANCER TREATMENT

Elena Ibáñez^{1*}, *Iranzu Lamberto*¹, *losu Jiménez*¹, *Daniel Plano*¹, *María Font*², *Juan Antonio Palop*¹, *Alfonso Calvo*³, *Carmen Sanmartín*^{1*}

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Nowadays, the *in vitro* and *in vivo* activity of a great variety of organic derivatives of Selenium¹ has provided new aims in drug

development of potential antitumoral agents. Setting as the principal aim the search and optimization of new structures with well determined characteristics, the design, synthesis and biological evaluation of new imidoselenocarbamates as potential antitumoral agents have been carried out. Therefore, the design has been based in a structural study of known cytotoxic molecules, which has provided a number of essential features for the activity²⁻⁵, among which the symmetry, a selenomethyl group as central element and an amide group as connection between the central element and the active heteroaromatic groups seem to be the most important. These heteroaromatic groups have been chosen by their known antitumoral activity they have by themselves. Satisfying our aims, the synthesis and biological evaluation of 20 derivatives with the following general structure are presented in this work.



To confirm how atoms with similar chemical properties, as Sulfur and Selenium, have a different biological activity, 10 imidoselenocarbamates and their sulfurated analogues are shown. The results confirm the different activity of the derivatives in all the studied cell lines. MCF-7 (breast) and HT-29 (colon) lines are the most sensible to this kind of compounds, reaching GI_{50} values of nM in some of the derivatives.

REFERENCES

1. Sanmartín, C.; Plano, D.; Palop, J.A. Selenium Compounds and Apoptotic Modulation: A New Perspective in Cancer Therapy. *Mini-Rev. Med. Chem.* 8, 1020-1031, 2008.
2. Plano, D.; Sanmartín, C.; Palop, J.A. Novel potent organoselenium compounds as cytotoxic agents in prostate cancer cells. *Bioorg. Med. Chem. Lett.* 17, 6853-6859, 2007.
3. Sanmartín, C.; Font, M.; Palop, J.A. Molecular Symmetry: A Structural Property Frequently Present in New Cytotoxic and Proapoptotic Drugs. *Mini-Rev. Med. Chem.* 6, 639-650, 2006.
4. Cubedo E, Cordeu L, Bandres E, Rebollo A, Malumbres R, Sanmartín C, Font M, Palop JA, Gacía-Foncillas J. New symmetrical quinazoline derivatives selectively induce apoptosis in human cancer cells. *Cancer Biol Ther.* 5(7),850-859, 2006.
5. Font M, Ardaiz E, Cordeu L, Cubedo E, García-Foncillas J, Sanmartín C, Palop JA. Structural characteristics of novel symmetrical diaryl derivatives with nitrogenated functions. Requirements for cytotoxic activity. *Bioorg Med. Chem.* 14(6), 1942-1948, 2006.

POSTER COMMUNICATIONS – PC.157

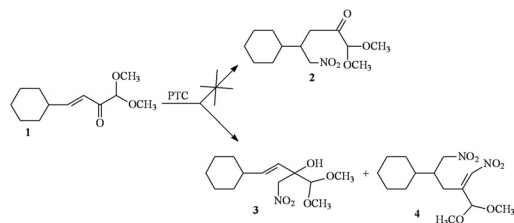
INVESTIGATION OF SYNTHESIS OF 4-CYCLOHEXYL-(S)-PROLINE - CRUCIAL INTERMEDIATE IN FOSINOPRIL PREPARATION

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Looking for an efficient, environment friendly and economical large scale laboratory synthesis of 4-cyclohexyl-(S)-proline – an intermediate in the preparation of Fosinopril – prodrug used in the treatment of hypertension and acute of chronic congestion heart failure, we proposed a three step reaction scheme, crucial step of which consists on Michael reaction between easy available ketoacetal **1** and nitromethane.

It is obvious, that proper selection of the base-solvent system used for reactions induced by bases is of crucial importance. We choosed Phase Transfer Catalysis - the most general, efficient and environment-friendly methodology of performing organic reactions in which organic anions react with organic substrates. According to this methodology reactions are performed in immiscible two-phase systems. One phase (inorganic) is a base for generation of organic anions. The second (organic) phase contains organic reactants, usually neat or sometimes in appropriate solvents. Upon introduction of a catalyst – tetraalkylammonium salt – continuous transfer of reacting anions, produced in the interfacial region, into the organic phase in the form of lipophilic ion pairs with the catalyst cation takes place. All further reactions occur in the organic phase. Earlier we applied this methodology for syntheses of some intermediates in preparation of Anastrozole and Sibutramine. We found, that instead of **2** a mixture of two products is formed in the reaction mentioned – namely **3** and **4**, their ratio being dependent on reaction time and temperature.



3 and **4** are highly functionalized compounds and may be used in syntheses of other products, possessing biological activity.

POSTER COMMUNICATIONS – PC.159

HOW THE RECRUITMENT OF COACTIVATORS REFLECTS THE CONFORMATION OF THE ESTROGEN RECEPTOR

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When it associates to estrogenic ligands, the estrogen receptor (ER) adopts an agonist conformation that allows its dimerization and phosphorylation. In addition, its interaction with estrogens confers ability to recruit coactivators. Briefly, coactivators are proteins required for transcription that engulf, through their LxxLL motif (where L correspond to leucine and x to any amino-acid) in a shallow hydrophobic binding site located at the surface of ER. It is of note that LxxLL motifs (or NR-boxes) adopt a α -helical conformation when associated with ER. The recruitment of LxxLL motifs is closely related to the orientation of the ER helix H12. Indeed, in the presence of antagonists, H12 occupies the LxxLL-binding site, preventing thereby the recruitment of coactivators. In contrast, in presence of agonists, H12 projects in distance of the LxxLL-binding site, allowing the recruitment of coactivators.

However, little is known about inter-relations existing between ligand-binding affinities, transcription and the recruitment of LxxLL motifs. To explore these relationships, we measured the effects of a panel of ligands on transcription as well as on the recruitment of LxxLL motifs. Data were compared to the ability of the ligands to compete with tritiated estradiol.

Transcriptional activities displayed by ligands were strongly associated with their ability to induce the recruitment of LxxLL motifs, particularly in the case of type I agonists (steroids, DES, flavones and isoflavones). In contrast, in the case of type II agonists (triphenylethylenes, cis-diphenylethylenes), transcriptional activities were less dependent upon the recruitment of LxxLL. This feature may explain why such derivatives are considered as partial agonists.

Our study suggests that the pharmacological evaluation of ER ligands of therapeutic interest should be tested not only with respect to transcription but also with respect to their ability to recruit LxxLL motifs. This approach may contribute to elucidate their mode of action as well as the related conformational status of ER. At least, our approach could be relevant to a better understanding of Structure-Activity Relationships associated with estrogens.

REFERENCES

1. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757.

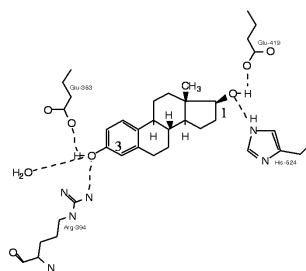
POSTER COMMUNICATIONS – PC.160

MODULATION OF THE STEROID ESTROGEN PHARMACOPHORE THROUGH INTRAMOLECULAR ELECTRONIC EFFECTS: APPLICATIONS TO STRUCTURE / AFFINITY RELATIONSHIPS

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Steroidal estrogens interact with the estrogen receptor (ER) to enhance transcription of target genes. This interaction requires various ER amino-acids among which the Arg-353 (helix H3), the Glu-394 (helix H6) and the His-524 (helix H11). In fact, the His-524 interacts with the hydroxyl in position 17 β of the steroid core structure of ligands whereas the Arg-353 and the Glu-394 interact with their phenolic hydroxyl in position 3 (with the contribution of a structure molecule of water) as shown in Scheme 1 [1]. The latter interaction appears as an absolute requirement for an efficient association with ER. Accordingly, it is usually considered that the presence of a phenolic group in a hydrophobic molecule of similar size than 17 β -estradiol is sufficient for ER recognition.



Scheme 1: Interaction of 17 β -estradiol within the ligand-binding pocket of the estrogen receptor.

A number of estradiol derivatives substituted in 11 β have been synthesized and their pharmacological profile extensively explored with respect to ER. Remarkably, these compounds are of great interest and exert a panel of actions through ER binding.

Remarkably, long hydrophobic chains exert an antagonist activity by engulfing within a pocket encompassing the helix H3 and different from the one accepting the ligand, limiting therefore

the flexibility of H3 through intermolecular hydrophobic contacts as well as the recruitment of coactivators [2]. In the case of short substituents in 11 β , the mechanisms by which ligands exert estrogenic effects are still obscure and do not appear to be related to such behaviour. By using mass spectrometry, we showed that the effects exerted by these short substituents are partially intramolecular [3]. Thus, the nature of the substituents as well as their stereochemistry may exert significant electronic effects that could modulate the acidity of the 3-phenolic hydroxyl. Therefore, we assume that such effects could influence the affinity as well as the ER-dependent transcriptional activity of steroid estrogens.

Our work suggests that Structure / Affinity Relationships studies associated with estrogens should not be restricted to the optimization of the intermolecular interactions by generating new anchoring points. Indeed, the optimisation of the anchoring strengths stabilizing ligand / ER association through indirect electronic effects should be also considered.

REFERENCES

1. Jacquot *et al.* Mini-Rev. Med. Chem. (2003); 3: 333-346;
2. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757;
3. Bourgoïn-Voillard *et al.* J. Am. Soc. Mass Spectrom. (2009); 20: 2318-2333.

POSTER COMMUNICATIONS – PC.161

MOLECULAR MECHANISMS THAT MODULATE ESTROGEN RECEPTOR-MEDIATED TRANSCRIPTION : A DYNAMIC APPROACH

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The estrogen receptor subtype α (ER α) is a nuclear transcription factor of 66 kDa implicated in various ligand-dependent (or independent) physiological and malignant processes such as breast cancer. ER α ligands activate (agonists) or repress (antagonists) transcription by modifying the conformational status of ER α as well as the recruitment of specific protein partners (coactivators and corepressors). With regard to steroidal ligands, both steric and electronic effects provoked by substitutions in position 11 β were found to influence the ER α conformation. Indeed, intramolecular electronic effects modulate the acidity of the 3-phenolic hydroxyl of these ligands [1] and regulate thereby the dynamic of the receptor's helix H3, which encompasses key residues important for the recruitment of co-activators. Indeed, the interaction between H3 and ligands is mediated through

hydrogen contacts between the Glu-353 (helix H3) / Arg-394 (helix H6) side chains and the 3-phenolic hydroxyl [2,3].

Recently, we found that these conformational modifications modify another surface region of ER α containing a type II β -turn [4]. In fact, this type II β -turn is subjected to a spatial reorientation depending upon the nature of the ligand, suggesting that it is a crucial regulatory region of ER α . An extensive study of this region reveals that it is surrounded by a mixed α -helix / polyproline II motif (residues 295 to 311) included within the autonomous regulatory function of the receptor. This motif, which corresponds to a regulatory platform subjected to a number of post-translational modifications (acetylation, phosphorylation, méthylation, SUMOylation, ubiquitylation), encompasses the third NLS of ER α , confers calmodulin-binding ability [5,6] and is sensitive to proteolysis. The corresponding peptide (ER α 17p, P₂₉₅LMIKRSKNSLALS₃₁₁), which interacts with holo-calmodulin in the micromolar range through a entropy-driven mechanism, associates with ER α [7] and the heat-shock protein Hsp70 [8], which is known to modulate receptor conformation and associated transcriptional activity. These properties may explain our finding, i.e that ER α 17p induces estrogenic responses exclusively in ER α -positive breast carcinoma cells and provokes the degradation of ER α through a proteasomal-dependent process. Curiously, this peptide inhibits the recruitment of the p160 co-activator motif LxxLL, suggesting that it may support an additional antagonist activity.

Thus, we have identified a new platform that could be useful for new therapeutic approaches. Functional and biomolecular characteristics of ER α , reported here, may contribute to the design of new estrogen antagonists devoted to the treatment of breast tumors.

REFERENCES

1. Bourgoïn-Voillard *et al.* J. Am. Soc. Mass Spectrom. (2009); 20: 2318-2333;
2. Jacquot & Leclercq *in* Estrogens: Production, Functions and Applications, Ed. Nova Science Publishers, Inc (2009);
3. Bourgoïn-Voillard *et al.* Biochem. Pharmacol. (2010); 79: 746-757;
4. Jacquot *et al.* J. Steroid Biochem. Mol. Biol. (2007); 104: 1-10;
5. Gallo *et al.* *in* Medicinal Chemistry Research Progress, Ed. Nova Science Publishers, Inc (2009);
6. Gallo *et al.* Mol. Cell. Endocrinol. (2008); 291: 20-26;
7. Gallo *et al.* Lett. Drug Design Discov. (2007); 4: 346-355;
8. Gallo *et al.* J. Steroid. Biochem. Mol. Biol. (2008); 109: 138-149.

POSTER COMMUNICATIONS – PC.162

HYDRATION SITE THERMODYNAMICS
EXPLAIN STRUCTURE-ACTIVITY
RELATIONSHIPS FOR TRIAZOLYPURINE
ANALOGUES BINDING TO THE A2A
ADENOSINE RECEPTOR*Christopher Higgs, Thijs Beuming, Woody Sherman*Schrodinger, Inc., 120 West 45th Street, 17th Floor, New York, New York 10036

A series of triazolypurine analogues shows interesting and uninituitive SAR against the A2A adenosine receptor. As the 2-substituted aliphatic group is initially increased to methyl and isopropyl there is a decrease in potency; however, extending the substituent to n-butyl and n-pentyl results in a significant gain in potency. This trend cannot be readily explained by ligand-receptor interactions, steric effects, or differences in ligand desolvation. Here, we show that a novel method for characterizing solvent thermodynamics in protein binding sites correctly predicts the trend in binding affinity for this series based on the differential water displacement patterns. In brief, small unfavorable substituents occupy a region in the A2A adenosine receptor binding site predicted to contain stable waters, while the longer favorable substituents extend to a region that contains several unstable waters. The predicted binding energies associated with displacing water within these hydration sites correlate well with the experimental activities.

KEYWORDS

A2A adenosine receptor, antagonist, GPCR, WaterMap, binding energy, desolvation

POSTER COMMUNICATIONS – PC.163

NOVEL CALPAIN INHIBITORS WITH
ANTICATARACT PROPERTIES*Rodrigo Alarcón, Adrián Luna, Mónica Ortiz,
Carlos F. Lagos and *Patricio Huenchunir*

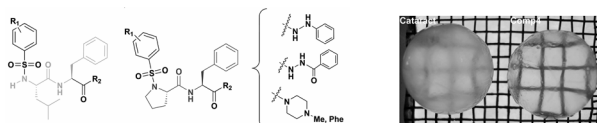
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Cortical cataract is associated with Ca²⁺ overload and protein loss, where an established feature is that there are major structural modifications to the water-soluble crystallins. It has become increasingly clear that the elevated levels of Ca²⁺ associated with some lens insults can induce crystalline proteolysis by calpains.

Calcium activated proteases calpains are known to occur in the lens, and the anterior epithelial layers and cortical regions of human cataract have been shown to contain calpain 2, a key enzyme implicated in the genesis of some forms of cataract. We have used a structure-based approach to design novel potential calpain 2 inhibitors, which might interfere with cataract progression.

To test the effect of test compounds on cataract formation, an in vitro bovine lenses assay was developed. Bovine lenses can be maintained in culture and remain transparent for at least 10 days. Incubation with CaCl₂ causes opacification in these lenses. Control and compound-treated lenses were pre-incubated in culture buffer, and their opacities determined by means of optical densitometry measurements, after cataract induction by addition of CaCl₂ (15 mM) and incubation for 20 h.

The synthesized calpain 2 inhibitors show IC₅₀ values in the micromolar range and prevent opacification of isolated bovine lenses when exposed to a CaCl₂ solution. Molecular modeling experiments show that these compounds assume an extended β -sheet-like structure shown by other calpain inhibitors.



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FONDEF Project N° D0811139 and CONICYT for CFL PhD fellowship.

POSTER COMMUNICATIONS – PC.164

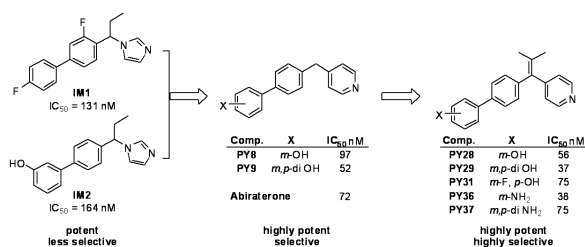
BIPHENYL METHYLENE HETEROCYCLES AS
POTENT CYP17 INHIBITORS FOR THE
TREATMENT OF PROSTATE CANCER: FROM
IMIDAZOLES TO PYRIDINES*Qingzhong Hu, Carsten Jagusch, Ulrike E. Hille,
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It is well known that the growth of up to 80 % of prostate carcinoma depends on androgen stimulation. Therefore, segregation of tumour cells from androgen will effectively prevent cancer cell proliferation. However, current standard therapy "combined androgen blockade" (CAB) often leads to resistance which can be associated with androgen receptor mutations. The mutated androgen receptor recognizes antagonists or glucocorticoids as agonists leading to the collapse of CAB therapy.

Therefore, inhibition of CYP17, which is the crucial enzyme in androgen biosyntheses, was proposed as a promising therapy because it totally blocks androgens formation in testes and adrenals, as well as inside cancer cells. The recent success in phase II and III clinical trials of steroidal CYP17 inhibitor Abiraterone demonstrates the benefits of CYP17 inhibition as therapy for castration-resistant prostate cancer. However, due to possible side effects associated with steroidal scaffolds, non-steroidal CYP17 inhibitors were designed and synthesized.

By mimicking the natural substrates, several series of biphenyl methylene heterocycles were designed as CYP17 inhibitors. Although potent inhibitors have been identified in imidazoles¹ showing favorable pharmacokinetic properties,² they were not sufficiently selective. After replacing imidazolyl by 4-pyridyl, improvements of activity and selectivity were observed. Inspired by the imidazole SARs that substituents on the A-ring and the methylene bridge showed profound influence on the activity, thorough investigation was performed on the pyridyl scaffold. It has been found that H-bond forming groups furnishing on the A-ring largely elevated the activity. As for the methylene bridge, flexible alkyl groups reduced potency, whereas conformation rigidifying *i*-propylidene groups significantly improved activity and selectivity. These efforts resulted in compounds more potent than or comparable to the drug candidate Abiraterone (IC₅₀ = 72 nM). Most of these potent compounds also exhibited better selectivity profiles toward CYP11B1, CYP11B2, CYP19 and hepatic CYP3A4 than Abiraterone. They can be considered as promising drug candidates after further validation *in vivo*.



REFERENCES

- Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Hartmann, R. W. et al. *Bioorg. Med. Chem.* **2008**, *16*, 7715–7727.
- Hu, Q.; Negri, M.; Olgen, S.; Hartmann, R. W. *ChemMedChem*. accepted.

POSTER COMMUNICATIONS – PC.166

NC-2500, A NOVEL AND ORALLY ACTIVE XANTHINE OXIDOREDUCTASE INHIBITOR

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Gout, a chronic disease that results from hyperuricemia, is one of the most painful forms of arthritis. Xanthine oxidoreductase (XOR) catalyzes the terminal steps in urate synthesis in humans and animals, thus XOR inhibitors are the mainstay of treatment for hyperuricemia in patients with gout. NC-2500, a thiazolopyrimidine derivative, is a novel, orally active inhibitor of XOR. NC-2500 inhibited XOR activity derived from bovine milk and urate production by rat, dog and human hepatocytes with an IC₅₀ of 10⁻⁹ mol/L. Besides, it exhibited a dose-dependent and highly potent plasma urate-lowering effect after oral administration in a rat model of oxonate-induced hyperuricemia and in normal dogs; the minimum effective dose was 0.3 and 0.1 mg/kg, respectively. The effect of NC-2500 was particularly marked in dogs whose metabolism of NC-2500 in *in vitro* studies was similar to that of humans and it was more than 10 times more potent than that of febuxostat. NC-2500 also exhibited unique pharmacokinetic properties such as enterohepatic circulation, low covalent binding to human liver microsomes and high non-renal elimination.

These characteristics of NC-2500 may offer improved treatment for hyperuricemia over allopurinol and febuxostat, which are the currently available XOR inhibitors, at a very low therapeutic dose and with a potential low incidence of idiosyncratic drug toxicity and serious hepatotoxicity.

POSTER COMMUNICATIONS – PC.167

A COMPREHENSIVE COMPUTATIONAL STUDY OF ASPARTIC PROTEASES IN DRUG DESIGN

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Proteolytic enzymes (also known as proteases) are key regulators of a number of important biological processes in virtually all organisms. The family of aspartic proteases, where the catalytic center is constituted by two aspartic acids, has received much interest from the biopharmaceutical sector. The most successful

case in pharmaceutical research is the HIV-1 protease and the HIV-PI (protease inhibitors of HIV), but several aspartic protease inhibitors are in preclinical research or clinical trials in many diseases, including: malaria (plasmeprin inhibitors), Alzheimer disease (β -secretase inhibitors) or hypertension (renin inhibitors). Other aspartic proteases are discussed as potential drug target.¹ Comparisons of inhibitor binding within the aspartic peptidase family has been a very important approach to improve the structure-based drug design, and this continues to be a fertile area.

This report is part of a project addressed to build a computational platform to describe and compare the binding modes of inhibitors to different aspartic proteases. As a first step, we report a virtual screening protocol for HIV-p on the basis of the LIE method for calculation of ligand affinities.

HIV-p/inhibitor complexes for which there is experimental structural and affinity data have been collected from the PDBbind database (<http://www.pdbbind.org>). The initial set was divided into a training set and a test set. LIE binding affinities were estimated for the test set, according to the general procedures described elsewhere,² on the basis of separate 0.5ns MD run of the ligand-protein complex and ligand-free states. A optimization of the LIE parameters preceded the application of the same procedure to the test set.

The LIE methodology demonstrated its utility in a VS protocol in order to discriminate high and low affinity ligands, with an associated error of less than 1 kcal/mol. Interestingly, for the present system under study (HIV-p), the optimal weighting factors of the corresponding electrostatic and non-electrostatic interactions in the LIE equation differed from the original parameters. In particular, an increase in the weight of the hydrophobic interactions was needed in order to better reproduce experimental results. Due to the relatively large size of the ligands, this results are in agreement with previous observations of LIE calculations on protein-protein interactions.³ The optimized model was consistently applied to the test set, and only a moderate increase up to 1.35 kcal/mol for the mean unassigned error was observed. A deeper investigation of the chemical nature of the ligands revealed that compounds with polyhydroxyl substitutions are most problematic. These observations are currently compared with a similar study on other proteases.

REFERENCES

1. Abbenante, G. and Fairlie, D.P. *Med Chem* 1, 71-104 (2005).
2. Aqvist, J., et al. *Protein Eng* 7, 385-391 (1994).
3. Almlöf M. et al. *Biophysical J* 90, 433-442 (2006).

POSTER COMMUNICATIONS – PC.168

ELECTRON-NUCLEAR QUINTESSENCE OF DRUGS THERAPEUTIC ACTION

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There are many different QSAR methods. All of them can be divided into two groups: the methods which consider molecular exterior and the methods considering molecular interior. For the first group of methods, the enough wide definition of exterior is used which can include not only molecular shape but also a distribution of charges, Van der Waals and electrostatic potentials, centers of potential hydrogen bonds and hydrophobic (or lipophilic) interactions etc. at the molecular surface. The significance of molecular exterior can hardly be underestimated. It is obvious that exterior determines molecular capability for intermolecular interactions. Though, frequently consideration of exterior is not enough for description of properties. The simple example of that is the pair of lists of cardboard and steel having the same form and the same sizes (i.e. the same exterior) but possessing by extremely different properties. Thus, the exterior shows the immediate properties of an object while the interior determines its potential capabilities, abilities for changes under the external influence. Therefore, in this work we suggested to use the combination of the methods – exterior-based BiS/MC [1] and interior-based ConGO [2] algorithms. The first one permits to determine conformational state of a molecule interacting with the target, while the second one is used for detailed study of the electron structure of the selected conformers. The distinguishing feature of the approach is the usage of quantum wave functions for description of molecular interior.

The work of the approach was demonstrated under consideration of DNA-antimetabolites, fXa inhibitors, inhibitors of 5-HT_{1A} and α_1 -AR receptors. The biological activities of the drugs were evaluated with very high quality – in all cases CrossR2 exceeded 0.90. The analysis of the sets permitted to determine pharmacophoric and antipharmacophoric fragments of drugs electronic structures. The consideration of fXa inhibitors showed the high quantum similarity of the most active molecules of the set while less active fXa inhibitors demonstrated considerable differences in their electron structures. Study of the molecules interacting with competing 5-HT_{1A} and α_1 -AR receptors revealed that intercalation of high active molecules is determined by folded conformation allowing their fitting into the receptor cavities without any problem. Interacting with the receptors, the molecules frequently have similar pharmacophoric electron fragments while their antipharmacophoric fragments defer significantly. The analysis of DNA-antimetabolites showed that in the case of the most active molecules the antipharmacophore fragments of the electron structure are, as a rule, located at distances exceeding the Van der Waals radii of atoms. The electron density is extremely low at such distances. Therefore, its negative influ-

ence on the activity level of high active compounds is reduced to the minimum. The points determining the antiparmacophore effect can be mostly attributed to methylene fragments of the structures. Probably these fragments of the molecules increase the structure volume interfering their intercalating into the receptor (DNA).

ACKNOWLEDGMENTS

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REFERENCES

1. Potemkin V., Grishina M. Principles for 3D/4D QSAR classification of drugs// *Drug Discovery Today*, 2008, V. 13 (21/22), 952 – 959.
2. Potemkin V.A., Grishina M.A. A new paradigm for pattern recognition of drugs// *Journal of Computer Aided Molecular Design*.- 2008.- 22.-P. 489-505

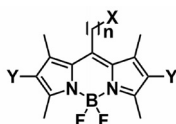
POSTER COMMUNICATIONS – PC.169

SYNTHESIS OF NOVEL FLUORESCENT-LABELED LIGANDS FOR STUDYING ADENOSINE RECEPTORS

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4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-s-indacene (BODIPY) dyes are useful tools to image processes in living cells due to their exceptional spectral properties, i.e. high absorption coefficients, fluorescence quantum yields ($\Phi > 0.5$) and peak intensities, photochemical stability, and their small size.^{1,2}



$n = 1, 2, 3, 4, 5, 10$; $X = Br, I, NH_2, OH, SH$; $Y = H, SO_3H$

Fig. 1: Functionalized BODIPY derivatives

In the present study we synthesized new, functionalized BODIPY derivatives, which fluoresce at approximately 500 nm and therefore do not interfere with biological fluorophores. Various functional groups (Br, I, NH_2 , SH, OH) were attached via alkyl spacers of different lengths to the BODIPY core in order to allow for their attachment to receptor ligands, or their integration into the pharmacophore of adenosine receptor ligands. All new, functionalized BODIPY derivatives showed high fluorescence quantum yields and absorption and emission wavelengths near 500 nm.

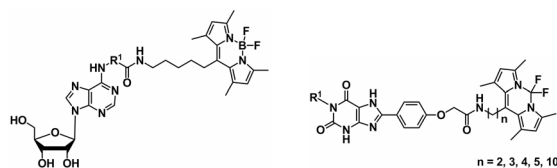


Fig. 2: Fluorescent-labeled adenosine receptor ligands

In a subsequent step we coupled these BODIPY dyes to adenosine and xanthine derivatives, respectively. The affinities of the obtained derivatives were determined in radioligand binding studies at membrane preparations cell lines expressing A_1 , A_{2A} , A_{2B} , or A_3 receptors, respectively.

Fluorescent A_1 -agonists and A_{2B} -antagonists were obtained, some of which show high potency (K_i values at low nanomolar concentrations) combined with receptor subtype-selectivity. The new fluorescent adenosine receptor ligands will be useful tools for studying adenosine receptor binding and function.

REFERENCES

1. Loudet, A.; Burgess, K. BODIPY dyes and their derivatives: synthesis and spectroscopic properties. *Chem. Rev.* **2007**, *107*, 4891-4932.
2. Middleton R.J.; Briddon S.J.; et al. New fluorescent adenosine A_1 -receptor agonists that allow quantification of ligand-receptor interactions in microdomains of single living cells. *J. Med. Chem.* **2007**, *50*, 782-793.

POSTER COMMUNICATIONS – PC.170

DISCOVERY OF POTENT AND SELECTIVE INHIBITORS OF DRUGGABLE CARBONIC ANHYDRASE ISOFORMS

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Carbonic anhydrases (CA, EC 4.2.1.1) are a family of metalloenzymes comprising various isoforms with different tissue and cellular localization. They play an important role in many physiological processes linked to the catalytic hydration of CO_2 to bicarbonate regulating respiration, pH, electrolyte secretion, biosynthetic reactions, bone resorption, calcification, etc. Furthermore, these enzymes are involved in several pathological pathways suggesting the development of carbonic anhydrase inhibitors (CAIs, Scheme 1) as new tools for diuretics, anticonvulsants, antiobesity and antitumor drugs. The most potent CAIs (see Figure) are characterized by the presence of a sulfon-

amide/sulfamate functionality coordinating the zinc ion and interacting with specific amino acid residues in the catalytic site.

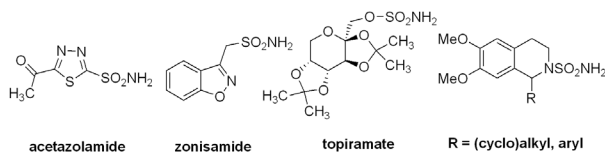


Figure. Carbonic anhydrase inhibitors (CAIs)

In previous works, we rationally designed and tested some isoquinolinesulfonamide derivatives as CAIs showing K_i values at nanomolar concentration as well as significant selectivity towards some druggable hCA isoforms (e.g. hCA VII, hCA IX, and hCA XIV).^[1,2] On this basis we herein report new studies addressed to deepen the structural understanding for carbonic anhydrase inhibition through SAR considerations, target-based computational approaches and crystallographic data of inhibitors in complexes with some selected hCA isoforms.

Research supported by MIUR- 20085HR5JK_002

REFERENCES

1. R. Gitto, S. Ferro, S. Agnello, L. De Luca, G. De Sarro, E. Russo, D. Vullo, C. T. Supuran, A. Chimirri, *Bioorg. Med. Chem.* **2009**, 17, 3659-3664.
2. R. Gitto, S. Agnello, S. Ferro, L. De Luca, D. Vullo, J. Brynda, P. Mader, C. T. Supuran, A. Chimirri, *J. Med. Chem.* **2010**, 53, 2401-2408.

POSTER COMMUNICATIONS – PC.173

ANALYSIS OF HIGH-ENERGY WATER SITES IN PROTEIN-PROTEIN INTERFACES: UNDERSTANDING AFFINITY, SELECTIVITY, AND APPLICATION TO DRUG DESIGN

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Schrödinger, Inc., 120 West 45th Street, 17th Floor, New York, New York 10036

PDZ domains have well known binding preferences for distinct C-terminal peptide motifs. For most PDZ domains, these motifs are of the form [S/T]-W-[I/L/V]. Although the preference for S/T has been explained by a specific hydrogen bond interaction with a histidine in the PDZ domain and the (I/L/V) is buried in a hydrophobic pocket, the mechanism for Trp specificity at the second to last position has thus far remained unknown. Here, we apply a method to compute the free energies of explicit water molecules and predict that potency gained by Trp binding is due to a favorable release of high-energy water molecules into bulk. The affinities of a series of peptides for both wild-type and mutant forms of the PDZ domain of Erbin correlate very well with the computed free energy of binding of displaced waters,

suggesting a direct relationship between water displacement and peptide affinity. Finally, we show a correlation between the magnitude of the displaced water free energy and the degree of Trp-sensitivity among subtypes of the HTRA PDZ family, indicating a water-mediated mechanism for specificity of peptide binding. In a subsequent example we apply the same method on the protein-protein interface of Bcl-xl and rationalize the SAR of ABT737 chemical series based on release of high-energy water molecules.

POSTER COMMUNICATIONS – PC.174

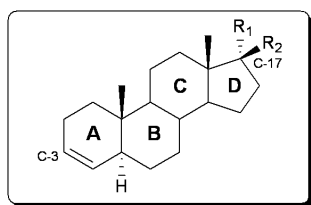
STRUCTURE-ACTIVITY RELATIONSHIPS ON D-RING MODIFIED 3-DEOXY-3-ANDROSTENES AS AROMATASE INHIBITORS

Carla L. Varela¹, Elisiário Tavares-da-Silva¹, Margarida M. D. S. Cepa³, Margarida Azevedo³, Cristina B. Amaral³, Georgina Correia-da-Silva^{2,3}, Natércia A. A. Teixeira^{2,3}, Fernanda Roleira¹

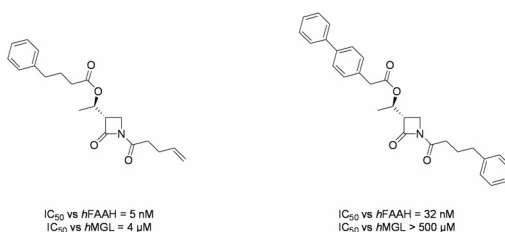
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Aromatase is a cytochrome P-450 enzyme that catalyses the aromatization of androgens, the final step in the biosynthesis of estrogens. Aromatase inhibitors (AIs) reduce the synthesis of estrogens and offer a therapeutic alternative for the treatment of estrogen-dependent cancers such as breast cancer.¹ Recently, the active site of aromatase has been elucidated, and the molecular basis for enzyme-substrate (androstenedione) interaction has been established,² which could lead to a more efficacious intervention at the level of estrogen production inhibition. Keeping this in mind, the design, synthesis and evaluation of new androstenedione-based inhibitors, containing into the A- and D-rings chemical key-features important for the enzyme-drug interaction, would greatly contribute for establishing new structure-activity relationships (SAR). These SAR are valuable tools for understanding the enzyme inhibition mechanism and to find more selective and potent AIs.

From the established SAR for steroidal AIs, it is known that the presence of oxygen functions at C-3 is not mandatory to have activity. On the contrary, some planarity in the A-ring and A/B-ring region seems to be important for aromatase inhibition.³⁻⁵ In this work, we present a structure-activity study, based on the synthesis and aromatase inhibitory activity evaluation, of steroid compounds having double bonds at C-3, which can confer the required planarity in the A/B-ring region, and different substituents at C-17. It was found that none of the 17-substituted 3-androstenes synthesized (**2-5**) is as good aromatase inhibitor as the 5 α -androst-3-en-17-one **1**.



- 1 R₁; R₂ (=O)
- 2 R₁; R₂ (=S)
- 3 R₁; R₂ (=NOH)
- 4 R₁ (H); R₂ (OH)
- 5 R₁ (H); R₂ (OCOCH₃)



REFERENCES

1. Chen, S. *Frontiers of Bioscience* **1998**, 3(9), 22. b) Blickenstaff, R. T. *Antitumor Steroids*, Academic Press (NY); **1992**.
2. Glosch, D.; Griswold, J.; Erman, M; Pangborn, W. *Nature* **2009**, 457, 219.
3. Nagaoka, M.; Watari, Y.; Yajima, H.; Tsukioka, K.; Muroi, Y.; Yamada, K.; Numazawa, M. *Steroids* **2003**, 68(6), 533.
4. Cepa, M.D.S.; Tavares da Silva, E.J.; Correia-da-Silva, G.; Roleira, F.M.F.; Teixeira, N.A.A. *J Med Chem* **2005**, 48, 6379.
5. Cepa, M.; Correia-da-Silva, G.; Tavares da Silva, E.J.; Roleira, F.M.F.; Teixeira, N. *Steroids* **2008**, 73, 1409.

POSTER COMMUNICATIONS – PC.175

SYNTHESIS AND PHARMACOLOGICAL STUDIES OF NOVEL HUMAN FAAH INHIBITORS, BASED ON THE β-LACTAM TEMPLATE

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A library of thirty β-lactams has been prepared from (3*R*,4*R*)-3-[(*R*)-1'-(*t*-butyldimethylsilyloxy)-ethyl]-4-acetoxy-2-azetidinone, and the corresponding de-acetoxy derivative, by sequential *N*- and *O*-functionalizations with various ω-alkenoyl and ω-aryltanoyl chains. All compounds were selective inhibitors of hFAAH versus hMGL, and IC₅₀ values in the nanomolar range (5–14 nM) were recorded for the best representatives. From time-dependant preincubation and rapid dilution studies, and from docking analyses in a homology model of the target enzyme, a reversible mechanism of inhibition of hFAAH is proposed.¹

REFERENCES

1. FELEDZIAK, M.; MICHAUX, C.; URBACH, A.; LABAR, G.; MUCCIOLI, G. G.; LAMBERT, D. M.; MARCHAND-BRYANERT, J. J. *Med. Chem.*, 2009, 52 (2), 7054-7068.

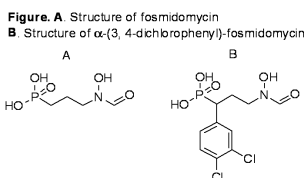
POSTER COMMUNICATIONS – PC.176

NOVEL α-ARYL SUBSTITUTED FOSMIDOMYCIN ANALOGUES AS MYCOBACTERIUM TUBERCULOSIS 1-DEOXY-D-XYLULOSE-5-PHOSPHATE REDUCTOISOMERASE INHIBITORS

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According to WHO, *Mycobacterium tuberculosis* (Mtb) the bacteria, which causes tuberculosis, infects one-third of the world's population causing 2 million deaths each year¹. Recently, it has been demonstrated that 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) is essential for *in vitro* growth of Mtb². The DXR enzyme is not present in humans, which makes it an interesting target for new and potentially selective antibacterial agents. Fosmidomycin, a natural antibiotic, which is a known inhibitor of *E. coli* and *P. falciparum* DXR, inhibits Mtb DXR with an IC₅₀ of 80 nM. Still, due to the lack of uptake it does not affect the growth or viability of Mtb. One of the approaches that can be used to improve the Mtb cell wall permeability is to increase the general lipophilicity of the compound. Therefore we identified α-(3, 4-dichlorophenyl)-fosmidomycin, previously reported as a *P. falciparum* DXR inhibitor³, as an interesting starting point for the development of novel Mtb DXR inhibitors. We have resynthesized the compound and demonstrated that it potently inhibits Mtb DXR (IC₅₀ = 150 nM).



However, when tested for activity on Mtb whole cells it lacked activity (MIC>32 μ g/ml).

X-ray crystallographic studies were therefore initiated and the Mtb DXR structure with the inhibitor was solved. Analysis of the structure suggested that an unexploited hydrated cavity near the 3, 4-dichlorophenyl ring could potentially be used for optimizing the binding of this structural class of compounds.

REFERENCES

1. WHO, <http://www.who.org>.
2. Brown, A. C.; Parish, T. *BMC Microbiol.* **2008**, 8, 78.
3. Haemers, T.; Wiesner, J.; Van Poecke, S.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Van Calenbergh, S. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1888-1891.

POSTER COMMUNICATIONS – PC.177

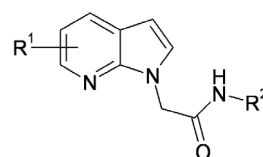
DISCOVERY OF 7-AZAINDOLES AS A NOVEL CLASS OF KCNQ2/KCNQ3 ACTIVATORS

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The KCNQ2/KCNQ3 channel (M-channel) is a voltage-gated potassium channel generating an outward rectifying K⁺ current leading to cell membrane hyperpolarization. M-channels are predominantly expressed in the CNS and in dorsal root ganglion (DRG) neurons and their role consist in controlling resting membrane potential, responsiveness to synaptic inputs and spike frequency adaptation. The current generated by the KCNQ2/KCNQ3 channels can be linked to neuronal hyperexcitability, in that their activation may convey anti-epileptic, analgesic and neuroprotective activity. Retigabine (Valeant Pharmaceuticals), a potent M-channel activator, is under approval by FDA and EMEA for the treatment of epilepsy and has also proven effective in animal models of neuropathic pain, anxiety and migraine.

In this poster, we would like to report the identification of a novel class of KCNQ2/KCNQ3 activators: the 7-azaindole acetamide derivatives. Full details of synthesis and structure-activity relationships are given as well as the evaluation of one derivative in an animal model of persistent pain.



POSTER COMMUNICATIONS – PC.178

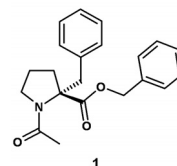
TOWARDS A KCC2 BLOCKER PHARMACOPHORE MODEL

¹Florence Lebon, ¹Cécile Pégurier, ¹Marie Ledecq, ¹Benoit Mathieu, ¹Nathalie Bosman, ¹Anne Frycia, ¹Sébastien Lengelé, ²Kashinath Dhurke, ²Ananda Kumar Kanduluru, ²Stéphane Meunier, ²Alain Wagner, ¹Christian Wolff and ¹Laurent Provins

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KCC2 is a cation-chloride co-transporter which acts as a modulator of inhibitory neurotransmission, both in the brain and in the spinal cord, hence its growing interest as a new target for neuronal hyperexcitability disorders such as epilepsy or neuropathic pain. We previously reported¹ the discovery and the optimization of a new family of potent and selective KCC2 blockers which can be represented by the lead compound **1**.

This poster describes the development of the first pharmacophore model for KCC2 blockers. Since no information was available concerning the structural requirement for binding to KCC2, a multi-disciplinary approach was used to identify the bioactive conformation of the lead compound **1**: several physico-chemical studies such as X-ray diffraction and NMR were combined to molecular modeling techniques, SAR analysis and synthesis of constrained analogues in order to determine a subset of potential bioactive conformations. These conformations were further compared to the conformational spaces of different chemical series of KCC2 blockers in order to identify the KCC2 pharmacophore which was validated by the discovery of more potent analogues.



The present project was supported by a grant from the Walloon Region (Belgium) – DG06 (Convention n° 5296).

REFERENCES

1. Pégurier, C; et al. *Bioorg. Med. Chem. Lett.* (2010), 2542.

POSTER COMMUNICATIONS – PC.179

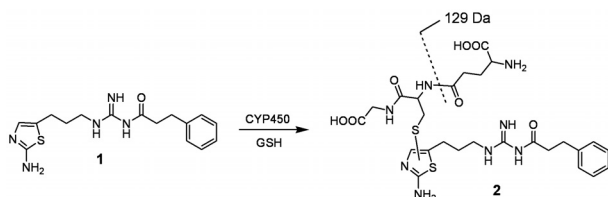
REACTIVE INTERMEDIATES IN THE BIOTRANSFORMATION OF AMINOTHIAZOLE-TYPE HISTAMINE H₂ RECEPTOR AGONISTS: DETECTION BY GLUTATHIONE TRAPPING USING LC/MS

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We previously reported that N^G-acylated 2-aminothiazol-5-ylpropylguanidines are potent and highly selective histamine H₂ receptor agonists.¹⁾ There is strong evidence that CYP450-mediated bioactivation of the 2-aminothiazole ring results in highly reactive intermediates, capable of covalent protein binding. Such reactions may be associated with idiosyncratic adverse drug reactions such as drug-induced hepatotoxicity.^{2), 3)} Therefore, the identification of the toxic potential of new chemical entities is mandatory at an early stage of drug discovery and development. In view of future pharmacological *in vivo* investigations, representative N^G-acylated 2-aminothiazol-5-ylpropylguanidines were studied with respect to the formation of reactive intermediates. We explored the toxic potential *in vitro*, using phenobarbital-induced rat liver microsomes and glutathione (GSH) as a trapping reagent for electrophilic reactive metabolites. Neutral loss (NL) scanning with a triple quadrupole LC/MS system was applied to detect putative covalent glutathione adducts. The NL of 129 Da corresponding to pyroglutamate is a common fragmentation pathway of glutathione-containing compounds.

As detected by NL scanning, the histamine H₂ receptor agonist AK471 (**1**) is activated by CYP450 enzymes to give a glutathione-containing trapping adduct (**2**), which was further characterized by MS/MS.



REFERENCES

1. Kraus, A., et al., Chem. Med. Chem. **2009**, 4, 232-240.
2. Subramanian, R., et al., Chem. Res. Toxicol. **2010**, 3, 653-663
3. Park, B. K., et al., Toxicology **2000**, 153, 39-60

POSTER COMMUNICATIONS – PC.180

STUDIES ON SYNTHESIS AND *IN VITRO* CYCLOOXYGENASE INHIBITORY ACTIVITIES OF DIARYL HETEROCYCLIC COMPOUNDS

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In this study, twenty-eight original of thirty-two compounds having 2-oxo-5H-furan, 2-oxo-3H-1,3-oxazole and 1H-pyrazole as central ring while 3-methyl-2-oxo-3H-benzoxazole and 4-sübstitutedphenyl attached were synthesized. Compounds were differentiated by substitution of phenyl ring with small groups such as OCH₃, Cl, F, SO₂CH₃ at *para* position.

Based on the literature, the compounds synthesized are expected to have COX inhibitory activity. The activities of the final fifteen compounds for possible COX inhibitory activity were performed by *in vitro* Purified Enzyme Assay at 10 µM concentration and the IC₅₀ values of the active compounds were calculated. A 2-oxo-5H-furan derivative Compound **7** and 1H-pyrazole derivatives Compound **25**, **26**, **27** have COX-2 IC₅₀ values as 0.325, 0.011, 0.011, 1.000 µM; COX-1 IC₅₀ values as 0.061, 0.004, 1.000 and 6.000 µM, respectively.

Molecular modelling studies which were performed on the binding site of COX-1 and COX-2 using the compounds having COX inhibitory activity, support the results of *in vitro* enzyme assay.

POSTER COMMUNICATIONS – PC.181

EFFECT OF CEREBROCRIST ON GLUCOSE AND LIPID METABOLISM, BODY AND ORGAN WEIGHTS, FOOD AND WATER INTAKE OF NORMAL RATS

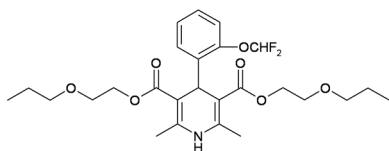
Janina Briede, Mara Stivrina, Dzintra Stoldere, Ilga Dabina, Brigita Vigante and Gunars Duburs

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Impaired carbohydrate and lipid metabolism, and hyperinsulinemia are the early pathogenic factors of obesity and Type 2 diabetes. In subjects with Type 2 diabetes in humans and rodent models there were observed: 1) resistance of adipose tissue,

muscle and liver to insulin; 2) excessive accumulation of lipids in nonadipose tissues as skeletal muscle, liver, heart, pancreatic beta cells that can lead to functional impairment these organs, body weight gain, polyuria, polydipsia etc.

In our previous study we showed that a new antidiabetic agent cerebrocrast (C), derivative of 1,4-dihydropyridine, normalized glucose levels in the organism, intensified and restored the action of insulin [1]. The aim of this work was: to investigate the effect of C on normal rats glucose and lipid metabolism, body weight, food and water intake.



C administration at doses of 0.05 and 0.5 mg/kg b. wt. (p.o.) for 3 consecutive days significantly increased glycogen content in the liver, decreased blood glucose and glycogen level in the kidney and heart. C partially inhibited fatty acid β -oxidation in liver mitochondria, and therefore favoured glucose oxidation and prevented the accumulation of lactate in the cells. C promoted lipolysis, thereby providing energy for tissue and cells, decreasing triacylglycerol content in organs, levels of β -hydroxybutyrate in blood.

C, during the experimental period (from 3 to 27 days after the last administration), decreased body weight by an average of approximately 32.3%, food intake by about 10-15% at the beginning of the experiments and by 22.6% at the end of the experiments. C has long-lasting effects on these parameters. These properties of C are important for treatment of Type 2 diabetes.

ACKNOWLEDGMENTS

This research work was supported by COST D34 and VP-05-07

REFERENCES

1. J. Briede, M. Stivrina, Dz. Stoldere, B. Vigante, G. Duburs Effect of cerebrocrast, a new long-acting compound on blood glucose and insulin levels in rats when administrated before and after STZ-induced diabetes mellitus. *Cell Biochem Funct* 2007; **25**: 673-680.

POSTER COMMUNICATIONS – PC.182

PLASMODIUM FALCIPARUM DNA GYRASE A AND ITS MOLECULAR DOCKING STUDY WITH CIPROFLOXACIN ANALOGUES

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59650 Villeneuve d'Ascq cedex, France. ² Université Lille Nord de France, Inserm U547, Institut Pasteur, 1 Rue du Pr Calmette, B.P. 245, 59019 Lille Cedex, France.

Structure-activity relationship studies were carried out by chemical modification of ciprofloxacin (Fig. 1), a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. The analogues combining bioorganometallic chemistry and the prodrug approach were evaluated for antimalarial activity. The novel compounds exhibited potent and improved in vitro antimalarial activity, being 10- to 100-fold more active than ciprofloxacin against *Plasmodium falciparum* chloroquine-susceptible and chloroquine-resistant strains.¹

As fluoroquinolones are thought to inhibit the plastid gyrase, the mode of binding of ciprofloxacin and its analogues to DNA gyrase was analyzed by means of docking calculations over the surface of the QRDR (quinolone resistance-determining regions) of GyrA.

On the basis of the results presented here, it will be possible to initiate rational drug design efforts around this fluoroquinolone scaffold for the treatment of malaria.

REFERENCES

1. Dubar, F., Anquetin, G., Pradines, B., Dive, D., Khalife, J., Biot, C. *J. Med Chem.* 2009, 52, 7954-7957.

This article has been selected for a highlight in Nature/SciBX.

POSTER COMMUNICATIONS – PC.183

NOVEL INHIBITORS OF ANGIOGENESIS THAT BLOCK THE PRODUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR INDUCED BY HYPOXIA

Alicia Zuazo¹, Daniel Plano², Elena Ansó³, Carmen Sanmartín², Enrique Domínguez², Iosu Jiménez², Juan A. Palop², Ana Rouzaut⁴, María Font¹, Juan J. Martínez Irujo³

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Tumour angiogenesis, the formation of new blood vessels from the preexisting vasculature, is stimulated by several growth factors. From these, the main regulator of physiological and pathological angiogenesis is the vascular endothelial growth factor (VEGF), which is highly stimulated by hypoxia-inducible-factor (HIF-1). VEGF is required for tumour growth, invasion and metastasis in many animals models. In this work we searched for molecules with the ability to inhibit the production of VEGF in tumoral cells induced by hypoxia. For this purpose, we analyzed the biological activity of about 200 structurally-related com-

pounds synthesized by the Department of Organic Chemistry and Pharmaceuticals. Specifically, we measured their effects on cell viability, cell proliferation and on the inhibition of hypoxia-induced VEGF secretion in the lung cancer cell line H157. We found that several imidoselenocarbamate derivatives effectively blocked the production of VEGF stimulated by hypoxia in the low micromolar range. These compounds did not inhibit the classical pathways that regulate HIF-1 α expression, such as PI3K-Akt or MAP-kinase pathways, suggesting that they regulate VEGF production by a HIF-1 independent mechanism. We are performing molecular modelling studies to establish structure-activity relationships that may help us to direct the search of more active derivatives. The knowledge of the mechanism of action of these compounds and their structure-activity relationships, would make possible the design of novel and more effective inhibitors of pathological angiogenesis.

POSTER COMMUNICATIONS – PC.184

NOVEL QUINAZOLINE DERIVATIVES AS CYTOTOXIC AGENTS IN SEVERAL CANCER CELL LINES

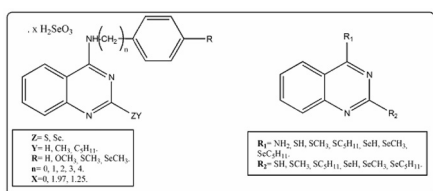
Esther Moreno^{1*}, Enrique Domínguez¹, Ylenia Baquedano¹, Daniel Plano¹, María Font², Ignacio Encío³, Juan Antonio Palop¹, Carmen Sanmartín¹

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Selenium is an essential trace element for human health. There are some indicators that selenium in doses above the physiological requirement has biological effects and can be applied in the prevention and therapy of several diseases^[1]. Epidemiological studies have suggested that low blood selenium levels were associated with an increased incidence and mortality from various kinds of cancer^[2]. Some potential mechanisms have been proposed for organoselenium compounds in cancer chemoprevention: antiandrogen activity, antioxidant functions, DNA repair^[3]...

In order to continue with the researched work of our group^[4,5], the synthesis and biological evaluation of new quinazoline derivatives have been proposed, according to the general structures:



Cytotoxic activity of all the compounds has been evaluated in four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and two non-malignant cell lines (184B5 and BEAS). For all of the cell lines, the following cytotoxic parameters have been determined: LD₅₀, TGI y GI₅₀. Several compounds show some cytotoxic activity, being more potent than some of the drugs currently used in clinical trials, such as: etoposide and cis-platine.

REFERENCES

- Behne, D.; Alber, D.; Journal Trace Elements in Medicine and Biology; in press.
- Chen, Y.; Hall, M.; Graziano, J. H.; Slavkovich, V.; Van Geer, A.; Parvez, F.; Ahsan, H.; Cancer Epidemiology Biomarkers Prevention; 16: 207, **2007**.
- Sanmartín, C.; Plano, D.; Palop, J. A.; Mini Reviews in Medicinal Chemistry; 8: 120-131, **2008**.
- Cubedo, E.; Cordeu, L.; Bandres, E.; Rebollo, A.; Malumbres, R.; Sanmartín, C.; Font, M.; Palop, J. A.; García-Foncillas, J.; Cancer Biology and Therapy; 5:7, 850-859, **2006**.
- Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J. A.; Bioorganic and Medicinal Chemistry Letters; 17, 6853-6859, **2007**.

POSTER COMMUNICATIONS – PC.185

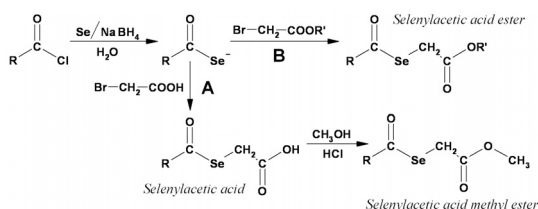
ESTERIFICATION IMPROVES BIOLOGICAL ACTIVITY OF SELENYLACETIC ACIDS

Enrique Domínguez^{1*}, Ylenia Baquedano¹, Esther Moreno¹, María Font², Daniel Plano¹, Alfonso Calvo³, Juan Antonio Palop¹, Carmen Sanmartín¹

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Selenium and its organic and inorganic derivatives fulfil a lot of biological functions and have been used in cancer prevention and chemotherapy¹. Several selenocompounds, like diphenyldiselenide² and Se-methylselenocysteine³, are under investigation because of their cytotoxic and anticarcinogenic properties. Due to these antecedents, our research group designed, synthesized and determined biological activity parameters of twenty-six novel selenocompounds with the general formula Ar/Het-CO-Se-CH₂-COOH.⁴ The five most active selenylacetic acids obtained had a good cytotoxicity activity in prostate cancer cell line (PC-3), but activity of the remaining ones was below our expectatives.

Consequently, we decided to study esterification, a logical structural modification of the previous compounds, and planned the chemical synthesis of carboxylic esters of selected selenylacetic acids. Methyl, *tert*-butyl and phenyl esters had been isolated and the compounds evaluated in PC-3 cancer cell line had, in general, better biological results than the corresponding acids.



Methyl esters chemical synthesis was carried out following the Fischer-Speier mechanism: the corresponding selenylacetic acid was solved in methanol and heated at boiling temperature in presence of hydrochloric acid (method A). Acids used as reagents are synthesized⁴ by reaction of the appropriate aryl or heteroaryl chloride with sodium hydrogen selenide in a first step and with α -bromoacetic acid in a second one. Non-methyl esters were obtained using a synthetic pathway similar to the method described above: in the alternative method B hydrogen selenide reacted with the pertinent α -bromoacetic acid ester instead of doing it with α -bromoacetic acid.

REFERENCES

1. Boosalis, M.G. *Nutr. Clin. Pract.* **2008**, 23(2): 152-160.
2. Rosa, R.M.; Picada, J.D.; Saffi, J.; Henriques, J.A.P. *Mu. Res.* **2007**, 628: 87-98.
3. Hurst, R.; Elliot, R.M.; Goldson, A.J.; Fainweather-Tait, S.J. *Cancer Lett.* **2008**, 269: 117-126.
4. Sanmartin, C.; Plano, D.; Domínguez, E.; Font, M.; Calvo, A.; Prior, C.; Encío, I.; Palop, J.A. *Molecules*, **2009**(14): 3313-3338.

POSTER COMMUNICATIONS – **PC.186**

A NEW CONCEPT FOR THE CONTROL OF PROTEASE ACTIVITY: SUBSTRATE-DEPENDENT MODULATION.

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IDE (Insulin-Degrading Enzyme) is an enzyme known for decades, yet remaining poorly understood. It is an ubiquitous, highly conserved Zinc metalloprotease that is involved in the clearance of many physiologically important peptides. There is a renewed interest for IDE since the last few years as both functional and genomic studies have placed IDE at the crossroads between type 2 diabetes and Alzheimer's disease. Indeed, amongst other peptides, insulin and β -amyloid are two cognate substrates of IDE. Moreover, the X-ray structure of IDE was solved by Tang et al. (1) in 2006. Initially, our project was to design inhibitors to study the role of IDE in cells and in normal and sick.

In the course of that project, we have discovered a series of drug-like modulators of IDE (2). X-ray analysis of ligand-enzyme complexes, revealed that unexpectedly these compounds are exosite ligands of IDE. Consistently with their peculiar binding mode, these compounds behave as inhibitors or activators of the enzyme in a substrate-dependent manner.

We present Structure-“Substrate-Profile”-Activity Relationships in the light of several X-ray structures.

At last some compounds were optimized for cell-permeation and results on several cell-based assays are presented and discussed.

The mode of action of our compounds opens new avenues both for the study of the function of IDE and for the design of therapeutic interventions.



Binding IDE in the exosite.

REFERENCES

1. Shen, Y., et al., Structures of human insulin degrading enzyme reveal a new substrate recognition mechanism, *Nature* 2006, 443,(7113), 870
2. Deprez-Poulain, R., et al. European Patent Ligands of Insulin Degrading Enzyme and their uses, March 2010.

POSTER COMMUNICATIONS – **PC.187**

ISOMERIC BENZOYLPIRROLYLACETIC ACIDS AS ALDOSE REDUCTASE INHIBITORS. AN IN SILICO STUDY

Vassilis J. Demopoulos

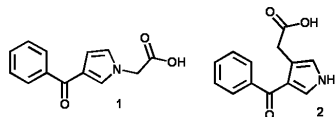
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Diabetes mellitus is a complex metabolic disorder and is one of the world's most common diseases and one of the biggest public health problems. The diabetic individual is prone to late onset complications that are largely responsible for the morbidity and mortality observed in the patients. Aldose reductase enzyme (AR, ALR2, E.C. 1.1.1.21) of the polyol metabolic pathway was first found to be implicated in the etiology of the secondary complications of diabetes. Aldose reductase inhibitors (ARIs) have therefore been noted as possible pharmacotherapeutic agents. Although several ARIs have progressed to the clinical trials' level, only one (Epalrestat) is currently on the market and only in

Japan. However, the inhibition of the polyol pathway is considered to be a promising approach to control diabetic complications.¹ In previous studies we have shown that (3-benzoylpyrrol-1-yl)acetic acid (1) is an active ARI in the micromolar range while its isomer, (4-benzoyl-1H-pyrrol-3-yl)acetic acid (2), is virtually inactive.^{2,3} In the present work the differences of 1 and 2 were studied via in silico methodologies. Thus, it was found that 1, in comparison with 2, exhibits a higher partitioning value (calculated logP), a higher acidity (calculated pKa), a lower molecular polar surface area (calculated TPSA), and a higher calculated van der Waals molecular surface area. It should be noted that previous reports have highlighted the importance of both lipophilicity and molecular surface area for ARI activity, while there is an anion binding pocket located in the active site of ALR2.^{1,4,5} Finally, the low energy structures of both compounds 1 and 2 were superimposed on the crystal structure of the known ARI zoporlestat. It was found that the derived RMS_(n=3) values were 2.33 and 5.01 respectively, indicating a significant better fit for compound 1 with zoporlestat. ALR2 is an enzyme showing pronounced induced-fit adaptation⁶, thus we propose that the results of the present work could comprise a useful tool for designing new and potent ARIs.

The following softwares, alone or in combination, were used in the above work: ClogP, Pallas, Molgen, MacSpartan and Hyperchem.

Scheme



REFERENCES

- Alexiou et. al., *Curr. Med. Chem.*, 16, 734-752 (2009),
- Demopoulos and Rekka, *J. Pharm. Sci.*, 84, 79-82 (1995).
- Nicolaou et. al., *J. Med. Chem.*, 47, 2706-2709 (2004),
- Alexiou et. al., *Bioorg. Med. Chem.*, 16, 3926-3932 (2008),
- Chrysanthakopoulos et. al., *QSAR Comb. Sci.*, 28, 551-560 (2009)
- Zentgraf et. Al., *Angew. Chem. Int. Ed. Engl.*, 46, 3575-3578 (2007).

POSTER COMMUNICATIONS – **PC.190**

ADENOSINE A₁ RECEPTOR: SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW PARTIAL AGONISTS

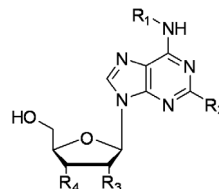
Diego Dal Ben¹, Michela Buccioni¹, Catia Lambertucci¹, Carmen Lammi¹, Gabriella Marucci¹, Sauro Vittori¹, Rosaria Volpini¹, Karl-Norbert Klotz², Gloria Cristalli¹

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Adenosine modulates cellular functions through the interaction with specific plasma membrane receptors classified in four subtypes as A₁, A_{2A}, A_{2B}, and A₃. Adenosine A₁ receptors (AA₁R) are present in cells throughout the body, and they mediate functions like slowing heart activity, reduction of lipolysis in adipose tissue, and inhibition of neuronal activity. Agonists of this subtype are useful in the treatment of acute and chronic disorders of heart rhythm such as atrial fibrillation, supraventricular tachycardia, and atrial flutter. Exposure to AA₁R partial agonists, which may selectively target a tissue and give functional response coupled to high AA₁R density, is preferred versus the use of full agonists due to the possibility to reduce unwanted side effects.¹

It is well known that the mono-substitution of adenosine at N⁶-position led to selective AA₁R ligands, while the lack of adenosine ribose hydroxyl groups produces the shift from full to partial activation of adenosine receptors.^{2,3}

Hence, a new series of N⁶-substituted 2'- or 3'-deoxyadenosine derivatives, bearing or not a chlorine atom in 2-position, have been synthesized. Preliminary biological results indicated that these compounds showed good affinity for AA₁R together with a sub maximal activation of the receptor.



R₁ = substituted (hetero)cycloalkyl
R₂ = H, Cl
R₃ / R₄ = H, OH

REFERENCES

- Dhalla, A. K.; Shryok, J. C.; Shreeniwas, R.; Belardinelli, L. Pharmacology and therapeutic applications of A₁ adenosine receptor ligands. *Curr. Top. Med. Chem.* **2003**, 3, 369-85.
- Vittori, S.; Lorenzen, A.; Stannek, C.; Costanzi, S.; Volpini, R.; Ijzerman, A. P.; von Frijtag Drabbe Kunzel, J. K.; Cristalli, G. N-Cycloalkyl derivatives of adenosine and 1-deazaadenosine as Agonists and Partial Agonists of the A₁ adenosine receptor. *J. Med. Chem.* **2000**, 43, 250-60.
- Cristalli, G.; Palle, V.; Zablocki, J. Partial and full agonists of A₁ adenosine receptors. US Patent 7163928, **2007**.

POSTER COMMUNICATIONS – **PC.191**

ADENOSINE RECEPTOR MODELING: THE IMPACT OF A_{2A} CRYSTAL STRUCTURE

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The evolution of the field of GPCRs modeling, including adenosine receptors (ARs), has depended on the availability of suitable receptor structural templates. For several years, modeling studies of GPCRs were based on the 3D structures of bacteriorhodopsin and bovine rhodopsin.¹ While the early models consisted basically on the transmembrane domains, the following studies led to improvement of the receptors structure by progressive insertion of intra- and extra-cellular domains and through refinement stages time by time more sophisticated. Rhodopsin-based modeling became the routine approach to develop adenosine receptor structural models. SAR and mutagenesis studies were not only target topics but also tools to refine theoretical models. The recent publication of the crystal structures of engineered adrenergic (beta1 and beta2)²⁻⁴ and adenosine A_{2A}R⁵ receptors confirm the helical arrangement similarity among the GPCRs, which can be explained in function of structural similarity of the interacting systems like G proteins or receptor kinases, but they also indicate a high variability in the GPCRs binding site leading to receptor diversity and ligand selectivity.

Comparison of rhodopsin- and adrenoceptor-based AR models with the AA_{2A}R crystal structure highlights deviations of modeling results from the experimental data, in particular in predicting the binding compound position and orientation, receptor extracellular domains fold, and the possible roles of water molecules in receptor-ligand interaction, but also it underlines the correctness of prediction of the role of key binding site residues in interaction with ligands. This data is supported by several docking and 3D-QSAR studies on ARs that allowed to design potent and often AR subtype selective ligands.

Even if it can be discussed the different utility for rational drug design of native and extensively engineered GPCRs, without doubt the AA_{2A}R crystal structure represents a breakthrough in X-ray field and provides a powerful tool to understand structural and functional properties of ARs.

REFERENCES

1. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000**, *289*, 739-45.
2. Warne, T.; Serrano-Vega, M. J.; Baker, J. G.; Moukhametzianov, R.; Edwards, P. C.; Henderson, R.; Leslie, A. G.; Tate, C. G.; Schertler, G. F. Structure of a β_1 -adrenergic G-protein-coupled receptor. *Nature* **2008**, *454*, 486-91.
3. Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258-65.
4. Hanson, M. A.; Cherezov, V.; Griffith, M. T.; Roth, C. B.; Jaakola, V. P.; Chien, E. Y.; Velasquez, J.; Kuhn, P.; Stevens, R. C. A specific cholesterol binding site is established by the 2.8 Å structure of the human β_2 -adrenergic receptor. *Structure* **2008**, *16*, 897-905.
5. Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y.; Lane, J. R.; Uzman, A. P.; Stevens, R. C. The 2.6 angstrom crystal structure of a human A_{2A} adenosine receptor bound to an antagonist. *Science* **2008**, *322*, 1211-7.

POSTER COMMUNICATIONS – PC.193

BIOLOGICAL ACTIVE PRENYLATED XANTHONES: RECENT PROGRESS IN SYNTHETIC METHODOLOGIES

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Xanthonic derivatives, namely prenylated xanthones have been reported to mediate some important biological activities, concerning a large variety of targets with therapeutic value [1-2]. This class of compounds has been found to exhibit interesting activities such as antibacterial, antifungal, PKC modulation and antitumor [1-2]. The presence of the prenyl groups becomes an important structural factor for the interaction of xanthones biological targets, taking into account the influence on the physico-chemical properties, including lipophilicity, and the overall stereochemistry of the molecule, which may create additional interactions [1]. On the other hand, the PKC isoenzymes are related to very important biological actions mainly in tumorigenesis. Clarification of the role of individual PKC isoforms in the cell has been difficult by the lack of selective PKC modulators [3].

In order to optimize the synthetic process to obtain compounds of this series and also to improve the yields, some new methodologies were followed, based on molecular modification strategies applied to 1,3-dihydroxy-2-methylxanthone (**1**) (Fig. 1) [4,5]. This compound was already evaluated as being active against the breast adenocarcinoma cell line (MCF-7) [4] as well as a PKC activator [6]. The experimental conditions involved microwave assisted organic synthesis (**1** via **A** to **2-3**; **3** via **B** to **4**) and heterogeneous catalysis using Amberlyst®15 as ion-exchange resin (**1** via **C** to **5-6**).

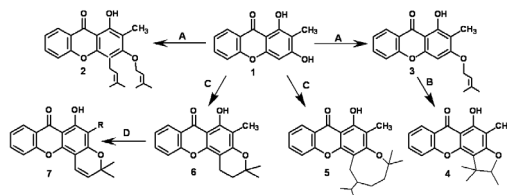


Figure 1:
A: Molecular Extension: Introduction of a lipidic chain
B: Molecular Rigidification: Introduction of an extra ring
C: Molecular Rigidification: Introduction of an extra ring
D: Molecular Rigidification: Inclusion of an insaturation

Compounds **2-4**, **6-7** were evaluated for their effect on the *in vitro* growth of three human tumor cell lines, MCF-7 (breast), NCI-H460 (non-small cell lung), SF-268 (central nervous system). Compound **5** is under study. It was also investigated the modulatory activity of some prenylated xanthones, on isoforms

α , β , δ , η and ζ of PKC. Xanthone **6** was found to exhibit a selective and potent growth inhibitory activity against the MCF-7 cell line, comparing with respective building block **1**, being able to become a new “hit” compound for this series. The results also showed that xanthone **1**, a non-selective PKC activator, gave rise to a new η -isoform-specific PKC inhibitor (**2**).

ACKNOWLEDGMENTS

FCT (I&D 4040/2007), FEDER, POCI for financial support.

REFERENCES

1. Pinto, M., Castanheiro, R., “Natural Prenylated Xanthenes: Chemistry and Biological Activities”, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, Índia, 2009, Chap. 17, pp.520-676.
2. Pinto, M., *et al.* *Curr. Med. Chem.* **2005**, *12*, 2517.
3. Webb, B., *et al.* *British J. Pharmacol.* **2000**, *130*, 1433.
4. Castanheiro, R., *et al.* *Bioorg. Med. Chem.* **2007**, *15*, 6080.
5. Pinto, M., Castanheiro, R. *Curr. Org. Chem.* **2009**, *13*(12), 1215.
6. Saraiva, L., *et al.* *Bioorg. Med. Chem.* **2002**, *10*, 3219.

POSTER COMMUNICATIONS – PC.194

PYRAZOLYLMETHYLCARBAMOYLMETHYL-PIPERAZINES AS CALCIUM CHANNEL BLOCKERS

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Calcium channels exist in nerve, muscle, and endocrine cells, and are responsible for a number of diseases. Of the subtypes of the calcium channels, we have been interested in the T-type channels that are known to be related to learning and memory, pain, epilepsy, etc. The first T-type calcium channel blocker, Mibefradil, was launched as an antihypertensive in 1997. The drug, however, was withdrawn in the next year due to drug-drug interaction.

Of many diseases related to the T-type calcium channels, we focused on neuropathic pain, and referring to the structure of Mibefradil and other CNS acting drugs, we designed pyrazole-containing structures that are expected to block T-type calcium channels selectively. A number of compounds were synthesized and evaluated for their biological properties.

In this presentation, we describe the synthesis of pyrazolyl-methylcarbamoylmethylpiperazines and their calcium channel blocking activities, along with other biological properties, as an attempt to find new drug candidates for neuropathic pain.

POSTER COMMUNICATIONS – PC.195

DISCOVERY OF SELECTIVE AND POTENTIAL T-TYPE CALCIUM CHANNEL MODULATORS: SYNTHESIS AND BIOLOGICAL EVALUATION OF PIPERAZINE ANALOGUES

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T-type calcium channels, also known as low-voltage-activated calcium channels play an important role in the regulation of neuronal excitability in the central and peripheral nervous system. Recent studies showed that modulation of T-type calcium channels involves several pathophysiological diseases such as epilepsy, pain, hypertension, congestive heart failure and cancer. Therefore, potent and selective inhibitors are requested to explore the fundamental function of T-type calcium channels in such disease states.

Based on three dimensional ligand-based pharmacophore model, we have designed and synthesized a series of new piperazine derivatives. Their inhibitory activities on T-type calcium channels have been evaluated using *in-vitro* assay against α_1G and α_1H subtypes by FDSS screening system. Furthermore, we have selected the compounds with high potencies and then analyzed their selectivities over hERG and N-type calcium channels as well as their stabilities in both rat plasma and human hepatic microsomes.

REFERENCES

1. S. J. Gu, J. K. Lee, A. N. Pae, H. J. Chung, H. Rhim, S. Y. Han, S.-J. Min, Y. S. Cho. *Bioorganic & Medicinal Chemistry Letters in press*.
2. Y. Oh, Y. J. Kim, S. H. Seo, J. K. Lee, H. Rhim, A. N. Pae, K.-S. Jeong, H. Choo, Y. S. Cho. *Bull. Korean Chem. Soc.* **2008**, *29*(10), 1881-1882.
3. H. S. Kim, Y. Kim, M. R. Doddareddy, S. H. Seo, H. Rhim, J. Tae, A. N. Pae, H. Choo, Y. S. Cho. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 476-481.

POSTER COMMUNICATIONS – PC.196

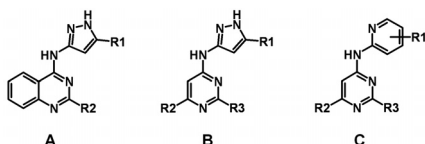
PKNB INHIBITORS AS ANTI-TUBERCULOSIS AGENTS

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Tuberculosis (TB) causes over 2 million deaths worldwide per year, and with the increasing prevalence of multi-drug resistant strains novel therapeutic approaches are urgently needed. PknB is one of 11 serine-threonine protein kinases found in *Mycobacterium tuberculosis* (*Mtb*) and has been shown to be essential for bacterial growth¹: therefore inhibition of PknB represents a potential therapeutic strategy for the treatment of TB.

A high-throughput screen of our compound collection against the PknB enzyme was conducted and hits were identified. The synthesis of analogues was performed and the structure-activity relationships around the template **A** (scheme 1) were investigated, which yielded inhibitors in the nanomolar range but with low efficacy against *Mtb* growth as determined in the Alamar Blue cell-based assay. Template modifications were made and potency against the enzyme was optimized around **B** and **C** (scheme 1) to give inhibitors in the low nanomolar range with improved physical properties. These compounds were shown to have Minimum inhibitory concentrations (MICs) in the low micromolar range against *Mtb*.



Scheme 1 Generalised structures of PknB inhibitors

REFERENCES

1. P. Fernandez et al., *Journal of Bacteriology*, **2006**, 188, 7778.

POSTER COMMUNICATIONS – PC.197

IDENTIFICATION OF ESSENTIAL AMINO ACID RESIDUES FOR PROTEIN-PROTEIN INTERACTION: APPLICATION OF A MICROWAVE PEPTIDE SYNTHESIZER

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In the framework of a collaborative project with different groups of the University of Leuven and the University of Edinburgh, a 13-mer peptide has been found active against the replication of the X (confidential) virus. In order to investigate the relevance of each residue in the protein-protein interaction responsible for the viral proliferation, different strategies have been applied such as Ala-scanning, fragmentation of the mother sequence, replacement with unnatural amino acid, cyclization.

Due to the relatively high number of peptides needed for this study and the complexity of the peptidic sequence, we decided to use a microwave peptide synthesizer, applying the Fmoc-strategy. This choice is due to the recent remarkable progress achieved in MASPOS (Microwave-Assisted Solid-Phase Organic Synthesis) which has led to a dramatic reduction of reaction times as well as to a higher purity of the products compared to conventional solid phase strategy. [1, 2, 3]

REFERENCES

1. Bacsa, B., Horváti, K., Bösze S., Andreae F. and Kappe C. O. *J. Org. Chem.*, **2008**, 73, 7532–7542.
2. Bacsa, B., Bösze S. and Kappe C. O. *J. Org. Chem.*, **2010**, 75 (6), 2103–2106.
3. Fara, M. A.; Diaz-Mochon, J. J.; Bradley, M. *Tetrahedron Lett.* **2006**, 47(6), 1011–1014.

POSTER COMMUNICATIONS – PC.198

SYNTHESIS AND BINDING AFFINITY OF NEW TETRALONE ANALOGUES OF HALOPERIDOL AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

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Schizophrenia is a complex disorder affecting approximately 1% of the population. For the treatment of this disease, classical (typical) neuroleptics such as haloperidol (**Fig. 1**) are currently used, but their use is associated with severe mechanism-related side effects, including induction of acute extrapyramidal symptoms (EPS), and they are ineffective against negative symptoms of schizophrenia [1].

The introduction of clozapine (**Fig. 1**) for treatment-resistant schizophrenia gave rise to a new group of atypical or non-classical antipsychotics which have no EPS and are effective against negative symptoms. These drugs exhibit potent antagonism at multiple receptor subtypes including serotonin and dopamine receptors, suggesting the implication of the serotonergic system in this pathology [2].

As part of our ongoing work to develop multi-target ligands for potential use as treatments for schizophrenia, we have studied the modulation of the butyrophenone system, with the aim of synthesizing a single molecule that can antagonize receptors of 5-HT₂ and D₂ families [3]. Therefore, we have obtained a variety of different butyrophenone analogues in which the rigidity of the haloperidol butyric chain was increased by its inclusion into a tetralone scaffold (**I**, **Fig.1**).

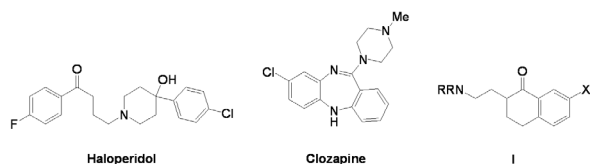


Figure 1

The synthetic route and the binding affinities of these new compounds on dopamine D₂ and serotonin 5-HT_{2A} and 5-HT_{2C} human receptors will be discussed in the communication.

ACKNOWLEDGMENTS

We thank the Spanish *Ministerio de Ciencia e Innovación* (Ref SAF2005-08025-C03 and SAF2009-13609-C04) for the financial support. L.C. is gratefully acknowledged to MICCIN for a predoctoral Fellowship (FPU).

REFERENCES

- Altar, A.; Martin, A. R.; Thurkauf, A. in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn.; Ed. D. J. Abraham, John Wiley & Sons, New Jersey, 2003, Vol. 6, p. 599.
- Marino, M. J.; Knutsen, L. J. S.; Williams, M. J. *Med. Chem.* **2008**, *51*, 1077.
- (a) Brea, J.; Rodrigo, J.; Carrieri, A.; Sanz, F.; Cadavid, M. I.; Enguix, M. J.; Villazon, M.; Mengod, G.; Caro, Y.; Masaguer, C. F.; Ravina, E.; Centeno, N. B.; Carotti, A.; Loza, M. I. *J. Med. Chem.* **2002**, *45*, 54. (b) Fontenla, J. A.; Osuna, J.; Rosa, E.; Castro, M. E.; Ferreira, T.; Loza, M. I.; Calleja, J. M.; Sanz, F.; Rodríguez, J.; Ravina, E.; Fueyo, J.; Masaguer, C. F.; Vidal, A.; de Ceballos, M. L. *J. Med. Chem.* **1994**, *37*, 2564. (c) Carro, L.; Ravina, E.; Domínguez, E.; Brea, J.; Loza, M. I.; Masaguer, C. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6059.

POSTER COMMUNICATIONS – PC.200

THE DISCOVERY OF CDP323, A NOVEL AND POTENT INHIBITOR OF THE INTEGRINS $\alpha 4 \beta 1$ (VLA-4) AND $\alpha 4 \beta 7$

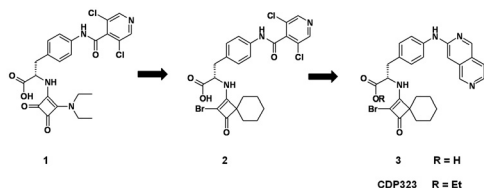
Julien Brown¹, Stuart Bailey¹, Stephen Brand¹, Ben de Candole¹, James Johnson¹, Paul Hale², Martyn Robinson², Tony Shock², Ailsa Webster², Lloyd King³, Ted Parton³, Tim Bourne⁴, Roly Foulkes⁴ and Neil Gozzard⁴

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The integrins are a family of heterodimeric cell-surface adhesion receptors which mediate trafficking of lymphocytes to sites of

inflammation. Substantial pharmacological evidence has implicated the integrins $\alpha 4\beta 1$ (VLA4) and $\alpha 4\beta 7$ in the pathogenesis of disorders including rheumatoid arthritis (RA), inflammatory bowel disease, asthma and multiple sclerosis (MS). As part of our anti-inflammatory programme we will describe the discovery of **CDP323**, a potential small molecule treatment for RA and MS.

The development of orally active $\alpha 4$ integrin antagonists, derived from the early peptide leads has been widely recognised as a key challenge in this field. We will discuss our progress starting from the previously reported¹ phenylalanine based squaric acid derivative **1**. As part of the optimisation process we evaluated the novel 3-amino-cyclobut-2-en-1-one scaffold as a versatile squaramide surrogate. Synthesis, SAR and *in vitro* profiling will be described. Optimisation of potency, *via* introduction of substituents at the 2 and 4 positions of the cyclobutenone, initially led to **2**. However, PK and oral bioavailability were markedly enhanced through replacement of the dichloropyridyl head group with a 2,7-naphthyridine leading to the wholly non-peptidic antagonist **3**. Prodrugs of **3** were designed leading to the ethyl ester, **CDP323**, as a development and clinical candidate. Compounds from this series are potent inhibitors of $\alpha 4\beta 7$ and $\alpha 4\beta 1$ dependent trafficking *in vivo* and significantly reduce clinical scores in a mouse collagen-induced arthritis model. **CDP323** has recently been evaluated in Phase II studies of MS.



REFERENCES

1. J.R. Porter et al, Bioorg. Med. Chem. Lett. **2002**, 12, 1015.

POSTER COMMUNICATIONS – PC.202

1-METHYL-3-PHENYLPYRROLIDINE AND 3-METHYL-1-PHENYL-3-AZABICYCLO[3.1.0]HEXANE AS MONOAMINE OXIDASE B SUBSTRATES

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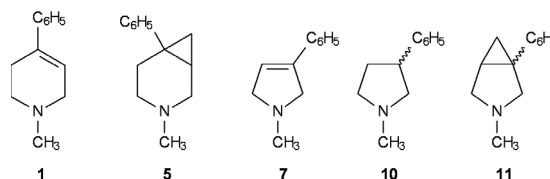
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It is well known that the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (**1**) acts as a substrate for monoamine oxidase B (MAO-B). The MAO-B substrate properties of the cyclic tertiary aminyl class of substrates are thought to be dependent on the presence of the allylic π -bond, the only known

exception being the 3,4-cyclopropyl analogue of MPTP, racemic 3-methyl-6-phenyl-3-azabicyclo[4.1.0]heptane (**5**). The π -bond characteristics of the cyclopropyl group may be responsible for the good substrate properties of **5**.

Another member of the cyclic tertiary allylaminyl class of MAO-B substrates, is the 5-membered ring MPTP analogue, 1-methyl-3-phenyl-3-pyrroline (**7**). In this study, we have synthesised and examined the interactions of the corresponding saturated pyrrolidinyl, [(±)-1-methyl-3-phenylpyrrolidine (**10**)] and 3,4-cyclopropyl, [(±)-3-methyl-1-phenyl-3-azabicyclo[3.1.0]hexane (**11**)], analogues of 1-methyl-3-phenyl-3-pyrroline with MAO-B.

The values of the steady-state kinetic parameters (K_m and V_{max}) for the baboon liver mitochondrial MAO-B catalyzed oxidation of the test substrates **10** and **11** were determined by measuring the initial rates of H_2O_2 production. Substrate **11** ($K_m = 148 \mu M$; $V_{max} = 16.9 \text{ nmol/min-mg protein}$) was shown to act as a good substrate for baboon liver MAO-B. An unexpected outcome was that the saturated pyrrolidinyl analogue **10**, also proved to be a MAO-B substrate ($K_m = 234 \mu M$; $V_{max} = 8.37 \text{ nmol/min-mg protein}$). This result suggests that the presence of an allylic π -bond or 3,4-cyclopropyl-fused system is not a prerequisite for cyclic tertiary amines to display MAO-B substrate properties and provides additional opportunities to map the nature of the interactions of MAO-B with tertiary aminyl substrates.



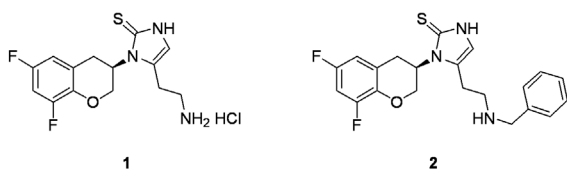
POSTER COMMUNICATIONS – PC.203

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL, N-BENZYLAMINOETHYL DERIVATIVES OF CHROMANYL IMIDAZOLETHIONE-BASED INHIBITORS OF DOPAMINE β -HYDROXYLASE

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BIA 5-453 (**1**), a peripherally-selective inhibitor of dopamine β -hydroxylase (DBH), is currently under clinical evaluation for the treatment of chronic heart failure and hypertension [1,2]. In the course of a search for peripherally-selective DBH inhibitors, novel N-benzyl derivatives **2** have been prepared and their biological activity has been studied [3].



REFERENCES

1. Beliaev, A.; Ferreira, H.; Learmonth, D. A.; Soares-da-Silva, P. Dopamine β -Monooxygenase – Mechanism, Substrates and Inhibitors. *Current Enzyme Inhibition* **2009**, 5, 27-43.
2. Beliaev, A.; Learmonth, D.A.; Soares-da-Silva, P. Synthesis and biological evaluation of novel, peripherally selective chromanyl imidazolethione-based inhibitors of dopamine β -hydroxylase. *J. Med. Chem.*, **2006**, 49, 1191-1197.
3. Soares-da-Silva, P.; Learmonth, D.A.; Beliaev, A. 1,3-Dihydroimidazole-2-thione derivatives as inhibitors of dopamine β -hydroxylase. PCT Int. Appl. WO2008/136695A1, 13 Nov 2008.

POSTER COMMUNICATIONS – PC.204

BIFUNCTIONAL HYBRIDES OF NITROGEN MUSTARDS WITH PEPTIDE “ADDRESS” ATTACHED TO THE 1,3,5-TRIAZINE SCAFFOLD

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Classical nitrogen mustards (NM) drugs do not operate selectively, causing high levels of inadvertent DNA damage in normal cells, toxic and mutagenic side effects, and in some cases, leading to secondary malignancies. The biological activity of these compounds is typically attributed to their ability to induce DNA-DNA cross-links, although, nitrogen mustards are able to produce also a variety of other lesions, including DNA-protein cross-links. The therapeutic efficacy of NM is limited by deactivation through reaction with nucleophiles such as water, proteins and thiols, leading to low potency and toxic side-effects. One of the most promising research pathway for improving the selectivity of cytotoxic agents involves formation of various nitrogen mustard conjugates which can target them to selected tumors.

Herein, in order to improve therapeutic efficacy of N-mustard, a series of bifunctional analogues were prepared by combination of alkylating fragments with peptide “address”, using 1,3,5-triazine [1] as scaffold molecule, expecting enhanced selectivity of modified structure. The hybrids were prepared on the Wang resin, starting with synthesis of peptide fragment followed by attachment of 2,4-dichloro-6-substituted-1,3,5-triazine scaffold to the N-terminal amino group. The effect of deactivation of chlorine atoms in triazine ring caused by the conjugation of nitrogen lone pair of electrons of amino group was diminished by using excessive amounts of

triazine and unpoling of substituent effect [2]. The final transformation into hybrid NM structure was done directly on the resin by treatment with excess of DABCO, followed by spontaneous decomposition of intermediate quaternary ammonium chloride.

ACKNOWLEDGMENT

This studies were supported by the grant N N405 355537 donated by M.S.H.E.

REFERENCES

1. Just recently, it has been reported that several relatively simple melamine analogues show antitumor activity as inhibitors of enzymes engaged in the cell proliferation cycle (topoisomerase, telomerase, histone deacetylase, phosphatidylinositol kinase, protein kinase CK2 etc.).
2. (a) Kolesińska, B.; Kamiński Z.J.; Wąsikowska, K.; Drozdowska, D. „A New Method of Nitrogen Mustards Synthesis”, Patent Appl. nr **P-385126** from 08. 05. 2008; (b) Kolesińska, B. Kamiński, Z.J. *Tetrahedron*, **65** (2009) 3573.

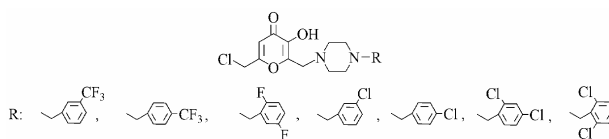
POSTER COMMUNICATIONS – PC.205

SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITIES OF CHLOROKOJIC ACID DERIVATIVES

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The most important part of the treatment program for epilepsy is anticonvulsant drugs. The search for antiepileptic compounds with more selective activity and lower toxicity is an area under intensive investigation. The development of antimicrobial agents to treat infections has been one of the most notable medical achievements of the past century. However these advances in medical care are threatened by resistance. Given almost all of the major classes of antibiotics have encountered resistance in clinical application. For these reasons, the research of new antimicrobial agents with novel modes of action represents a main target in chemotherapy. In our previous studies, Mannich bases of kojic acid, chlorokojic acid and allomaltol derivatives were synthesized. Their anticonvulsant, antiviral and antimicrobial activities were evaluated¹⁻³.



In this study, seven new 6-chloromethyl-3-hydroxy-2-substituted 4H-pyran-4-one derivatives were synthesized. The structures

of the compounds were identified by IR, ^1H -NMR, ESI-MS and elemental analysis data. Anticonvulsant activities were examined by maximal electroshock and subcutaneous Metrazol-induced seizure tests. Neurotoxicity was determined by the rotorod test. All these tests were performed in accordance with the procedures of the Antiepileptic Drug Development program⁴. Compound bearing 2,5-difluorobenzyl moiety showed the highest anticonvulsant activity at all doses at 4h against scMet-induced seizure test. Compounds also screened for their *in-vitro* antibacterial and antifungal activities. Standard strains of *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecalis*, and *B. subtilis* with their pathogens from clinical isolates as well as fungi such as *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* were used to ascertain the antimicrobial activity whereas ampicilline, gentamicine, levofloxacin, ketoconazole, and fluconazole was used as the standard positive control for antibacterial and antifungal activities, respectively. Microbroth dilution method recommended by Clinical and Laboratory Standards Institute is used. According to the results, 3- and 4-trifluorobenzyl derivatives showed significant antibacterial activity against Gram-negative (MIC: 8-16 $\mu\text{g/ml}$) and Gram-positive (MIC: 4-16 $\mu\text{g/ml}$), respectively. Important outcome of the exhaustive screening of all the new candidates in the present experiment was that the introduction of piperazine containing Mannich groups at the 6-position of chlorokojic acid nucleus significantly improved the biological activity. This study is supported by *H.U. Scientific Research Fond*.

REFERENCES

1. Aytemir MD, Çalıř Ü, Özalp M, Arch Pharm Chem Life Sci, 337, 281-288, 2004.
2. Aytemir MD, Çalıř Ü, Arch Pharm Chem Life Sci, 343, 173-181, 2010.
3. Aytemir MD, Özçelik B, Med Chem Res, DOI 10.1007/s00044-010-9338-x.
4. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Epilepsia 19, 409-428, 1978.

POSTER COMMUNICATIONS – PC.207

BZM055, A PET/SPECT TRACER CANDIDATE FOR STUDYING FTY720 BRAIN DISTRIBUTION AND MYELIN IMAGING

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FTY720 (fingolimod, Gilenia®) is a sphingosine 1-phosphate (S1P) receptor modulator that showed significant improvement in relapse rate and MRI outcomes after oral administration to multiple sclerosis (MS) patients.^[1] This drug prevents lymphocyte egress from lymph nodes,^[2] leading to a reduced infiltration

of lymphocytes into the central nervous system (CNS).^[3] Preclinical evidence also suggests that FTY720 may provide additional neuroprotection to the CNS via modulation of cerebral S1P receptors.^[4]

To further study the therapeutic action of FTY720, it would therefore be useful to quantify its pharmacokinetics and organ distribution in patients. As it has already been shown in rodents that FTY720 accumulates in myelin sheaths,^[5] we reasoned that an imaging agent based on this drug might also allow to image myelin and study disease progression, in addition to following the pharmacokinetics of FTY720 in the brain of MS patients.

FTY720 does not contain any atom whose Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) radioisotope would have a half-life compatible with its pharmacokinetic properties. It is therefore not possible to study this drug with such imaging techniques without modifying it slightly. With this in mind, we defined the criteria that would make a radiolabeled derivative of FTY720 an adequate surrogate of the drug, mimicking its pharmacokinetics and organ distribution, but also its affinity and selectivity for S1P receptors, as well as having similar overall physicochemical properties.

Furthermore, FTY720 itself is inactive at S1P receptors, but is phosphorylated *in vivo* by sphingosine kinase-2 to (S)-FTY720-phosphate, which is the active moiety binding to S1P receptors. Any derivative mimicking FTY720 should hence also be a substrate of SphK2, and be phosphorylated in a similar manner. Besides measuring phosphorylation rates of tracer candidates, we synthesized their phosphate analogues to enable the comparison of their affinity for S1P receptors, and adequately compare their overall properties to (S)-FTY720-phosphate.

From all candidate tracers profiled according to these criteria, we selected BZM055 for PET/SPECT radiolabeling and further characterization, as it is the closest mimic of the drug. Its profile is described in the poster.

REFERENCES

1. L. Kappos et al. N. Engl. J. Med. 2010, 362(5), 387.
2. a) M. Matloubian et al. Nature 2004, 427(6972), 355; b) S. Mandala et al. Science 2002, 296(5566), 346.
3. a) A. Compston et al. Lancet 2008, 372(9648), 1502; b) I. Bartholomaeus et al. Nature 2009, 462 (7269), 94; c) V. Brinkmann. Pharmacol. Ther. 2007, 115(1), 84; d) S.J. Jackson et al. Neurology 2009, 72 (Suppl. 3), A377 (P308.047); e) V.E. Miron et al. Ann. Neurol. 2008, 63(1), 61-71; f) Coetho RP et al. J. Pharmacol. Exp. Ther. 2007, 323(2), 626-635; g) C. Barske et al. Neurology 2008, 70 (11 suppl 1)
4. V. Brinkmann, Br. J. Pharmacol. 2009, 158(5), 1173-1182.
5. C.A. Foster et al. J. Pharmacol. Exp. Ther. 2007, 323(2), 469-475

POSTER COMMUNICATIONS – PC.208

IN VITRO AND IN VIVO EFFECTIVENESS OF NEUROPROTECTIVE L-GLUTAMIC ACID DERIVATIVES FOR THE POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE¹

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Solid phase synthesis was employed to obtain a series of L-glutamic acid derivatives,^{1,2} which exhibit attractive *in vitro* and *in vivo* biological activities for the potential treatment of Alzheimer's disease. At submicromolar concentrations, they efficiently inhibit acetylcholinesterase (AChE), displace propidium from the peripheral anionic site of the AChE, and protect neurons against damage caused by oxidative stress, okadaic acid, calcium overload, and beta-amyloid peptide (Aβeta). They show no toxicity and could be able to penetrate into the central nervous system, according to an *in vitro* blood-brain barrier assay.³ A selected glutamic acid derivative shows neuroprotection against oxygen-glucose deprivation in rat hippocampal slices. In animal models, this L-glutamic acid derivative shows neuroprotection in mice subjected to a focal cerebral ischemia by photothrombosis or to an intrahippocampal injection of kainate. Consequently, it is expected that these multifunctional compounds could be able to improve cognitive impairment, diminish the oxidative damage caused by mitochondrial free radicals, and delay the degenerative process related to the excessive deposition of Aβeta in patients suffering Alzheimer's disease.

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REFERENCES

- Conde, S.; Rodríguez-Franco, M. I.; Arce, M. P.; González-Muñoz, G. C.; Villarroya, M.; López M. G.; García, A. G. Novel dicarboxylic amino acid derivatives and the use thereof in the treatment of neurodegenerative diseases. PCT/ES2008/070221; WO 2009/074706 A1.
- Arce, M. P.; Rodríguez-Franco, M. I.; González-Muñoz, G. C.; Pérez, C.; López, B.; Villarroya, M.; G. López M.; García, A. G.; Conde, S. Neuroprotective and cholinergic properties of multifunctional glutamic acid derivatives for the treatment of Alzheimer's disease. *J. Med. Chem.*, **2009**, 52, 7249–7257.
- Fernández-Bachiller, M. I.; Pérez, C.; Campillo, N. E.; Páez, J. A.; González-Muñoz, G. C.; Usán, P.; García-Palomero, E.; López, M. G.;

Villarroya, M.; García, A. G.; Martínez, A.; Rodríguez-Franco, M. I. Tacrine – Melatonin Hybrids as Multifunctional Agents for Alzheimer's Disease, with Cholinergic, Antioxidant, and Neuroprotective Properties. *ChemMedChem* **2009**, 4, 828-841.

POSTER COMMUNICATIONS – PC.209

SYNTHESIS OF NOVEL ANTIMITOTIC ANALOGUES OF COMBRETASTATIN

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Combretastatins are natural products with antimitotic activity. They act by binding to tubulin at the colchicine site. One of the combretastatins problems is their low aqueous solubility. To improve this solubility we have synthesized and evaluated analogues with a pyridine moiety¹ replacing the trimethoxyphenyl ring.

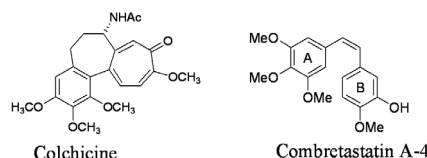


Figure 1. Structure of colchicine and combretastatin A-4.

New pyridine combretastatins have been synthesized by means of Wittig reaction between the corresponding phosphonium salt and 2,6-dimethoxypyridine-4-carbaldehyde, which was synthesized from citrazinic acid in four steps. In order to test the capability of the methoxylated pyridine ring to substitute for the A or B rings of combretastatin, we have combined it with either a 2-naphthyl², or a p-methoxyphenyl, or a 3,4,5-trimethoxyphenyl ring. Dihydrocombretastatins have also been obtained by hydrogenation of the bridge double bonds.

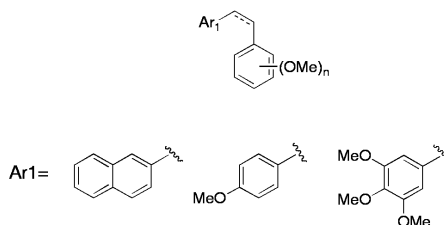


Figure 2. Structure of the synthesized analogues

These compounds have been assayed for antimitotic activity but none of them has shown higher potency in tubulin polymerization inhibition than the 3,4,5-trimethoxyphenyl combretastatin analogues, which indicates that it seems to be necessary for the activity of these compounds.

ACKNOWLEDGMENTS

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REFERENCES

1. Lozano, M. Aramburu, L. Degree Dissertations. University of Salamanca, 2007, 2009.
2. Maya, A. B.; Pérez-Melero, C.; Mateo, C.; Alonso, D; Fernández, J. L.; Gajate, C.; Mollinedo, F; Peláez, R; Caballero, E.; Medarde, M. Further Naphthylcombretastatins. An Investigation on the Role of the Naphthalene Moiety. *J. Med. Chem.* **2005**, 48, 556-568.

POSTER COMMUNICATIONS – PC.210

SYNTHESIS, BIOLOGICAL ACTIVITY, AND DOCKING STUDIES OF NOVEL ACETAMIDINES AS SELECTIVE MODULATORS OF NITRIC OXIDE SYNTHASE

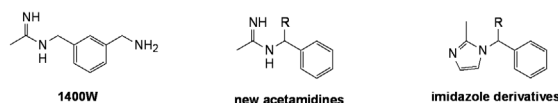
Rosa Amoroso, Alessandra Ammazalorso, Barbara De Filippis, Maria Luigia Fantacuzzi, Letizia Giampietro, Fabio Lannutti, Cristina Maccallini, Simona Masella, Antonia Patruno, Nazzareno Re

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Nitric oxide (NO) is an important mediator involved in the regulation of many physiological and pathological processes. [1] The formation of NO is catalyzed by the enzyme nitric oxide synthase (NOS) via the NADPH- and O₂-dependent oxidation of L-arginine. Three distinct isoforms of NOS have been identified: the constitutive endothelial (eNOS) and neuronal (nNOS), predominantly expressed in the vascular endothelium and in the nervous system, respectively, and the inducible (iNOS), which generates high levels of NO that modulates inflammation through multiple pathways and plays an important role in the regulation of immune reactions. When highly regulated, the production of NO is beneficial to the host organism. It is, however, believed that a number of disease states arise from excessive or inappropriate production of NO, including cerebral ischemia, septic shock, and arthritis. It has been proposed that overexpression of iNOS contributes to the progression of inflammatory diseases such as rheumatoid arthritis and osteoarthritis. [2] Moreover, there is evidence that also NO overproduction by nNOS plays a key role in these pathologies, and recently published studies reported promising results from chronic inhibition of nNOS as an adjunct therapeutic strategy for motor nerve repair. [3] Selective inhibition of iNOS and nNOS would be a useful therapy for such diseases. [4]

Recently, we have synthesized and evaluated a series of acetamides structurally related to N-(3-(aminomethyl)benzyl)acetamide (1400W); among them, compounds characterized by removal of the 3-aminomethyl group and addition of a methyl or an ethyl group on the benzylic carbon connected to

the acetamide nitrogen, showed good inhibitory potencies and high selectivities toward iNOS. [5] Based on the obtained results, other acetamides and imidazole derivatives were synthesized and biologically evaluated, and a docking study was also performed to shed light on the effects of the structural modifications on the interaction with the NOS.



REFERENCES

1. R. Bruckdorfer. *Molecular Aspects of Medicine*, **2005**, 26, 3-31
2. P. Ghafourifar, E. Cadenas. *Trends in Pharmacological Sciences*, **2005**, 26, 190-195.
3. L. Zhou, D.-Y. Zhu, *Nitric Oxide - Biology and Chemistry*, **2009**, 20, 223-230.
4. R. Domenico, *Current Pharmaceutical Design*, **2004**, 10, 1667-1676.
5. C. Maccallini, A. Patruno, N. Besker, J. I. Ali, A. Ammazalorso, B. De Filippis, S. Franceschelli, L. Giampietro, M. Pesce, M. Reale, M. L. Tricca, N. Re, M. Felaco, R. Amoroso. *Journal of Medicinal Chemistry*, **2009**, 52, 1481-1485.

POSTER COMMUNICATIONS – PC.211

SYNTHESIS AND EVALUATION OF NEW 1-BENZOYLPIPERIDINES

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Phenstatins are cytotoxic compounds that bind tubulin at the colchicine site. Although they are more polar than combretastatins, their water solubility can still be improved. As trimethoxyphenyl ring is considered necessary for keeping the cytotoxic and antimitotic potency, we have replaced the other aromatic ring by modified piperidine rings in order to increase the solubility.

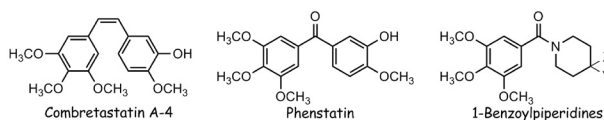


Figure 1. Structure of combretastatins, phenstatins and benzoylpiperidines.

New 1-benzoylpiperidines have been obtained by amide bond formation among the corresponding aromatic acids (3,4,5-trimethoxybenzoic acid, 2,3,4-trimethoxybenzoic acid, 2-(3,4,5-trimethoxyphenyl)acetic acid, 3,4,5-trimethoxycycanic acid) and different piperidines or tetrahydrocarbolines¹.

The ^1H and ^{13}C NMR signals of the nitrogenated methylenes of some piperidines are broadened or missing due to hindered rotation of the amide bond in the NMR time scale.

The activity of these new compounds has been tested by performing the “*in vitro*” tubulin polymerization inhibitory and cytotoxic assays.

In addition, docking studies at the tubulin colchicine site have been carried out to compare the rings disposition of the 1-benzoylpiperidines with respect to the podophyllotoxin pose at the site.

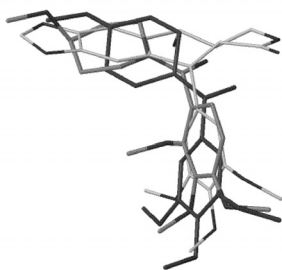


Figure 2. Superposition of podophyllotoxin (green carbons), (4-methoxypiperidin-1-yl)(3,4,5-trimethoxyphenyl) methanone (pink carbons) and (4-methoxypiperidin-1-yl)(2,3,4-trimethoxyphenyl) methanone (grey carbons).

ACKNOWLEDGMENTS

Financial support came from Spanish MCINNSAF 2008-04242 and Junta de Castilla y León SA067A09.

REFERENCES

1. Rodríguez, M., Master's dissertation, University of Salamanca, 2010.

POSTER COMMUNICATIONS – PC.212

A THEORETICAL STUDY OF A FAMILY OF 2-BENZYL-3-METHYLQUINOXALINE 1,4-DI-*N*-OXIDE DERIVATIVES

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The quinoxaline derivatives are a class of compounds that show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, and insecticidal). The oxidation of both nitrogens of this heterocyclic system, needed for obtaining quinoxaline 1,4-di-*N*-oxide derivatives, greatly increases the number of biological properties [1]. In fact, recent studies have demonstrated that quinoxaline 1,4-di-*N*-oxides are endowed with antimycobacterial, antiprotozoal, and anticandida activities as well as mutagenic properties, depending on specific chemical features [1].

In our continuing efforts to find new compounds with therapeutic activities, a series of 2-benzyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives was proposed, synthesized and published [2]. This work recorded the IR and ^1H -NMR spectra of the new species as part of their characterization studies, but no reference to structural parameters was given.

In order to further describe the chemistry of aforementioned compounds, hybrid density functional calculations were performed on them, following a similar methodology as previously described [3]. The conformational space of each one of the ten 2-benzyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives analyzed was scanned using a classical molecular dynamics module of the HyperChem package for the simulation (MM+ Molecular Mechanics Force Field). The 40 lowest-energy molecular conformations of each molecule obtained showed total energies that were mostly grouped, as evidenced by their flat (quinoxaline) structures. These conformations were subjected to further geometry optimization in Gaussian03 program using the density functional theory of B3LYP/6-31G(d,p), and in this way, the most stable conformation resulting from each derivative is selected. The harmonic vibrational frequencies were calculated using the same level of theory. In addition, the isotropic chemical shifts for hydrogen atoms were also calculated, with the isotropic magnetic shielding tensor obtained at the B3LYP/6-311+G(2d,p) level.

In brief, optimized geometries, harmonic vibrational frequencies, and ^1H chemical shifts were reported and compared with experimental data when available. The systematic-theoretical study carried out enabled us to obtain geometric data in quinoxaline 1,4-di-*N*-oxide derivatives that did not pose experimental values, and suggested the preferential conformations that these biological systems would adopt during their interaction with the substrate.

REFERENCES

1. Carta, A.; Corona, P.; Loriga, M., *Curr. Med. Chem.* **2005**, 12(19): 2259-2272.
2. Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A., *Molecules*. **2008**, 13(1): 86-95.
3. Pis Diez, R.; Duchowicz, P.R.; Castañeta, H.; Castro, E.A.; Fernández, F.M.; Albesa, A.G., *J. Mol. Graphics Model.* **2006**, 25: 487-494.

POSTER COMMUNICATIONS – **PC.214**THE LIMITATIONS OF DATA SETS IN
PREDICTION OF DRUG SOLUBILITY

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Drug candidates with poor aqueous solubility run a higher risk of late-stage development failures. The prospect of enabling early screening of combinatorial libraries for such inadequately soluble compounds has prompted many researchers to pursue the development of an *in silico* model for the prediction of solubility, yielding numerous models in the past decade. When challenged with diverse drug structures, however, the predictive capability of the majority of reported models diminishes considerably¹. It has been recognized that the accuracy of experimental data used in model training can be the main limiting factor in achieving good prediction reliability^{2,3}.

For the investigation of the impact of experimental data accuracy on model training, three data sets were used in our study: Data Set 1 (n=319) consisted of solubility values carefully compiled from various literature sources; Data Set 2 (n=319) that was created by substituting solubility values for 28 compounds from Data Set 1 with those determined under standardized experimental conditions; and Data Set 3 (n=375) which was created by expanding Data Set 2 with 56 additional compounds for which uniform experimental data were obtained using the same standardized protocol. All structures were optimized using the AM1 semi-empirical method and then were used for the calculation of a large number of molecular descriptors in CODESSA software. Descriptor selection was performed using the heuristic method which was also applied for establishing one-parameter and multiparameter correlations.

The significance of the selected descriptors was then compared between the three data sets. Constitutional, electrostatic and quantum-chemical (QM) descriptors appeared to be the most significant for drug solubility in each of the data sets. Interpretability of descriptors was found to be favourable: constitutional descriptors accounted for the overall hydrophobicity and aromaticity of the molecule while the electrostatic and QM descriptors were derived from polar surface areas, relative electrostatic charges and H-bond donor/acceptor surface areas, reflecting the presumed solvation mechanism. In one-parameter correlation, the order of significance of the selected descriptors was not largely affected by the changes in experimental data, but there was an overall improvement in individual descriptor correlations found for Data Set 3. When considering the established multiparameter equations for the three data sets, reflecting actual model development more closely, significant differences were observed. The selection of most significant descriptors have been different for three Data sets, and correlation coefficients in multiparameter equations increased with percentage of uniform experimental data used.

Despite significant advances made in developing new modelling techniques, aqueous solubility remains a difficult property to predict. In this work, we have shown that the usage of high-quality, uniformly determined experimental solubility data for drug-like compounds can significantly improve the statistical performance of prospective models. Our findings are in agreement with the growing consensus that improving prediction reliability will require the use of diverse *drug-like* sets with more accurate data.

REFERENCES

1. J. Taskinen and U. Norinder, in *Cprehensive Medicinal Chemistry II* (vol. 5), Elsevier, 2007, p. 627
2. C. A. S. Bergström, *Basic Clin. Pharmacol. Toxicol.* 96 (2005) 156
3. B. Fallner and P. Ertl, *Adv. Drug Delivery Rev.* 59 (2007) 533

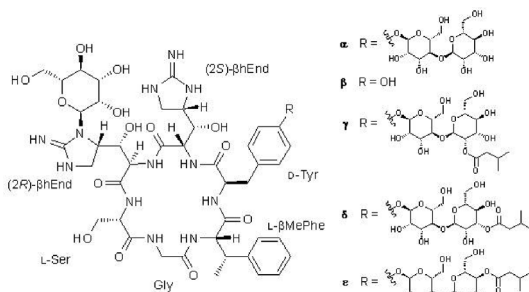
POSTER COMMUNICATIONS – PC.215

TOWARDS THE TOTAL SYNTHESIS OF MANNOPEPTIMYCINS

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Increasing incidence of resistance against therapeutically used antibiotics makes the development of new agents for the treatment of bacterial infections an urgent need. The Manno-peptimycins (MPs), a group of lipoglycopeptides, represent a promising lead-structure for such an undertaking. Some members of this family exhibit an extraordinary activity against various resistant bacterial strains.^[1] Preliminary studies have shown that their mode of action is the inhibition of peptidoglycan biosynthesis by selectively binding to Lipid II and thereby inhibiting its assembly.



Structure of the Manno-peptimycins α - ϵ .

The cyclic hexapeptide core of the MPs contains the unprecedented C2-epimeric amino acids (3S,4S)- β -hydroxy-enduracidines (β hEnd).

Additionally, the (2R)- β hEnd carries an α -linked mannose residue on its C4 nitrogen. The structures of the natural MPs differ in their modification of the tyrosine side-chain. All active compounds (MP γ - ϵ) carry a dimannose residue that is esterified with isovaleric acid.

New and convenient syntheses for both β hEnds were developed^[2] and introduction of the mannosides can be achieved in a high yielding and synthetically useful manner. With those building blocks to hand formation of the hexapeptide and its cyclization can be pursued. This would constitute the first total synthesis of a member of the MP family.

A reliable and convenient chemical synthesis of this promising new lead structure should enable us to gain a deeper insight into the mode of action of the MPs. We plan to vary selected structural elements in order to determine the accompanying changes in antibacterial activity (structure activity relationship). At the same time, investigation of a putative Lipid II/MP complex utilising 2D-nmr techniques should allow the direct observation of potential interactions on a molecular level. By combining these two approaches, Lipid II complexation of MP derivatives may be related to their in vitro activities in MIC assays.

REFERENCES

1. H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen, G. T. Carter, *J. Am. Chem. Soc.* **2002**, 124, 9729–9736
2. C. J. Schwörer, M. Oberthür, *Eur. J. Org. Chem.* **2009**, 35, 6129–6139.

POSTER COMMUNICATIONS – PC.216

HIGHLY POTENT AND SELECTIVE INHIBITORS OF 17 β -HYDROXYSTEROID DEHYDROGENASE TYPE 1 (17 β -HSD1) NOVEL, POTENTIAL THERAPEUTICS FOR BREAST CANCER AND ENDOMETRIOSIS

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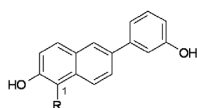
Breast cancer is the most frequent cancer type among women and the most common cause of cancer death. Endometriosis affects approximately 20 % of the female population and can lead to infertility. Both endometriosis and a high percentage of the breast tumours are estrogen-dependent, i.e. growth and proliferation are stimulated by estrogens. Present endocrine therapies - aromatase inhibitors, GnRH-analogues or anti-estrogens - result in a radical and unspecific reduction of estrogenic effects throughout the body leading to severe side-effects like osteoporosis or induction of endometrium carcinomas.

17 β -HSD1 catalyses the reduction of the weak estrogen estrone (E1) to the highly potent estradiol (E2). The enzyme is expressed in different organs like ovaries, breast, endometrium and is pres-

ent in breast cancer tissue as well as in endometriosis. Inhibition of 17 β -HSD1 could selectively reduce the E2-level in specific tissues thus allowing for a novel, targeted approach in the treatment of these diseases resulting in less side-effects compared to established therapies.

As 17 β -HSD2 catalyses the reverse reaction – decreasing the level of active E2 – it should not be affected by inhibitors of the type 1 enzyme. Furthermore, to avoid intrinsic estrogenic effects, 17 β -HSD1 inhibitors should not bind to the estrogen receptors (ERs) α and β .

The combination of a structure- and ligand-based drug design approach led to the development of the previously described steroidomimetics hydroxyphenylnaphthols¹⁻³ as potential inhibitors of the target enzyme. Structural optimisations have been performed by introduction of substituents in the 1-position of the naphthalene core (compound **A**) and led to the identification of new highly potent inhibitors of 17 β -HSD1 with IC₅₀ values in the low nanomolar range displaying good selectivity toward 17 β -HSD2 and ERs α and β .



Compound **A**

REFERENCES

1. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzter, L.; Scherer, C.; Müller Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J Med Chem.* **2008**, *51*, 2158-2169.
2. Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. *J Med Chem.* **2008**, *51*, 4685-4698.
3. Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. *Mol Cell Endocrinol.* **2009**, *301*, 205-211.

POSTER COMMUNICATIONS – PC.218

THE DISCOVERY OF AZD5672, AN ORALLY AVAILABLE CCR5 ANTAGONIST FOR RHEUMATOID ARTHRITIS

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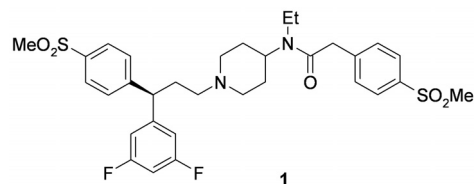
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The chemokine receptor CCR5 has been proposed to be important in the pathogenesis of rheumatoid arthritis (RA).^{1,2} Here we describe the discovery of a series of potent, selective antagonists

of the human CCR5 receptor and its optimisation to the oral clinical candidate AZD5672.

Previous investigations led to the discovery of the pre-clinical candidate **1**. While acceptable in other aspects, compound **1** showed moderate inhibition of the hERG ion channel which is associated with a risk of adverse cardiac events. Therefore subsequent optimisation efforts focused on reducing the activity at the hERG channel while maintaining the excellent potency, selectivity and oral PK properties of compound **1**. Exploration of a related chemical series achieved this goal with the identification of AZD5672. Further profiling of AZD5672 in healthy human volunteers demonstrated it had properties suitable for testing of the clinical hypothesis in RA patients in a Phase IIb clinical trial. The identification of a suitable physical form and improvements to the synthetic route to facilitate kilogram-scale production of AZD5672 will also be described.⁴



REFERENCES

1. Haringman, J.J.; Smeets, T.J.M.; Reinders-Blankert, P.; Tak, P.P. Chemokine and chemokine receptor expression in paired peripheral blood mononuclear cells and synovial tissue of patients with rheumatoid arthritis, osteoarthritis, and reactive arthritis. *Ann. Rheum. Dis.* (2006); *65*: 294-300.
2. Katschke, K.J. Jr.; Rottman, J.B.; Ruth, J.H.; Qin, S.; Wu, L.; LaRosa, G.; Ponath, P.; Park, C.C.; Pope, R.M.; Koch, A.E. Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis. *Arthritis Rheum.* (2001); *44*: 1022-1032.
3. Cumming, John G.; Brown, Simon J.; Cooper, Anne E.; Faull, Alan W.; Flynn, Anthony P.; Grime, Ken; Oldfield, John; Shaw, John S.; Shepherd, Emma; Tucker, Howard; Whittaker, David. Modulators of the human CCR5 receptor. Part 3: SAR of substituted 1-[3-(4-methanesulfonylphenyl)-3-phenylpropyl]-piperidinyl phenylacetamides. *Bioorganic & Medicinal Chemistry Letters* (2006), *16*(13), 3533-3536.
4. Brock, S.; Hose, D.R.; Moseley, J.D.; Parker, A.J.; Patel, I.; Williams, A.J. *Org. Proc. Res. & Dev.* (2008), *12*, 496-502.

POSTER COMMUNICATIONS – PC.219

POSTER COMMUNICATIONS – PC.220

DESIGN AND OPTIMIZATION OF A
SUBSTITUTED AMINO PROPANAMIDE SERIES
OF RENIN INHIBITORS FOR THE TREATMENT
OF ESSENTIAL HYPERTENSION

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The renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of extracellular fluid and blood pressure¹. Since the cleavage of angiotensinogen by the renin enzyme represents the first and rate limiting step of the RAAS cascade, the maximum benefit that can be gleaned from the modulation of this pathway should come in the form of a direct renin inhibitor². In this regard, research towards the discovery of renin inhibitors with *in vitro* and *in vivo* profiles suitable for clinical development was initiated and this effort culminated in the identification of a novel structural series. In this presentation, the key SAR of this family of substituted amino propanamide renin inhibitors; including the strategies employed to overcome the challenges pertaining to limited oral bioavailability, covalent binding and time-dependent CYP3A4 inhibition, will be discussed³.

REFERENCES

1. Zaman, M. A.; Oparil, S.; Calhoun, D. A. *Nat. Rev. Drug Discovery* **2002**, *1*, 621.
2. (a) Cooper, M. E. *Am. J. Hypertens.* **2004**, *17*, 16S. (b) Norris, K.; Vaughn, C. *Expert Rev. Cardiovasc. Thera.* **2003**, *1*, 51.
3. Chen, A.; Bayly, C.; Benzençon, O.; Richard-Bildstein, S.; Dubé, D.; Dubé, L.; Gagné, S.; Gallant, M.; Gaudreault, M.; Grimm, E.; Houle, R.; Lacombe, P.; Laliberté, S.; Lévesque, J.-F.; Liu, S.; MacDonald, D.; Mackay, B.; Martin, D.; McKay, D.; Powell, D.; Remeň, L.; Soisson, S.; Toulmond, S. *Bioorg. Med. Chem. Lett.* (2010) doi: 10.1016/j.bmcl.2010.02.036.

WITHDRAWN

POSTER COMMUNICATIONS – PC.221

MULTI-NUCLEAR NMR AS A METHOD FOR *EX VIVO* DRUG METABOLISM STUDIES

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Metabolic studies of the activation of nucleoside phosphoramidate prodrugs in different biological media (i.e. pig liver carboxylesterase, CEM cell extracts, human serum, mouse serum) and the isolation of subsequent metabolites have been determined by the use of HPLC.^{1,2} Most recently, the use of ³¹P rather than ¹H and ¹³C NMR has been used to evaluate the stability of potential prodrugs in a biological environment. This technique provides a useful tool to monitor the degradation process of organic molecules and the formation of metabolic intermediates.³ We now wish to report the study of metabolic stability by ³¹P NMR carried out in biological media (human serum) on a small library of ProTides with different esters and possible metabolites synthesized in our lab. Enzymatic studies were performed in order to investigate if our ProTides can be metabolised by esterase type enzyme using Carboxypeptidase Y. Moreover chemical stability was evaluated in a buffer at both acidic and basic pH. NMR methodology has proved to be a useful, quick and inexpensive tool to gather preliminary information on ProTide metabolism. Moreover the use of multi-nuclear NMR has allowed us to discriminate which drugs would be viable for further progression *in vivo*.

REFERENCES

1. Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. Characterization of the activation pathway of phosphoramidate triester prodrugs of stavudine and zidovudine. *Mol. Pharmacol.* 1999, **56**, 693-704
2. Valette, G.; Pompon, A.; Girardet, J. L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A. G.; Périgaud, C.; Gosselin, G.; Imbach, J. L. Decomposition pathways and *in-vitro* inhibitory effects of isoddA pronucleotides: Toward a rational approach for intracellular delivery of nucleoside 5'-monophosphates. *J. Med. Chem.* 1996, **39**, 1981-1990
3. Bibbo, R. Design and synthesis of novel agents with potential against osteoarthritis, *PhD Thesis* 2008, Cardiff University

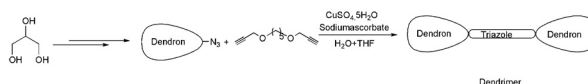
POSTER COMMUNICATIONS – PC.223

SYNTHESIS OF NOVEL GLYCEROL-BASED DRUG DELIVERY VEHICLES AND ENCAPSULATION STUDIES WITH HYDROPHOBIC DRUG MOLECULES

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One of the major challenges faced by pharmaceutical industries is increasing solubility of poorly water soluble drugs¹. Amphiphilic dendrimers have attracted interest over the past few years due to capability of solubilising hydrophobic drug molecules in aqueous medium². Glycerol is an inexpensive³ and biocompatible⁴ molecule which we have utilized for the construction of novel drug delivery vehicles (dendrimers) through "Click chemistry"⁵. These dendrimers possess hydrophilic free hydroxyl groups at the periphery whereas the interior is relatively hydrophobic and were completely characterized by NMR (1D and 2D) and MALDI-TOF spectral analysis. Encapsulation studies were carried out with a model hydrophobic drug molecule. The presentation will discuss details of this work.



REFERENCES

1. E. Atef, A. A. Belmonte, *Eur. J. Pharm. Sci.* 2008, **35**, 257-263.
2. a) S. R. Meyers, F. S. Juhn, A. P. Griset, N. R. Luman, M. W. Grinstaff, *J. Am. Chem. Soc.* 2008, **130**, 14444-14445; b) A. Schmitzer, E. Perez, L. I. Rico, A. Lattes, S. Rosca, *Langmuir* 1999, **15**, 4397-4403; c) J. Sun, M. Ramanathan, D. Dorman, G. R. Newkome, C. N. Moorefield, P. S. Russo, *Langmuir* 2008, **24**, 1858-1862; d) B.K.Cho, A. Jain, J. Nieberle, S. Mahajan, U. Wiesner, S. M. Gruner, S. Tuerk, H. J. Raeder, *Macromolecules* 2004, **37**, 4227-4234; e) D. Joester, M. Losson, R. Pugin, H. Heinzelmann, E. Walter, H. P. Merkle, F. Diederich, *Angew. Chem., Int. Ed.* 2003, **42**, 1486-1490; f) L. Degoricija, M. A. Carnahan, C. S. Johnson, T. Kim, M. W. Grinstaff, *Macromolecules* 2006, **39**, 8952-8958.
3. a) D. T. Johnson, K. A. Taconi, *Environ. Prog.* 2007, **26**, 338-348; b) M. Pagliaro, R. Ciriminna, H. Kimura, M. Rossi, P. C. Della, *Angew. Chem., Int. Ed.* 2007, **46**, 4434-4440.
4. a) H. Frey, R. Haag, *Rev. Mol. Biotechnol.* 2002, **90**, 257-267; b) A. L. Sisson, D. Steinhilber, T. Rossow, P. Welker, K. Licha, R. Haag, *Angew. Chem., Int. Ed.* 2009, **48**, 7540-7545.
5. H. C. Kolb, K. B. Sharpless, *Angew. Chem., Int. Ed.* 2002, **41**, 2596-2599.

POSTER COMMUNICATIONS – PC.224

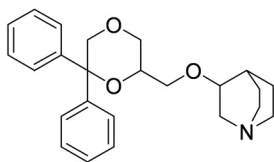
COPRESENCE OF 1,4-DIOXANE AND QUINUCLIDINE NUCLEI IN THE DESIGN OF NOVEL MUSCARINIC ANTAGONISTS

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The five muscarinic receptors are involved in many physiological functions and, therefore, the potential therapeutical applications of muscarinic agonists and antagonists are numerous. However, the high homology of the orthosteric binding sites of the different subtypes (amino acid sequence identity > 60%) makes particularly difficult the achievement of selective ligands (1). Candidates which are more likely to discriminate among subtypes are obviously antagonists because of their ability to interact with sites in the extracellular loops or with accessory residues of the receptor. In the search for novel muscarinic antagonists we opted for molecules bearing the 1,4-dioxane nucleus, which has proved to be a suitable scaffold for targeting muscarinic receptors (2). Such a scaffold, substituted in position 6 with two phenyl rings, has been linked by an oxymethylene bridge to a quinuclidine nucleus, which is present in numerous muscarinic agents as well. The presence of this nucleus has the advantage of including in the final compound a second stereogenic center, thus achieving a more selective effect, after the separation of the four enantiomers.

The synthesis of the novel compounds, the characterization of their structure, and the preliminary pharmacological results will be discussed.



REFERENCES

1. Eglén, R. M. Muscarinic receptor subtype pharmacology and physiology. *Prog. Med. Chem.* **2005**, 43, 105-136.
2. Piergentili A.; Quaglia, W.; Del Bello, F.; Giannella, M.; Pigni, M.; Barocelli, E.; Bertoni, S.; Matucci, R.; Nesi, M.; Bruni, B.; Di Vaira, M. Properly substituted 1,4-dioxane nucleus favours the selective M₃ muscarinic receptor activation. *Bioorg. Med. Chem.* **2009**, 17, 8174-8185 and references therein.

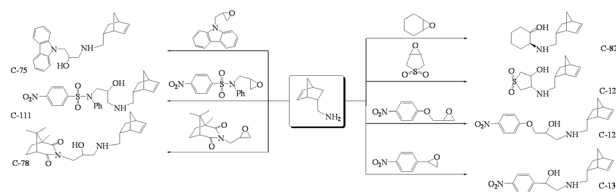
POSTER COMMUNICATIONS – PC.225

SYNTHESIS, ANALGESIC AND ANTICONVULSIVE ACTIVITY OF AMINOALCOHOLS BASED ON BICYCLO[2.2.1]HEPT-5-ENE-ENDO-2-YLMETHYLAMINE

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The present work deals with neurotropic activity of seven novel aminoalcohols C-75, C-111, C-78, C-82, C-123, C-121 and C-13, synthesized from 9-(oxiran-2-ylmethyl)-9H-carbazole, 4-nitro-N-(oxiran-2-ylmethyl)-N-phenylbenzenesulfonamide, glycidylcamphorimide, epoxycyclohexane, 3,4-epoxysulfolane, 4-nitrophenyl glycidyl ether, 2-(4-nitrophenyl)oxirane and stereochemical purity *endo*-aminomethylnorbornene (bicyclo[2.2.1]hept-5-ene-*endo*-2-ylmethylamine) in 2-propanol with yields 60-93% (Figure). Aminoalcohols as the products of regioselective aminolysis of epoxides (according to the Krasusky rule) have been obtained. The structures of individual products have been confirmed by IR spectra, NMR ¹H, ¹³C spectra, using experiments that involve homonuclear and heteronuclear scalar coupling interactions (COSY, NOESY, HMQC, HMBC), and mass spectrometry.



Experiments have been carried out on both genders adult white mice at the doses of 1/10 LD₅₀. Acute toxicity of compounds (LD₅₀) has been measured by Litchfield & Wilcoxon method in Prozorovsky V.B. (1962) modification and varies within 375-1196 mg/kg (they are considerably less toxic than *endo*-aminomethylnorbornene, LD₅₀ 331 mg/kg). The most toxic compound is aminoalcohol C-111 with N-phenylbenzenesulfonamide fragment. Investigation of analgesic activity has been carried out by determination of pain threshold by thermal irritation method (hot plate) at 55°C. Anticonvulsive activity has been studied by corazole spasm test. The activity has been calculated in percentage in relation to control group of animals.

All aminoalcohols by analgesic activity essentially surpass initial amine. For aminoalcohols C-82, C-111 and C-121 the most intense activities are analgesic (+104-127%) and for C-78 – anticonvulsive activity (+213%). For compounds C-111, C-121 and C-123 it is typical

proconvulsive action at a level -14–(-35)%. Compound C-75 show medium analgesic (+39%) and anticonvulsive activity (+18%).

The detection of correlation between chemical structure and biological activity in frame amine row defines an opportunity of the directed synthesis of new active neurotropic agents.

POSTER COMMUNICATIONS – PC.226

SYNTHESIS AND BIOLOGICAL EVALUATION OF SYMMETRIC AND ASYMMETRIC BIVALENT DOPAMINE D2 RECEPTOR LIGANDS

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A growing body of evidence indicates that GPCRs exist as homomers or heteromers. Thus, the role of dimerization/ oligomerization in receptor function is under extensive investigation.^[1] Inventing a functional complementation assay, Han et al.^[2] were able to show, that two protomers of the dopamine D2 receptor are necessary to initiate signalling through one G-protein. Interestingly, agonist binding to a single protomer is sufficient for G-protein activation. Furthermore, the two protomers are able to allosterically influence each other. An enhanced functional response was achieved by agonist binding to one protomer and inverse agonist binding to the second protomer; whereas agonist binding to the second protomer blunted signalling. More generally, the protomer crosstalk depends on the various activation states adopted by each protomer in a GPCR dimer and is believed to influence the G-protein dependent and G-protein independent signalling pathways. Thus, the possibility that homodimers can adopt multiple active states, accounts for the design of symmetric (both pharmacophores are the same) and asymmetric (pharmacophores differ in their activation pattern) bivalent ligands, as potential tools for the selective addressing of different signalling pathways.

Herein we present the design of three types of bivalent dopamine D2 receptor ligands consisting of antagonist-antagonist, agonist-agonist and antagonist-agonist as well as their monomeric counterparts. The recognition elements were connected via copper-catalyzed azide alkyne cycloaddition (CuAAC) to obtain 1,2,3-1H-triazoles containing alkyl chain linkers. The ligands were extensively investigated at the D2R for their binding affinities and intrinsic activities in various functional assays, intending to detect their influence on different signalling pathways.

REFERENCES

1. Milligan G. *Mol. Pharmacol.* **2004**, 66, 1-7.
2. Han Y.; Moreira, I. S.; Urizar, E.; Weinstein, H.; Javitch, J. A. *Nat Chem Biol.* **2009**, 5, 688-95.

POSTER COMMUNICATIONS – PC.227

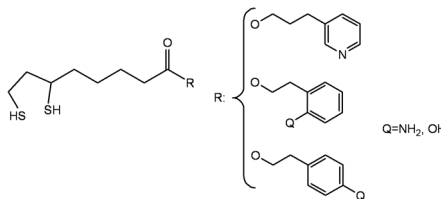
SYNTHESIS AND PHARMACOCHEMICAL STUDY OF NOVEL LIPOIC ACID DERIVATIVES WITH PUTATIVE ACTIVITY AGAINST NEURODEGENERATIVE ALZHEIMER'S TYPE DISEASE

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Senile Dementia Alzheimer's Type (SDAT) is the third cause of morbidity in the western societies. Attempts to find satisfactory drugs for SDAT are a challenge, since there are no efficient drugs for this condition, and the currently applied pharmacotherapy cannot arrest the progress of the disease. However, there are many pathobiochemical changes in the demented brain, which could be used as a basis for designing better agents against SDAT.

Using two or three of these changes, e.g. oxidative stress, neurotrophic agent dysregulation and inflammation, we designed, synthesised and pharmacochémically studied some lipoic acid esters in the hope that they possess beneficial properties. Lipoic acid served as and antioxidant and neurotrophic agent, and the alcohols used were 3-(pyridin-3-yl)propan-1-ol as a neurotrophic structure, 2-(2-aminophenyl)ethanol and 2-(4-aminophenyl)ethanol as potential antioxidants. Synthesis was conducted by conventional or novel methods and structures were identified by modern spectroscopic techniques (IR, ¹H and ¹³C NMR, DEPT NMR) and elemental analysis.



Structures of the synthesised final compounds

They were tested for antioxidant activity assessed as inhibition of lipid peroxidation of hepatic microsomal membranes, induced by the FeSO₄ – ascorbate system, and as interaction with the stable radical DPPH. Lipid peroxidation was determined spectrophotometrically as thiobarbituric acid reactive material. Antiinflammatory action was expressed in vitro as inhibition of lipoxygenase and in vivo as paw edema reduction. Many of the tested compounds demonstrated encouraging results. We believe that multicausal diseases like SDAT can better be treated by polyfunctional compounds^[1, 2], like the above structures incorporating neurotrophic and antioxidant properties.

REFERENCES

1. Doulgeris CM et al., *Bioorg Med Chem Lett.* 16 (2006), 825-829.
2. Tsiakitzis KC et al., *J. Med. Chem.* 52 (2009), 7315-7318.

POSTER COMMUNICATIONS – **PC.228**

HETEROMERIC DOPAMINE - NEUROTENSIN RECEPTOR INTERACTIONS CONTROL THE BINDING AFFINITIES OF DOPAMINERGICS

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Neurotensin (NT) is a tridecapeptide that is widely distributed in the central nervous system, as well as in the gastrointestinal tract. In many regions of the CNS, NT acts as neurotransmitter and neuromodulator by activating the G-protein coupled neurotensin receptors (NTS1 and NTS2).

Several lines of evidence based on neurochemical, behavioral and anatomical data suggest an interaction of NT with neurotransmitter systems in the CNS, especially the mesolimbic and nigrostriatal dopaminergic pathways. According to these findings, NT is discussed as endogenous antipsychotic agent and might influence the pathophysiology of brain diseases such as schizophrenia, Parkinson's Disease and Alzheimer's Disease. The modulating effect of neurotensin on striatal dopamine D₂ receptors most likely is mediated by activation of NTS1 and, subsequently, causes an attenuation of dopaminergic signalling. [1]

Here, we present a co-expression study of the dopamine receptors (D_{2L} or D₃) and the neurotensin receptors (NTS1 or NTS2). One dopamine receptor and one neurotensin receptor subtype were transiently co-transfected in HEK293 cells, generating four different *in vitro* models for heteromeric GPCR systems. Binding affinities of the dopamine receptor antagonist spiperone and the agonist 7-OH-DPAT, as indicative parameters of the modulating effect of NT on these heteromers, were investigated. When measuring the affinities of the radioligands [³H] spiperone and [³H] 7-OH-DPAT to the dopamine receptors in the absence and presence of NT, a clearly reduced binding of the dopamine agonist could be observed with NT. These results confirm the modulating effect of neurotensin on the dopamine receptors in our *in vitro* systems. Heteromeric systems of GPCRs as models to investigate the molecular interactions of receptor heteromers are presented and exploited for the evaluation of novel dopamine receptor agonists.

REFERENCES

1. E. B. Binder, B. Kinkad, M. J. Owens, C. B. Nemeroff Pharmacol. Rev. **2001**, 53, 453-486.

POSTER COMMUNICATIONS – **PC.229**

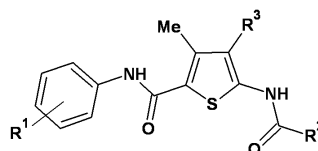
SYNTHESIS OF THIOPHENE CARBOXAMIDE-TYPE TRPV1 ANTAGONISTS

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Being potent antagonists of TRPV1 drug-like thiophene carboxamides were identified as feasible starting points for further synthetic modifications; regions of the thiophene molecule were changed in a systematic way by simple, few-step syntheses.

It was aimed to improve the potency as well as the metabolic stability of thiophenes by modifications at three positions of the molecule (as it is designated in the figure below). The SAR for the series obtained from these structural modifications suggested that 4-substituted-phenylcarbamoyl moiety and also the R³ group have a determinant role in TRPV1 activity; the presence of the R²-amido moiety believed responsible for the intensive non-CYP metabolism observed in human liver microsomes. As a result, a new class of potent and metabolically stable TRPV1 antagonists were obtained.

POSTER COMMUNICATIONS – **PC.230**

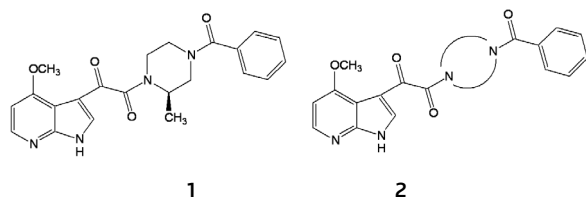
SYNTHESIS, COMPUTATIONAL STUDIES AND HIV-1 INHIBITORY ACTIVITY OF NEW ANTIVIRAL COMPOUNDS

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The AIDS disease is caused by the Human Immunodeficiency Virus (HIV), a retrovirus with RNA genome.^[1] The UNAIDS Report 2009 estimated that 33.4 million people worldwide were living with HIV at the end of 2008.^[2] Since the AIDS pandemic remains

one of the leading global public health threats, the discovery and development of new antiretroviral drugs with reduced toxicity, enhanced potency, different mechanisms of action, and reduced prevalence of adverse drug-drug interactions remain a very high priority. In particular, a promising area of investigation is the identification of agents that inhibit viral attachment and entry into host cells^[3] in order to block HIV infection at the early stages. The azaindole derivative BMS-37806 **1**, discovered at Bristol-Myers Squibb,^[4] has been shown to interfere with the HIV-1 entry process, inhibiting the interaction between the viral gp120 envelope glycoprotein and its cellular CD4 receptors.^[5-7] In this context we have synthesized analogs of compound **1** introducing different changes in the chemical structure instead of the methylpiperazine group. The new compounds of general formula **2** were synthesized and their biological activity evaluated. Finally, in an attempt to correlate the geometrical features of these compounds to their HIV-1 inhibitory activity, a modeling study was carried out to ascertain their conformational preferences. The chemistry, the computational and NMR studies, and biological data will be discussed in the poster.



REFERENCES

1. R. C. Gallo, L. Montagnier, *New Engl. J. Med.* **2003**, 349, 2283–2285.
2. WHO/UNAIDS, AIDS Epidemic Update December 2008.
3. a) V. Briz, E. Poveda, V. Soriano, *J. Antimicrob. Chemother.* **2006**, 57, 619–627; b) S. Rusconi, et al. *Curr. Drug Targets Infect. Disord.* **2004**, 4, 339–355; c) F. Shaheen, et al., *Curr. Opin. Infect. Dis.* **2004**, 17, 7–16; d) I. Markovic, *Curr. Pharm. Des.* **2006**, 12, 1105–1119.
4. T. Wang, et al. *J. Med. Chem.* **2003**, 46, 4236–4239.
5. P. F. Lin, et al. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 11013–11018.
6. R. S. Veazey et al. *Nature* **2005**, 438, 99–102.
7. D. Colombo, S. Villa, L. Solano, L. Legnani, F. Marinone Albini, L. Toma. *Eur. J. Org. Chem.*, **2009**, 3178–3183.

POSTER COMMUNICATIONS – **PC.231**

TRICYCLIC PYRIDAZINONE DERIVATIVES: DEVELOPMENT ON NEW STAT3 INHIBITORS

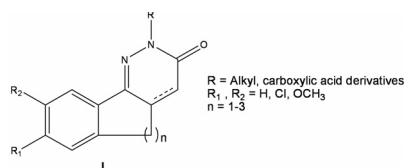
Arianna Gelain¹, Daniela Masciocchi¹, Stefania Villa¹, Alessandro Pedretti¹, Fiorella Meneghetti¹, Daniela Barlocco¹, Byoung-Mog Kwon², Shin Dae-Seop², Laura Legnani³ and Lucio Toma³

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Signal transducer and activator of transcription 3 (Stat3) is a latent cytoplasmic protein that transmits the signals from the cell surface to the nucleus¹ and participates directly in gene regulation by binding to response elements in gene promoters effecting transactivation. Stat3 plays a crucial role in cell growth and survival but the abnormal regulation of its functions is closely linked to tumorigenesis²; in fact, its constitutive activation by aberrant upstream tyrosine kinase activities is evident in a broad spectrum of human solid and blood tumours. Moreover the inhibition of Stat3 induces apoptosis in tumour cells selectively³⁻⁴, which validates Stat3 as a promising cancer drug target. During our ongoing researches on non peptidic small molecules that could directly interact with Stat3, we focused our attention on the tricyclic pyridazinone skeleton **1**. In particular, we explored the effects of substituents both at N-2 and at different positions of the aromatic ring as well as the size of the central ring.



Synthesis, molecular modeling studies, X-ray analysis and biological data will be presented.

REFERENCES

1. Costantino, L.; Barlocco, D. *Curr. Med. Chem.*, **2008**, 15 (9), 834-843.
2. Aggarwal, B.B.; Sethi, G.; Ahn, K.S.; Sandur, S. K.; Pandey, M.K.; Kunnumakkara, A.B.; Sung, B.; Ichikawa, H. *Ann. N. Y. A. S.*, **2006**, 1091, 151-169.
3. Bowman, T.; Broome, M. A.; Sinibaldi, D.; Wharton, W.; Pledger, W. J.; Sedivy, J. M.; Irby, R.; Yeatman, T.; Courtneidge, S. A.; and Jove, R. *PNAS*, **2001**, 98 (13), 7319-7324
4. Turkson, J.; Ryan, D.; Kim, J. S.; Zhang, Y.; Chen, Z.; Haura, E.; Laudano, A.; Sebti, S.; Hamilton, A. D. and Jove, R. *J. Biol. Chem.*, **2001**, 276 (48), 45443-45455

POSTER COMMUNICATIONS – **PC.232**

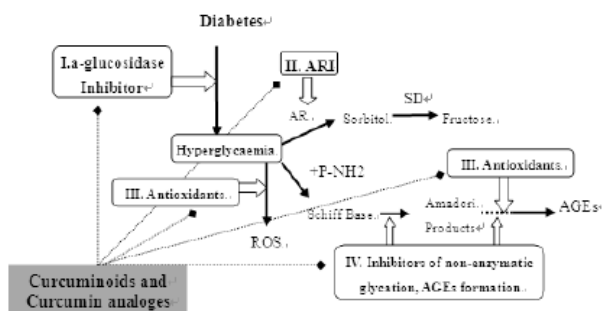
INTERVENTION ON MULTIPLE TARGETS OF DIABETIC COMPLICATIONS BY CURCUMINOIDS AND CURCUMIN ANALOGUES

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Diabetes mellitus is a major and growing public health problem throughout the world. The increased flux of glucose results in long-term complications which include neuropathy, nephropathy, retinopathy, cardiovascular disease and cataract formation. It is reported that multiple mechanisms have been implicated in the development of diabetic complications, and three of them appear to play a prominent role (Scheme 1): (i) increased flux of glucose through the polyol pathway; (ii) increased advanced glycation end-product (AGE) formation and (iii) increased oxidative stress [1]. Although a considerable number of therapeutic agents against diabetic complications are available, none is optimal and can achieve a satisfactory effect on its own. Thus, combinations of complementary agents have been suggested and play an increasing role in the treatment of the disease [2]. In the same way, it is more effective if one compound can exhibit more activities on the multiple mechanisms of diabetic complications.

Curcumin, a natural product isolated from Rhizome of the perennial herb *Cucuma Longa L* (turmeric), is a yellow pigment and a known antioxidant in food system. It has already been reported to possess diverse biological activities such as anti-inflammatory, antitumor, antihepatotoxic, anti-HIV. We previously studied the inhibitory activities of natural curcuminoids and synthesized curcumin analogues against aldose reductase (AR) and α -glucosidase [3,4], and found that the ortho-dihydroxy curcumin analogues possessed much more potential bioactivities for both the targets than that of curcumin. Recently, it was reported that curcumin showed potential effects on diabetic animal model as an aldose reductase inhibitor (ARI) [5]. This prompted us to synthesize a new series of novel curcumin analogues with ortho-dihydroxy moiety and evaluated their inhibitory activities against α -glucosidase, aldose reductase, advanced glycation end products (AGEs), and antioxidation ability. The results showed that some curcumin analogues exerted highly potential effects against all above mentioned targets. It suggests that curcumin analogues can be leading compounds to develop an effective drug intervening multiple targets in preventing diabetic complications.



Scheme 1. Possible pharmacological preventions of diabetic complications.

REFERENCES

1. Mark, S.M. The American Journal of Medicine, 123(3), S3-S11 (2010)
2. Kyselova, Z., Stefek, M., Bauer, V. Journal of Diabetes and Its Complications, 18, 129-140 (2004).
3. DU, Z.Y., LIU R.R., SHAO W.Y., et al. Eur. J. Med. Chem. 42(2), 213-218 (2006).

4. DU, Z.Y., BAO, Y.D., LIU, Z., et al. Archiv der Pharmazie, 339(3), 123-128 (2006).
5. Muthenna, P., Suryanarayana, P., Gunda, S.K., et al., FEBS Letters, 583, 3637-3642 (2009).

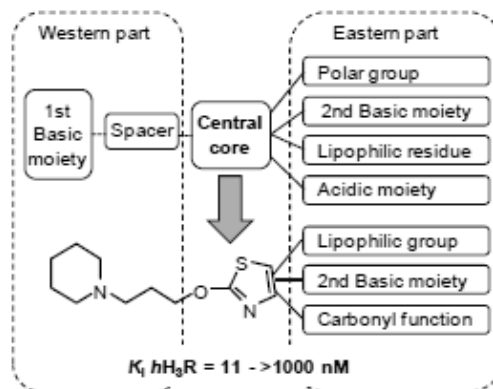
POSTER COMMUNICATIONS – PC.233

THIAZOLE DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS

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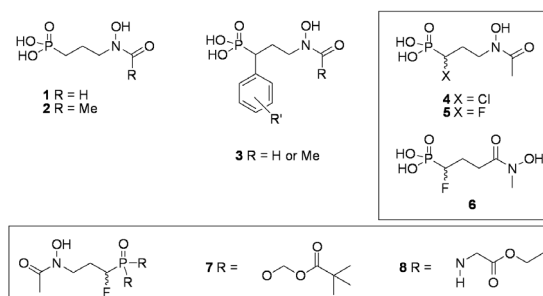
Biogenic amines, such as histamine, play important roles as chemical mediators in human physiology. Histamine mediates its numerous central and peripheral effects *via* four known G protein-coupled receptor subtypes (hH_1R - hH_4R). Human histamine H_3 receptors (H_3R) are acting as autoreceptors on synthesis and liberation of histamine as well as heteroreceptors on modulating the release of several other neurotransmitters (e.g. acetylcholine, dopamine, glutamate, noradrenalin, serotonin, GABA). Due to a distinct expression pattern in the central nervous system (CNS) and resulting involvement in several neuronal functions, e.g. vigilance, attention and learning, the hH_3R are attractive targets for the treatment of CNS disorders like schizophrenia, epilepsy, depression, Alzheimer's disease and sleep disorders.¹



Antagonist H_3R ligands follow a general pharmacophore blueprint, containing a basic moiety, mostly a tertiary amine, linked by a spacer to a central core substituted by a variety of structural elements providing different physicochemical properties.² Based on the well established 1-(3-phenoxypropyl)amine pharmacophore, offering an aromatic ring system in the core region, we investigated the acceptance of polar core elements. Therefore the phenyl ring was replaced by polar thiazole groups.

The thiazole derivatives could be obtained by Hantzsch-thiazole synthesis.^{3,4} A diversity of structural motifs is used as substituents to optimize the *in vitro* receptor binding properties, which ranging from low nanomolar binding strength to completely loss of affinity.

We successfully introduced polar thiazole groups as new central core element in the *h*H₃R antagonist pharmacophore and could establish a new structural class of *h*H₃R antagonists/inverse agonists.



REFERENCES

1. Tiligada, E. et al., *Expert Opin. Investig. Drugs*. 2009, 18, 1519-31.
2. Sander, K. et al., *Bioorg. Med. Chem. Lett.* 2010, 20, 1581-4.
3. Hantzsch, A. et al., *Chem. Ber.* 1888, 21, 938-41.
4. Wiley, R.H. et al., *Organic Reactions*, 1951, 6, 367-409.

POSTER COMMUNICATIONS – PC.234

SYNTHESIS AND EVALUATION OF α -HALOGENATED ANALOGUES OF FR900098 AND THEIR PRODRUGS AS ANTIMALARIALS

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Fosmidomycin (**1**) and its acetyl congener FR900098 (**2**) inhibit DOXP reductoisomerase, a vital enzyme in the non-mevalonate pathway for isoprenoid biosynthesis, and are good leads for further development of new antimalarials. Previously, it has been shown that introduction of an aromatic group in α -position of the phosphonate increases the *in vitro* cellular activity of these analogues.^{1,2}

Assuming that this increase in potency was due to the electron withdrawing effect of the aromatic ring causing a decrease in pKa of the phosphonate, we anticipated that introduction of a halogen atom in α -position of the phosphonate could accomplish the same effect without the steric implications that come with the aromatic rings. Therefore, we synthesized two halogenated retrohydroxamates **4** and **5** as well as the fluorinated hydroxamate **6** via halogenation of a suitable phosphonate precursor. Analogues **4-6** showed submicromolar activity *in vitro* (*P. falciparum* K1 and GHA) as well as good *in vivo* activity in a *P. berghei* mouse model.

To address the high polarity of these phosphonates, resulting in poor bioavailability and cell penetration, we also synthesized and evaluated the bis(pivaloyloxymethyl) **7** and bis(ethoxycarbonylmethylamino) **8** prodrugs of α -fluoro-analogue **5**.

REFERENCES

1. Haemers, T.; Wiesner, J.; Van Poecke, S.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Van Calenberg, S. *BIOORG. MED. CHEM. LETT.* **2006**, 16, (7), 1888-1891.
2. Devreux, V.; Wiesner, J.; Jomaa, H.; Rozenski, J.; Van der Eycken, J.; Van Calenberg, S. *J. ORG. CHEM.* **2007**, 72, (10), 3783-3789.

POSTER COMMUNICATIONS – PC.235

AUTOMATION OF THE CHARMM GENERAL FORCE FIELD FOR COMPUTER-AIDED DRUG DESIGN

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The CHARMM Force Field [1] is widely used in computer-aided drug design for the study of biological macromolecules. The high demand for simulations of these biological systems in interaction with drug candidates motivated development of the CHARMM General Force Field (CGenFF), an organic force field aimed at simulating drug-like molecules in a biological environment represented by the CHARMM biomolecular force field. [2] To facilitate the use of CGenFF, an algorithm was developed that automatically assigns atom types, bonded parameters and charges to an arbitrary molecule. The atom typer is rule-based and programmable, making it easy to update the atom typing scheme as the force field grows. Assignment of bonded parameters is based on substituting atom types in the definition of the desired parameter. A penalty is associated with every substitution and the existing parameter with the lowest total penalty is chosen as an approximation for the desired parameter; the "penalty score" is returned to the user as a measure for the accuracy of the approximation. Charges are assigned using an extended bond-charge increment scheme that should be able to

capture short- and medium-range inductive and mesomeric effects. [3] The resulting program's functionality will be accessible on the web, which makes it possible to apply CGenFF on arbitrary molecules in a matter of seconds. [4] Although the program should ideally accept any molecule, the quality of the resulting parameters (as indicated by their "penalty scores") will vary depending on whether a similar compound has been parametrized before. Therefore, an effort has been started to build a computational engine with the capability of automatically validating and/or optimizing parameters for drug-like model compounds of the user's interest in the framework of CGenFF, thus providing high-quality force field parameters for computer-aided drug discovery. [4]

REFERENCES

1. A. D. MacKerell et al., *J. Phys. Chem. B* **1998**, *102*, 3586-3616
2. K. Vanommeslaeghe et al., *J. Comput. Chem.* **2010**, *31*, 671-690
3. K. Vanommeslaeghe et al., *in preparation*
4. NSF Award #0823198; <https://www.paramchem.org/>

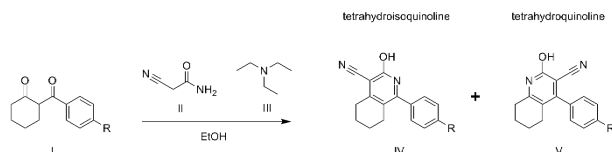
POSTER COMMUNICATIONS – PC.236

ON THE REACTION OF PARA-SUBSTITUTED 2-BENZOYL CYCLOHEXANONES WITH CYANOACETAMIDE

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In the synthesis of a more complex heterocyclic system, we explored 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) as an important intermediate. The reaction of substituted 2-benzoyl cyclohexanone (I) with cyanoacetamide (II) and triethylamine (III) in ethanol at room temperature has been reported to lead to the formation of 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) (1). However, depending on the nature of the *para* substituent on the phenyl ring, formation of regioisomeric 2-hydroxy-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile can compete (V).



No molecular determinants for the regiochemistry have been described. Therefore, we undertook efforts to gain more insights in the mechanism of this reaction. We investigated the effect of different *para*-substituents and their influence on the observed ratio between formed tetrahydroisoquinoline and tetrahydro-

quinoline products. For the characterization and quantification of precursors and products we used an array of techniques, including GC-MS and 2D NMR. We postulate that the electron-donating or -withdrawing properties of the *para* substituents directly determine the ratio of product formation.

REFERENCES

1. Rosowsky, A.; Papathanasopoulos, N. Pyrimido[4,5-*c*]isoquinolines. Synthesis and biological evaluation of some 6-alkyl-, 6-aralkyl-, and 6-aryl-1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinolines as potential folate antagonists. *J. Med. Chem.*, **1974**, *84*, 1140-1443

POSTER COMMUNICATIONS – PC.237

MULTIFUNCTIONAL NEW COMPOUNDS FOR THE TREATMENT OF DEGENERATIVE CONDITIONS WHICH IMPLICATE INFLAMMATION

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used agents for the treatment of inflammation. However, they possess major and even life-threatening side effects, such as damage to the gastric mucosa, prolongation of bleeding time and renal failure.

A number of approaches have been pursued to minimize these adverse effects of NSAIDs. It is well known that reactive oxygen species are implicated in inflammation. Furthermore, since cyclooxygenase (COX) catalysis involves radical intermediates, a radical scavenger would be considered to interfere with the COX reaction. Thus, antioxidant properties seem to be advantageous for anti-inflammatory agents. Derivatives bearing the 3,5-di-*tert*-butyl-4-hydroxybenzylidene moiety with antioxidant activity have been found to be dual inhibitors of COX-2 and 5-lipoxygenase (5-LOX) as well as to suppress interleukin-1 production. In addition, we have found that derivatives of 2,6-di-*tert*-butyl phenol which are amines or amides of thiomorpholine, morpholine and proline residues reduce inflammation and offer protection against free radical attack by direct inhibition of lipid peroxidation as well as by inhibiting LOX, which is considered to play a significant contributory role in the generation of cellular oxidative stress. Considering the above studies, we designed some new derivatives of 2,6-di-*tert*-butyl phenol with known NSAIDs.

Neurodegenerative disorders of Alzheimer's type are one of the top causes of morbidity. There is a series of pathobiochemical changes in the demented brain, which could be used as a starting point for rational design of molecules for the particular condition, such as oxidative stress, inflammation, reduction of nerve growth factor, increased apoE₄ and hypercholesterolemia. Thus, we describe the synthesis of derivatives of NSAIDs esterified with 3-(3-hydroxypropyl)pyridine expected to offer neurotrophic

and/or neuroprotective activity Furthermore, we have found that this moiety enhances anti-inflammatory action.

These compounds were tested in vitro and in vivo for the evaluation of their antioxidant and anti-inflammatory activities. They were found to inhibit in vitro rat hepatic microsomal membrane lipid peroxidation, to exert anti-inflammatory activity, assessed as paw edema reduction and inflammation enzyme inhibition, and to reduce total cholesterol, LDL-cholesterol and triglyceride levels in the plasma of dyslipidemic rats. Thus, compounds combining anti-inflammatory and antioxidant structural features may be proven useful for the treatment of conditions which implicate inflammation.

POSTER COMMUNICATIONS – PC.238

DISCOVERY OF INHIBITORS OF *SCHISTOSOMA MANSONI* NAD⁺ CATABOLIZING ENZYME

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Schistosoma mansoni flatworm is the agent of the human intestinal schistosomiasis disease. *Schistosoma mansoni* NAD⁺ catabolizing enzyme (*SmNACE*) is a distant homolog of mammalian CD38 enzymes. [1] It efficiently converts NAD⁺ into Adenosine diphosphoribose (ADPR), but lacks the ADPR cyclase activity. [2] It is localized to the outer tegument of the adult parasite hosted in human blood vessels, and most probably scavenges the host extracellular/circulating free NAD⁺, therefore impacting NAD⁺-dependent pathways of the human immune system.

To discover *SmNACE* inhibitors, we carried out a virtual screening of commercially available compounds libraries (ca. 2 millions of molecules). Our approach combined docking using a homology model of *SmNACE* and scoring using machine learning and interaction fingerprinting. [3] It has benefited from the preliminary analysis of CD38 apo- and holo- structures, which revealed the rigidity of the catalytic site as well as the key determinants for binding of NAD⁺-like ligands. We selected and experimentally tested 103 compounds, and found four enzyme inhibitors at low micromolar concentrations. Among hits, taxifolin, a natural product of the favonoid family, has an inhibitory constant of 10 μ M.

A parallel experimental screening of an "in-house" chemical library of 6160 molecules [4] yielded only two confirmed hits, cyanidin and delphinidin, with respective inhibitory constants of 1.2 μ M and 3.2 μ M. Cyanidin and delphinidin are both anthocyanidins, which are chemically similar to flavonoids. Docking poses suggest that anthocyanidins and flavonoids can mimic the nicotinamide ribosyl part of NAD⁺.

REFERENCES

1. Goodrich, S. P.; Muller-Steffner, H.; Osman, A.; Moutin, M. J.; Kusser, K.; Roberts, A.; Woodland, D. L.; Randall, T. D.; Kellenberger, E.; LoVerde, P. T.; Schuber, F.; Lund, F. E., *Biochemistry* 2005, 44, (33), 11082-97.
2. Kuhn, I.; Kellenberger, E.; Rognan, D.; Lund, F. E.; Muller-Steffner, H.; Schuber, F., *Biochemistry* 2006, 45, (39), 11867-11878.
3. Marcou, G.; Rognan, D., 2007, 47, (1), 195-207.
4. <http://chimiotheque-nationale.enscm.fr/>

POSTER COMMUNICATIONS – PC.239

DESIGN AND SYNTHESIS OF AZAISOFLAVONES AND THEIR INHIBITORY ACTIVITIES OF NO PRODUCTION IN ACTIVATED MICROGLIA

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Nitric oxide (NO) produced by nitric oxide synthase (NOS) is involved in diverse biological functions including regulation of vascular relaxation, long-term potentiation in neurons and the cytotoxic action of macrophages. The family of NOS is classified as inducible form (iNOS) and two constitutive forms such as neuronal enzyme (nNOS) and endothelial enzyme (eNOS). The overproduction of NO by iNOS is responsible for the severe hypotension, cell cytotoxicity, tissue damage, the pathogenesis of septic shock, various inflammatory processes and cancer. Thus selective inhibition of iNOS may offer possible treatments for such diseases. Many strategies to control NO levels have been applied and mainly directed towards regulation of iNOS by the control of expression level and/or enzymatic activity of iNOS. Several classes of iNOS inhibitors, such as amino acid analogues, amino heterocycles, amidines, guanidines, isoquinolinamines and isothiourea, were reported. Despite the identification of various iNOS inhibitors, there is still strong need for development of selective and low toxic molecules.

Isoflavones are polyphenolic compounds that are widespread in legume plants. Isoflavones exhibit a variety of biological properties and may play a role in the prevention of cancers, coronary heart diseases, menopausal symptoms and osteoporosis. In addition, some isoflavones have been reported to exhibit anti-inflammatory and neuroprotective functions by inhibition of microglia activation. In spite of abundant reports of isoflavones, structural requirements and mechanisms for their anti-inflammatory effects have not been fully understood. Therefore, there is a strong demand for the various chemical tools. Although many synthetic variations of isoflavones have been reported, relatively few quinolone analogs, so called aza-isoflavones which were replaced oxygen of isoflavone skeletons with nitrogen, have been described.

Herein we report design and synthesis of new aza-isoflavones. The prepared compounds were evaluated their inhibitory activities of NO production and iNOS expression in BV-2 microglia cell lines. Several compounds showed significant inhibitory activities of NO production. Among these compounds, *N*-ethyl trihydroxy azaisoflavone was the most potent with IC_{50} 7.83 μ M for inhibition of NO production. This compound inhibited expression of iNOS in LPS-induced BV2 cells. This result suggests that the *N*-ethyl trihydroxy azaisoflavone inhibited the production of NO by suppressing the expression of iNOS.

^a As a first approach, one compound was considered an inhibitor if it inactivated TcTIM by more than 50 % at 100 μ M concentration.

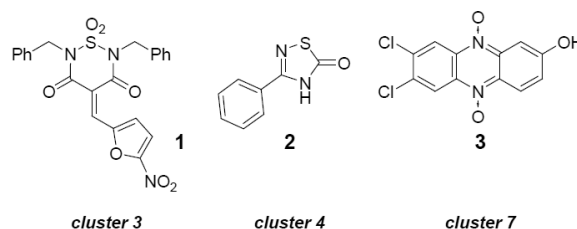


Figure 1

POSTER COMMUNICATIONS – PC.240

T. CRUZI TRIOSEPHOSPHATE ISOMERASE INHIBITORS DERIVED FROM A SCREEN HIT STUDY

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Chagas' disease (CD) is the third largest neglected disease in Latin America, affecting at least 15 million people.¹ The current drugs exhibit unacceptable side effects, do not eliminate the pathogen in the chronic stage, and ineffective due to parasite resistance. Therefore, development of new drugs against CD is urgent. *Trypanosoma cruzi* triosephosphate isomerase (TcTIM) is a ubiquitous glycolytic enzyme that catalyzes the interconversion between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The enzyme exhibits high catalytic rates only in its dimeric form, but each inactive monomer has all three catalytic residues. Recently, we have identified small molecules capable of inhibiting TcTIM by perturbing the interactions between its two subunits, which also have antiproliferative activity against *T. cruzi*.²

Along this line, we have designed a high-throughput assay to identify TcTIM inhibitors studying a 250 compound library that includes other known *T. cruzi* antiproliferative leads from our laboratory.³ The compounds belong to the following twelve clusters: 1) benzofuroxanes; 2) furanes- and thiophenes; 3) 1,2,6-thiadiazines; 4) 1,2-thiazoles; 5) nitroalkenes; 6) furoxanes; 7) 1,4-dioxidoquinoxalines and 5,9-dioxido-phenazines; 8) flavonoids; 9) *N*-oxidebenzimidazoles; 10) indazoles; 11) 1,2,4-triazines; 12) miscellaneous derivatives. The initial results, gathered according to the corresponding cluster, are summarised in the Table. From the dose-response study, compounds **1-3** (Figure 1), belonging to clusters 3, 4, and 7, were identified as new hits for TcTIM inhibition.

cluster	1	2	3	4	5	6	7	8	9	10	11	12	Total
Number of evaluated compound	50	59	19	9	4	25	11	6	18	12	10	28	251
Number of TcTIM inhibitors ^a	13	18	10	7	3	10	5	1	1	0	0	5	73

ACKNOWLEDGMENTS

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REFERENCES

1. J. Rodrigues Coura, S.L. Castro, *Mem. Inst. Oswaldo Cruz*, 2002, **97**, 3.
2. a) V. Olivares-Illana, R. Pérez-Montfort, F. López-Calahorra, M. Costas, A. Rodríguez-Romero, et al., *Biochemistry*, 2006, **45**, 2556; b) V. Olivares-Illana, A. Rodríguez-Romero, I. Becker, M. Berzunza, J. García, R. Pérez-Montfort, N. Cabrera, F. López-Calahorra, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, *PLoS Neg. Trop. Dis.*, 2007, **1**, e01.
3. H. Cerecetto, M. González, *Mini Rev. Med. Chem.*, 2008, **8**, 1355.

POSTER COMMUNICATIONS – PC.241

DESIGN AND SYNTHESIS OF PENTACYCLIC TRITERPENE LIBRARIES WITH MULTIPLE TARGETING ANTITUMOR ACTIVITY

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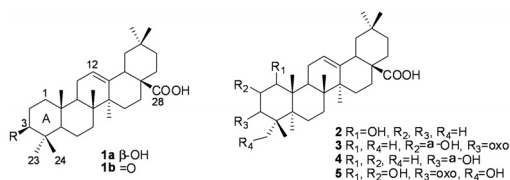
Multitargeting agents are believed to be crucial in the treatment of complex, multiple gene related diseases, such as cancer, neurodegenerative diseases, etc. To discover multitargeting agents, molecules with scaffolds based on privileged structure are of interests to be explored.

Pentacyclic triterpenoids have been known to exert their antitumor actions through various mechanisms, but usually exhibiting moderate activity [1]. We have found that 3-oxo OA (**1b**) exhibited antitumor and angiogenesis inhibitory activity [2]. So we chose to work on this type of privileged structure, in order to search for multitargeting antitumor leads, through the design and parallel synthesis of diverse libraries of oleanic acid (**1a**), with an emphasis on its A ring modified analogues.

The derivatization at A ring of **1** was first realized by introduction of hydroxyl groups in liquid phase, leading to the 1-OH (**2**), 2 α -OH (**3**), 3 α -OH (**4**), 23-OH (**5**) analogs, enabling us to introduce

diverse substituents to various positions on triterpene scaffold. For the library design, we selected a series of carboxylic acids from MDL® Available Chemical Directory, and grouped them by using many structural parameters, such as hydrophobic, electrostatic and stereoscopic factors, as descriptors, to ensure the selected synthetic building blocks from each group are diverse in the chemical space. Those parameters were also used for theoretical prediction of the reactivity order of those building blocks in the esterification reactions. Then we explored the solid phase³ parallel synthesis to prepare a 60 membered compound library of the **1** and **4** analogues. The inhibition of tumor cells proliferation, endothelial cells migration, and angiogenesis for those analogues were tested, and some of them demonstrated better activity than **1** in one or more assays.

Our study demonstrated the usefulness of triterpene scaffolds bearing diverse substituents in the search for multitargeting leads in cancer chemotherapy. Further investigations are warranted, and undergoing in our labs.



REFERENCES

- Wang, S.-R.; Fang, W.-S. *Curr. Top. Med. Chem.* 2009, 9: 1581-1596.
- Huang, D.; Ding, Y.; Li, Y.; Zhang, W.; Fang, W.; Chen, X. *Cancer Lett.* 2008, 233: 289-96.3) Pathak, A.; Singh, S. K.; Biabani, M. A. F.; Kulshreshtha, D. K.; Puri, S. K.; Srivastava, S.; Kundu, B. *Comb Chem High Throughput Screening* 2002, 5: 241-248.

POSTER COMMUNICATIONS – PC.243

HITTING THE RIGHT SPOTS: INTEGRATIVE STRUCTURE-BASED VIRTUAL SCREENING AGAINST INTENDED AND UNDESIED PROTEIN DRUG TARGETS

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To be effective and safe, drug molecules must discriminate successfully the intended protein target from alternative proteins present in the human body. We have developed an integrative structure-based molecular design strategy in which not only intended target-selective hot spots but also unfavorable anti-target-specific interactions are simultaneously considered for the construction of protein-ligand interaction fingerprint (IFP) models. Our virtual screening method enables the identification of target-selective ligands among large chemical databases while steering ligand design away from undesired anti-targets.

Applying this approach to sequentially and structurally unrelated targets and anti/targets, such as G protein-coupled receptors (GPCRs) and cytochrome P450 enzymes (CYPs), is especially challenging. The molecular backgrounds of ligand cross-reactivity between these pharmaceutically relevant systems are not well understood, and general strategies to design selective ligands are lacking. By combining our *in silico* models with experimental site-directed mutagenesis and structure-activity relationship studies we aim to identify the molecular determinants of ligand selectivity and promiscuity.

POSTER COMMUNICATIONS – PC.244

GUANIDINE CONTAINING BENZOFUROXAN AND BENZIMIDAZOLE 1,3-DIOXIDE SCAFFOLDS AS CRUZIPAIN-T. CRUZI INHIBITORS

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Chagas' disease (CD) is the third largest neglected disease in Latin America, affecting at least 15 million people with more than 25 million at risk of infection.¹ Current drugs exhibit unacceptable side effects, do not eliminate the parasite in the chronic stage, and display resistance. For that, development of new drugs against CD is urgent. One of our recent focuses has been the development of multitarget-directed ligands as anti-*T. cruzi* agents, using free-radical releasing moieties linked to cruzipain (CP)-inhibitor- pharmacophores (**1-3**, Fig. 1).²

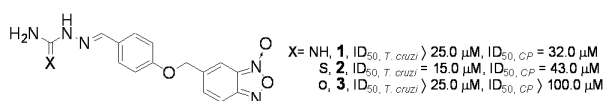


Figure 1

We selected parent compound **1** and benzofuroxan and benzimidazole 1,3-dioxide as core scaffolds linked to guanidine-pharmacophore aiming to produce new anti-*T. cruzi* agents with CP-inhibition capability. The designed compounds (**4-11**) were synthesized following the strategy shown in Fig. 2.

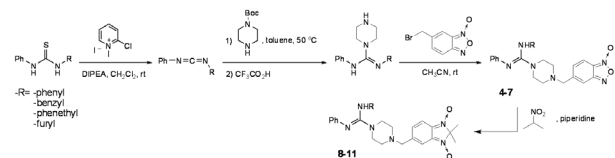


Figure 2

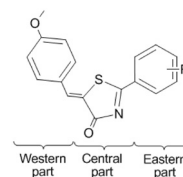
Their anti-proliferative activity was determined *in vitro* against *T. cruzi*, Tulahuen 2 strain, and the unspecific cytotoxicity was evaluated using human blood red (hrb) cells. The inhibition of CP was assayed *in vitro* and ligand-enzyme interactions were studied in terms of docking and NMR analysis. The new guanidine derivatives displayed good anti-*T. cruzi* activity, with IC_{50} values lower than Nifurtimox and **1**, and selectivity indexes ($IC_{50,hrb}/IC_{50,T.cruzi}$) higher than 11. The CP-inhibition was modest, increasing at pH 5.0 and the TI values, from the NMR experiments, and docking analysis clearly indicated that the main interactions with the enzyme are established by the piperazine and the aromatic moieties of the ligands.

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REFERENCES

1. J. Rodrigues Coura, S.L. Castro, *Mem. Inst. Oswaldo Cruz*, 2002, **97**, 3.
2. a) W. Porcal, P. Hernández, M. Boiani, G. Aguirre, L. Boiani, A. Chidichimo, J.J. Cazzulo, N.E. Campillo, J.A. Paez, A. Castro, R. L. Krauth-Siegel, C. Davies, M.A. Basombrio, M. González, H. Cerecetto, *J. Med. Chem.*, 2007, **50**, 6004; b) W. Porcal, P. Hernandez, L. Boiani, M. Boiani, A. Ferreira, A. Chidichimo, J.J. Cazzulo, C. Olea-Azar, M. González, H. Cerecetto, *Bioorg. Med. Chem.*, 2008, **16**, 6995.



Taking the so-called “western part” and the “central core ring” moiety of the compounds constant we investigated the possibility for variations on the substitution pattern of the phenyl moiety as “eastern part” with different electron-releasing and -withdrawing moieties as well as with small and larger substituents of different lipophilic properties. The results of the activity assays using a cell-free 5-LO assay and on whole cells (PMNL cells)² demonstrated the possibility of large variation maintaining inhibitory potency, respectively.

On the one hand the study shows the possibility for large structural variation maintaining affinity, but also clearly demonstrates the difficulties in significantly increasing their antagonist potencies at 5-LO.

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REFERENCES

1. Rådmark et al., *TrendsBiochemSci* **2007**, 32, 332-41.
2. B. Hofmann et al., *ChemMedChem* **2008**, 3, 1535-38.
3. E.M. Zayed et al., *Pharmazie* **1985**, 3, 194-7.

POSTER COMMUNICATIONS – PC.246

THIAZOLONE DERIVATIVES AS INHIBITORS OF 5-LIPOXYGENASE

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The enzyme 5-lipoxygenase (5-LO) plays an important role in the metabolism of arachidonic acid (AA). It catalyzes the reaction of AA into the leukotrienes (LT). Inhibition of 5-LO is a promising option for the treatment of inflammatory diseases, such as asthma or rheumatic arthritis¹. Actually, two drugs admitted for therapy targeting the LT pathway are on the market only: the antagonist of CysLT₁ receptor Montelukast (Singulair®), and the 5-LO inhibitor Zileuton (Zyflo®). So far Zileuton, a direct 5LO inhibitor of the iron-ligand type, is only admitted in the USA. Both drugs are marketed for the therapeutic indication of asthma bronchiale. Due of further medical need for alternative treatment options, the development of new direct inhibitors at 5-LO is of special interest.

Based on very recently disclosed lead structures² we designed and synthesized novel derivatives of thiazolone-containing compounds as direct inhibitors of 5-LO. The disubstituted thiazolone compounds were comfortably prepared by a three component one-pot-synthesis procedure using *p*-methoxybenzaldehyde, thioglycolic acid and differently substituted benzonitriles³.

POSTER COMMUNICATIONS – PC.247

PYRIMIDO[4,5-*d*]AZEPINES AS POTENT AND SELECTIVE 5-HT_{2C} RECEPTOR AGONISTS: THE DISCOVERY OF PF-3246799

Mark D. Andrews, J. Blagg, T. K. Brabham, P. E. Brennan, A. Bridgeland, A. D. Brown, P. J. Bungay, K. M. Conlon, N. J. Edmunds, P. V. Fish, K. J. af Forselles, C. P. Gibbons, M. Green, G. Hanton, M. Holbrook, A. S. Jessiman, G. McMurray, L. R. Roberts, J. A. Root, R. I. Storer, M. R. Sutton, R. V. Ward, D. Westbrook, G. A. Whitlock

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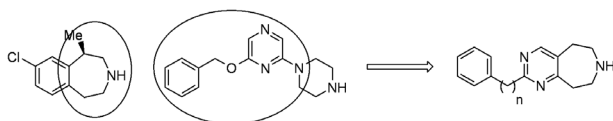
The neurotransmitter serotonin (5-HT) mediates its effects through at least 14 different receptor subtypes that have been classified into seven major families, 5-HT₁₋₇. The 5-HT₂ family has three members 2A, 2B and 2C and unlike 5-HT_{2A} and 5-HT_{2B} receptors, the expression of 5-HT_{2C} receptors appears to be restricted to the central nervous system (CNS). 5-HT_{2C} receptor agonists have become attractive drug targets that have potential use in the treatment of a number of conditions including obesity, schizophrenia, sexual dysfunction and urinary incontinence. For these indications, selectivity over 5-HT_{2A} and 5-HT_{2B} agonism is a key objective as 5-HT_{2A} agonists can potentially be hallucinogenic and have cardiovascular (CV) effects, while 5-

HT_{2B} agonism has been associated with heart valvulopathy and pulmonary hypertension.

The search for potent and selective 5-HT_{2C} agonists has identified lorcaserin (APD-356; Arena) which has been filed with the FDA for the treatment of obesity; vabicaserin (SCA-136; Wyeth/Pfizer), and several other small molecule 5-HT_{2C} agonists that are reported to be in early clinical development.

Pfizer have disclosed several new templates as 5-HT_{2C} receptor agonists and some of these compounds have been progressed to clinical trials. As part of our efforts to identify potential 5-HT_{2C} agonist drug candidates, we adopted a strategy of exploring multiple chemical templates in order to increase our chances of having compounds survive to become advanced clinical candidates. This presentation discloses novel pyrimido[4,5-d]azepines as potent and selective 5-HT_{2C} receptor agonists,¹ and includes:

- The identification of the lead series by "template hopping".
- Discussion of changes in structural features and physicochemical properties that effect CNS permeability.
- The exciting preclinical profile of PF-3246799, including efficacy data in animal models, pharmacokinetic and safety data and a comparison with the profile of lorcaserin.



REFERENCES

1. Pyrimido[4,5-d]azepine derivatives as 5HT_{2C} receptor agonists. M. D. Andrews, J. Blagg, P. Brennan, P. V. Fish, L. R. Roberts, R. I. Storer, G. A. Whitlock. **2008**, WO 117169.

POSTER COMMUNICATIONS – PC.248

DISCOVERY OF D-ASPARTATE LIGASE INHIBITORS BY VIRTUAL SCREENING

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D-Aspartate ligase is an enzyme involved in the peptidoglycan assembly pathway of Gram-positive bacteria, such as multiresistant enterococci and appears as an attractive target for the design of new narrow-spectrum antibacterial agents active against multiresistant *E. Faecium*. Aspartate ligase is a member

of the ATP-grasp protein superfamily, which includes a diverse set of enzymes catalyzing the ATP-dependent formation of carbon-nitrogen bond referred to as carboxylate-amine ligases. The role of aspartate ligase in peptidoglycan synthesis is to ligate the β-carboxylate of D-Asp to the ε-amino group of L-Lys in the nucleotide precursor UDP-*N*-acetylmuramyl-pentapeptide.

Virtual screening of ZINC database guided by 3D shape and electrostatics surface similarity searching using the ROCS and EON software programs was performed based on the structure of β-D-Asp-phosphate and dipeptide L-Lys-β-D-Asp. All known promiscuous inhibitors and compounds containing metal ions or reactive groups were excluded and a total of 50 compounds from virtual screen of 240000 compounds were selected for biological evaluation. By this approach we were able to identify the first inhibitors of D-aspartate ligase, which are a good starting point for further studies on our target enzyme.

REFERENCES

1. Staudenbauer WL, Strominger JL: Activation of D-Aspartic acid for incorporation into peptidoglycan, *J. Biol. Chem.*, **1972**, 247, 5095-5102
2. Staudenbauer WL, Willoughby E, Strominger JL: Further studies of the D-Aspartic acid-activating enzyme of *Streptococcus faecalis* and its attachment to the membrane, *J. Biol. Chem.*, **1972**, 247, 5289-5296
3. Bellais S, Arthur M, Dubost L, Hogunnet J, Gutmann L, Heijenoort J, Legrand R, Brouard J, Rice L, Mainardi J: Asl_{fm}, the D-aspartate ligase responsible for the addition of D-aspartic acid onto the peptidoglycan precursor of *Enterococcus faecium*, *J. Biol. Chem.*, **2006**, 281, 11586-11594

POSTER COMMUNICATIONS – PC.249

NICOTINIC ACID DERIVATIVES AS PRECURSORS OF THIOPEPTIDE ANTIBIOTICS

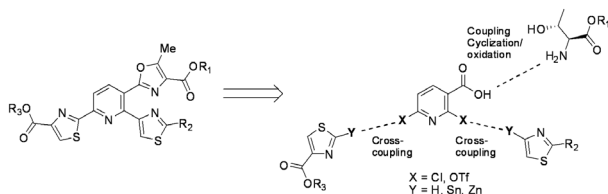
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The thiopeptide class of antibiotics¹ has been the centre of much attention during the last years due to the need of new antibacterial agents required to face the antibiotics resistance by strains of bacteria that were not thought to be a threat these days. These natural products, which have been extracted from both soil and marine microorganisms, are highly active inhibitors of protein synthesis in Gram-positive bacteria, and in many cases are effective even against methicillin-resistant *Staphylococcus aureus*, a strain usually resistant to most conventional treatments. Thus, thiopeptides represent a source of new structural diversity and modes of action that offer a unique opportunity to study the protein synthesis and ribosomal machinery in bacteria.



Properly functionalised nicotinic acid derivatives serve as precursors of the 2,3,6-trisubstituted thiopeptide antibiotics of the d series. The adequate halogen substitution pattern on the pyridine and the thiazole cross-coupling partners allow for the synthesis of the desired natural products in a highly convergent manner, thus granting access to analogues that should permit the study of the Structure-Activity Relationships of this family of compounds.

REFERENCES

1. Bagley, M. C.; Dale J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, 105, 685. (b) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, 46, 7930.

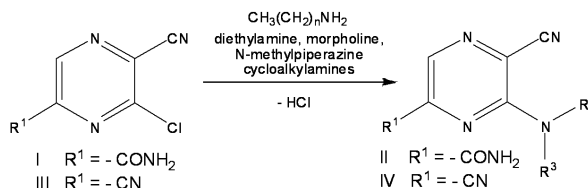
POSTER COMMUNICATIONS – PC.250

SYNTHESIS AND ANTI-INFECTIVE PROPERTIES OF SOME PYRAZINAMIDE ANALOGUES

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The widespread of Mycobacteria strains resistant to current treatment methods is an important epidemiological problem recognized by WHO¹. Pyrazinamide, the first line antituberculous substance, is used as a template model for compounds involved in this research project. Some pyrazinamide analogues, 3-arylaminopyrazine-2,5-dicarbonitriles, have already demonstrated good antimycobacterial and/or antifungal activity in a previous study². 6-chloro-5-cyanopyrazine-2-carboxamide (**I**) treated with various non-aromatic amines yielded **N-substituted 6-amino-5-cyanopyrazine-2-carboxamides (II)**. Similarly, the dehydration product of (**I**), 3-chloropyrazine-2,5-dicarbonitrile (**III**), yielded **N-substituted 3-aminopyrazine-2,5-dicarbonitriles (IV)**. Prepared compounds were chemically characterized and screened for antimycobacterial (*Mycobacterium tuberculosis* Rv H37, *M. tuberculosis* wild stem, *M. kansasii*, *M. avium* 80/72, *M. avium* 152/73) and antifungal activity (*in vitro* testing against *Candida albicans*, *C. tropicalis*, *C. crusei*, *C. glabrata*, *Trichosporon asahii*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and *Absidia corymbifera*).



Starting compound (**III**) possessed better antimycobacterial effect (MIC = 100 µg.ml⁻¹) against non-typical Mycobacteria (*M. avium* 80/72, *M. avium* 152/73) than pyrazinamide (MIC > 200 µg.ml⁻¹). Some other compounds of type (**II**), e.g. 6-heptylamino-5-cyanopyrazine-2-carboxamide (**II**, R¹ = -CONH₂, R² = heptyl, R³ = H) exhibited equal or slightly better activity (MIC ≤ 12.5 µg.ml⁻¹) against *Mycobacterium tuberculosis* Rv H37 than pyrazinamide.

3-heptylamino-5-cyanopyrazine-2,5-dicarbonitrile (**IV**, R¹ = -CN, R² = heptyl, R³ = H) showed a specific activity against *Trichophyton mentagrophytes* (MIC = 3.9 µmol.l⁻¹, standard ketoconazole MIC = 0.98 µmol.l⁻¹), and did not inhibit the growth of other tested fungi species (MIC > 500 µmol.l⁻¹).

This study was supported by the Grant Agency of Charles University (**BCH/120509**). The presentation was supported by ZENTIVA, k.s., Czech Republic.

REFERENCES

1. Anti-tuberculosis drug resistance in the world, Fourth Global Report, WHO/HTM/TB/2008.394, Accessed at: http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf, November 5th, 2008
2. Palek, L.; Dvorak, J.; Svobodova, M.; Buchta, V.; Jampilek, J.; Dolezal, M. *Arch. Pharm.* **2008**, 341, 61

POSTER COMMUNICATIONS – PC.251

A STUDY ON NEW INHIBITORS OF BHMT AND BHMT-2

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Betaine-homocysteine S-methyltransferase (BHMT) and betaine-homocysteine S-methyltransferase 2 (BHMT-2) are enzymes expressed in the liver and kidney of mammals where they are responsible for the conversion of homocysteine into methionine. Both BHMT and BHMT-2 are Zn-metalloenzymes and the Zn-cation was shown to be essential for their activity.^{1,2} While BHMT catalyzes a methyl transfer from betaine to L-Hcy to form dimethylglycine and L-Met, BHMT-2 methylates L-Hcy using S-methyl-L-methionine (SMM), Fig. 1.

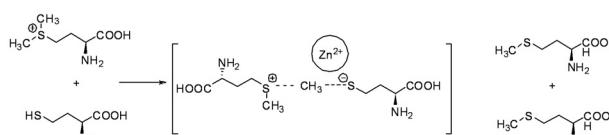


Fig. 1: Reaction catalyzed by BHMT-2

Recently, we synthesized and tested a series of strong BHMT inhibitors mimicking the hypothetical transition state.³ In contrary, BHMT-2 is a much less studied enzyme and up to date, no potent inhibitors are available. A series of rationally designed inhibitors can be very useful tools to study the physiological functions of the BHMT-2 *in vivo* and to understand the structural factors that govern substrate, product or inhibitor binding and/or the catalysis of the enzyme.

Herein, we present a series of first inhibitors of BHMT-2 developed recently in our laboratory and compare their properties to BHMT inhibitors described previously.

REFERENCES

1. Pajares, MA; Perez-Salab, D: Betaine-homocysteine S-methyltransferase: Just a regulator of homocysteine metabolism? *Cell. Mol. Life Sci.* **2006**, 63, 2792-2803.
2. Szegedi, S. S; Castro, C; Koutmos, M; Garrow, TA: Betaine-homocysteine S-methyltransferase-2 is an S-methylmethionine-homocysteine methyltransferase. *J. Biol. Chem.* **2008** 283(14), 8939-8945.
3. Vaněk, V; Buděšínský, M; Kabeleová, P; Šanda, M; Kožíšek, M; Hančlová, I; Mládková, J; Brynda, J; Rosenberg, I; Koutmos, M; Garrow, TA; and Jiráček, J: Structure-Activity Study of New Inhibitors of Human Betaine-Homocysteine S-Methyltransferase. *J. Med. Chem.* **2009**, 52, 3652-3665.

POSTER COMMUNICATIONS – PC.252

STRUCTURAL AND THEORETICAL STUDY OF HIV-1 INTEGRASE INHIBITION: ELUCIDATION OF QUINOLONYL DIKETOACID BINDING MODE

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Human immunodeficiency virus (HIV-1) is the causal agent of acquired immunodeficiency syndrome (AIDS). The mortality and the morbidity of HIV-1 infection led to the design of antiviral agents targeting different steps in the viral replication process. HIV-1 integrase is an especially attractive target for HIV

chemotherapy because the enzyme is essential for viral infectivity by catalyzing the insertion of viral DNA into the host cell genome through a metal ion dependent process^[1].

More recently, some diketoacid derivatives containing the quinolone scaffold have been reported^[2,3]. Based on this series, we synthesized two quinolone compounds containing a β -diketoacid/ester group as chelating moiety (Figure 1). Both compounds were evaluated for their enzymatic and antiviral activity, acidic compound **2** being more potent than ester compound **1**. X-ray diffraction, UV-visible spectroscopy studies and theoretical calculations were performed in order to identify the most stable conformations of the diketoacid moiety and to study the chelation mode. In order to characterize the binding mode of compound **2**, a model of the enzyme was built by using the very recent crystal structure of PFV integrase^[4]. Based on these docking studies, pharmacomodulations were proposed in order to increase the inhibitory potency of the series of HIV-1 integrase inhibitors.

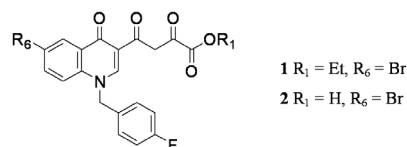


Figure 1

REFERENCES

1. Delelis, O. *et al.* *Retrovirology* 2008, 5,114.
2. Di Santo, R. *et al.* *J. Med. Chem.* 2006, 49, 1939.
3. Di Santo, R. *et al.* *J. Med. Chem.* 2008, 51, 4744.
4. Hare, S. *et al.* *Nature* 2010, 464, 232.

POSTER COMMUNICATIONS – PC.253

SYNTHESIS OF NUCLEOSIDE 5'-PHOSPHONATE ANALOGUES AS POTENTIAL P2Y₂ RECEPTOR LIGANDS

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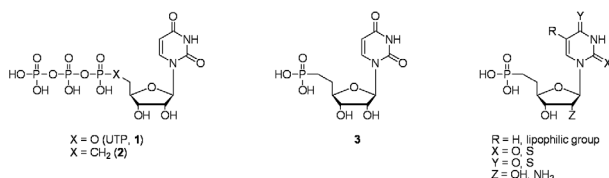
P2Y receptors are a family of eight class A GPCRs that respond to different nucleotides. The human P2Y₂ receptor is activated equipotently by both UTP and ATP. This receptor subtype is distributed in a broad range of tissues¹ and plays important physiological roles in epithelial cells of the lung, gastrointestinal tract and the eye. P2Y₂ receptors are targets for several therapeutic approaches, some of which are currently being explored. Agonists are promising for cystic fibrosis, cancer and dry eye syndrome^{1,2} while P2Y₂ antagonists exhibit anti-inflammatory³ and neuroprotective effects.⁴

The natural ligand UTP (**1**), as well as most reported analogs thereof, are liable to enzymatic degradation at the surface of the airways. This results in a relative short time of action when these molecules are given by means of inhalation. Changing the α -phosphate group for an isosteric phosphonate could raise up the metabolic stability.²

While we initially focused on a diphosphophosphonate mimic of UTP (**2**), it was accidentally discovered that the synthetic precursor **3** was also capable of activating the P2Y₂ receptor, possibly through an allosteric mechanism, while being inactive at the P2Y₄ receptor.²

From the growing series of UTP analogs that have been screened on P2Y receptors, it is known that a 2-thio and a 4-thio modification raise the affinity for the P2Y₂ receptor, while the former also enhances the selectivity vs the P2Y₄ receptor.⁵

Here, we decided to combine these uracil modifications and/or the selectivity-enhancing 2'-amino-2'-deoxy substitution with a 5'-phosphonate group. The synthesis of 5'-modified 5'-phosphonate analogues is another goal.



REFERENCES

1. Kellerman, D.; Evans, R.; Mathew, D.; Shaffer, C. *Adv. Drug Deliv. Rev.* (2002), **54**, 1463-1474.
2. Cosyn, L. et al. *Bioorg. Med. Chem. Lett.* (2009), **19**, 3002-3005.
3. Morse, D. M.; Smullen, J. L.; Davis, C. W. *Am. J. Physiol. Cell. Physiol.* (2001), **280**, 1485.
4. Volonte, C. et al. *Neuropharmacology* (1999), **38**, 1335-1342.
5. Ko, H. et al. *Bioorg. Med. Chem.* (2008), **16**, 6319-6332.

POSTER COMMUNICATIONS – PC.254

HEMODYNAMIC EFFECTS OF LEVOSIMENDAN DURING ISCHEMIA/REPERFUSION IN THE ISOLATED PERFUSED RAT HEART

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Levosimendan exerts a coronary and systemic vasodilator effect through its K_{ATP} channel-opening properties and this action may be of specific interest in the setting of myocardial ischemia. The aim of this experimental study was to investigate whether levosimendan has protective effects on myocardial ischemia-reperfusion injury.

After anesthesia rats heart were removed placed in ice-cold Krebs-Heinseleit and mounted on Langendorff apparatus. Hearts were assigned into three groups: first, after 30 min ischemia in order to induce, ischemic postconditioning 3 times 10 second ischemia separated by 10 second reperfusion protocol was used; second, control; and third, 10^{-7,8,9} mol levosimendan was added to the reperfusion fluid after 30 min ischemia in order to induce chemical postconditioning. In all these groups, 30 min global ischemia was followed by 120 min reperfusion. In all experiments, hemodynamic parameters (LVDP, LVEDP, $\pm$ dP/dt, RPP) were evaluated at 30th and the 120th minutes of reperfusion. Left ventricular pressure was measured with a water-filled balloon. After ischemia, the heart tissues were dyed with TTC for infarct size measurement.

LVDP, LVEDP and $\pm$ dP/dt values were similar to the pre-ischemia in ischemic postconditioning and levosimendan (10⁻⁸mol) groups at 30th minute of reperfusion. However at the 120th minute, the values were reduced in the same groups. The infarct size was significantly reduced in levosimendan and ischemic postconditioning groups compared to the control group.

The results showed that, levosimendan and ischemic postconditioning may provide cardioprotection over the myocardial ischemia reperfusion injury.

POSTER COMMUNICATIONS – PC.255

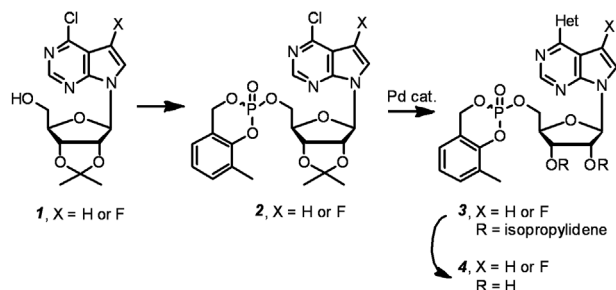
SYNTHESIS AND BIOLOGICAL ACTIVITIES OF CYCLOSAL-PRONUCLEOTIDES DERIVED FROM 6-HETARYL-7-DEAZAPURINE RIBONUCLEOSIDES

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The usage of prodrugs plays a key role in antiviral and antitumor drug development. The *cycloSal* approach is a successful method used for improvement of antiviral activities of nucleosides.¹ Recently, a series of 6-hetaryl-7-deazapurine nucleosides with cytotoxic activity in nanomolar range was synthesized in our group.² The aim of this project was to improve the activity of these derivatives using *cycloSal*-phosphate prodrugs. The 2',3'-isopropylidene 6-chloro-7-deazapurine ribonucleosides **1** were converted to *cycloSal*-pronucleotides **2**. Using Suzuki and Hille cross-coupling reactions of *cycloSal*-pronucleotides **2** with hetarylboronic acids or -stannanes a series of isopropylidene protected 6-hetaryl-7-deazapurine pronucleotides **3** was prepared. These were further deprotected to give the derivatives of free pronucleotides **4**. All the compounds were obtained as 1:1 mixtures of diastereomers as a result of non-stereoselective introduction of the centre of chirality to phosphorus atom. All 12 target compounds **4** were tested for their *in vitro* cytotoxic activity on various cell lines. Mostly, the compounds showed similar or

lower activities compared to the parent nucleosides. Only in the case of three 7-fluoro pronucleotides the cytotoxic activities were improved by one order of magnitude.



This work is a part of the research project from the Academy of Sciences of the Czech Republic Z4 055 0506. It was supported by the Ministry of Education, Youth and Sports (grant 1M0508) and by Gilead Sciences, Inc

REFERENCES

1. Meier C.: Eur. J. Org. Chem. 2006, 1081.
2. Nauš P., Pohl R., Votruba I., Džubák P., Hajdúch M., Ameral R., Birkus G., Wang T., Ray A.S., Mackman R., Cihlar T., Hocek M.: J. Med. Chem. 53, 460 (2010).

experiments, STD NMR, and by monitoring the $^1\text{H}/^{13}\text{C}$ chemical shift changes of MurD selectively labeled with ^{13}C at the methyl groups of Ile, Val, and Leu upon binding of ligands².

REFERENCES

1. Humljan J., Kotnik M., Contreras-Martel C., Blanot D., Urleb U., Dessen A., Solmajer T., Gobec S. *J. Med. Chem.* **2008**, 51: 7486-7494.
2. Hajduk P. J., Augeri D. J., Mack J., Mendoza R., Yang J., Betz S. F., Fesik S. W. *J. Am. Chem. Soc.* **2000**, 122: 7898-7904.

POSTER COMMUNICATIONS – PC.257

CONFORMATIONAL INSIGHTS INTO A RIGID BODY C-TERMINAL DOMAIN MOVEMENT OF MurD LIGASE

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Bacterial resistance is a worrying phenomenon that urges the need to discover new antibacterial drugs. Since peptidoglycan is a unique component of the cell wall of almost all eubacteria, its biosynthetic pathways represent an attractive targets for new drugs.¹ ATP dependant enzymes of the Mur ligase family have an essential role in peptidoglycan biosynthesis as the stepwise addition of amino acids to the UDP-*N*-acetyl muramic acid to yield UDP-MurNAc pentapeptide is catalyzed by them. MurD (UDP-*N*-acetylmuramoyl-L-alanine: D-glutamate ligase) is the second enzyme in the series of Mur ligases, and catalyzes the addition of D-glutamic acid to UDP-*N*-acetylmuramoyl-L-alanine (UMA).²

MurD has been structurally very well characterized as several crystal structures of apo-enzyme and enzyme co-crystallized with natural substrates have been solved.^{3,5} Structures reveal that a rigid body rotation of the MurD C-terminal domain occurs and its position defines the so called closed and open conformation of the protein.^{4,5} Previous structural studies revealed that, MurD co-crystallized with UMA and 5'-adenylylimido-diphosphate (AMPPNP: A non-hydrolyzable analogue of ATP), was in the closed conformation⁴, on the contrary MurD co-crystallized with UMA or in its apo-form was in the open conformation⁵.

We discovered two new conformations of MurD, which are not in line with previous findings: Closed conformation of apo-MurD and a structure of MurD in an open conformation co-crystallized with UMA and AMPPNP.

POSTER COMMUNICATIONS – PC.256

NOVEL RIGIDIFIED INHIBITORS OF MURD

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MurD is the second enzyme in the series of Mur ligases. It catalyses the addition of D-glutamic acid to the cytoplasmic intermediate UDP-*N*-acetylmuramoyl-L-alanine (UMA). A series of naphthalene *N*-sulfonyl-D-Glu derivatives previously synthesized and the high-resolution crystal structures of MurD from *E.coli* in complex with four small molecule inhibitors revealed details of the binding mode in the active site of MurD¹. An insight into structure-activity relationship presented a solid foundation for optimization of inhibitors of this structural class and was the main reason for the synthesis of novel sulfonamide compounds where a rigid surrogate of D-glutamic acid moiety was incorporated in the structure.

Three compounds displayed significant inhibitory activity, with an IC_{50} values of 38, 46, and 50 μ M, respectively. In addition these compounds were extensively studied with high resolution NMR spectroscopy, in order to determine binding to the enzyme and the location of compounds in protein binding site. This was done by using several different NMR techniques, such as trNOE

New structural information on MurD represents an important contribution to design of novel inhibitors by implementation of computer-aided approach. Furthermore, the loop which is important for the C-terminal domain movement could be used as a potential target of novel allosteric inhibitors, which could prevent the C-terminal domain to rotate and consequently inactivate the enzyme's function.

REFERENCES

1. van Heijenoort J. *Nat. Prod. Rep.* 200; 18(5): 503-19.
2. Barreteau H, Kovač A, Boniface A, Sova M, Gobec S, Blanot D. *FEMS Microbiol. Rev.* 2008; 32(2): 168-207.
3. Bertrand JA, Auger G, Fanchon E, Martin L, Blanot D, van Heijenoort J, Dideberg O. *EMBO J.* 1997; 16(12): 3416-25.
4. Bertrand JA, Auger G, Martin L, Fanchon E, Blanot D, Le Beller D, van Heijenoort J, Dideberg O. *J. Mol. Biol.* 1999; 289(3): 579-90.
5. Bertrand JA, Fanchon E, Martin L, Chantalat L, Auger G, Blanot D, van Heijenoort J, Dideberg O. *J. Mol. Biol.* 2000; 301(5): 1257-66.

POSTER COMMUNICATIONS – PC.259

NEW PHENYLALKYLAMINES AS ISOFORM SELECTIVE HCN-CHANNELS BLOCKERS

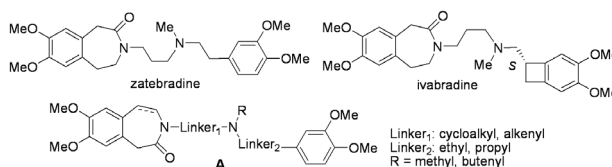
M. Novella Romanelli,^{1,4} Michele Melchiorre,¹ Luca Guandalini,¹ Martina Del Lungo,² Silvia Dei,² Dina Manetti,¹ Serena Scapecchi,¹ Elisabetta Teodori,¹ Elisabetta Cerbai,² Laura Sartiani,² Alessandro Mugelli,² Andras Varro,³

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The "funny current" (I_f) plays a major role in the generation of pacemaker activity and in the regulation of heart rate. [I_f] is carried by the HCN (Hyperpolarization-activated Cyclic Nucleotide gated) channels, a family of membrane proteins formed by four members (HCN1-4) which are differently distributed within tissues such as heart and nervous system. [2] Beside heart, HCN channels regulate rhythmic activity in other excitable cells such as neurons and photoreceptors, they have a role in dendritic integration, long term potentiation, synaptic transmission. [3] The different isoforms vary in their distribution within tissues; therefore, isoform-selective substances could be important pharmacological tools to study the channels in native tissues, and may be useful to design safe drugs. [3]

I_f can be blocked by the so-called «specific bradycardic agents», a group of structurally-diverse drugs among which are zatebradine and ivabradine. Ivabradine is the only specific bradycardic agent available for therapy: in 2005 it has been approved by EMEA for the treatment of stable *angina pectoris*. However, this drug, as well as its congener zatebradine, is not able to discriminate among

HCN channel isoforms. [4] In order to search for isoform-selective I_f blockers, new phenylalkyl amines structurally related to zatebradine have been prepared (general formula **A**). These compounds were tested for their I_f blocking ability on HEK293 cells stably expressing the HCN1, HCN2 and HCN4 channels. It was found that structural manipulations can lead to molecules showing isoform selectivity, the best selectivity profile being toward HCN1 or HCN4. The most interesting compounds were further characterized on heart by electrophysiological studies.



REFERENCES

1. DiFrancesco, D. *Pharmacol. Res.* **2006**, 53, 399-406.
2. Herrmann, S.; Stieber, J.; Ludwig, A. *Pflugers Arch. Eur. J. Physiol.* **2007**, 454, 517-522
3. Wahl-Schott, C.; Biel, M. *Cell Mol. Life Sci.* **2008**,
4. Stieber, J.; Wieland, K.; Stockl, G.; Ludwig, A.; Hofmann, F. *Mol. Pharmacol.* **2006**, 69, 1328-1337

POSTER COMMUNICATIONS – PC.260

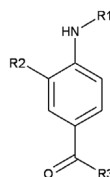
SYNTHESIS OF NOVEL BENZOIC ACID DERIVATIVES AS POTENTIAL ANTICHAGAS AGENTS

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Chagas disease or American Trypanosomiasis is caused by *Trypanosoma cruzi*, and is one of the most important parasitic diseases in Latin America. Currently chemotherapy is only based in nifurtimox or benznidazole, but it has limited efficacy in the chronic stage of the disease and produce toxic side effects. Parasite proteins as trypanothione reductase, trans-sialidase and cruzain cysteine protease among others are key enzymes in the metabolism of *Trypanosoma cruzi* and recently have been proposed as potential drug targets for Chagas disease. Particularly, trans-sialidase from *Trypanosoma cruzi* (TcTS) has emerged as a potential drug target for pharmacological treatment of Chagas disease (1-2). As a part of our ongoing research in the design and synthesis of new antichagas drug candidates, here, we report a series of novel benzoic acid derivatives

(Scheme 1.) that were tested *in vitro* against the trypomastigote form of *Trypanosoma cruzi* (NINOA strain).



Scheme 1. General structure of novel benzoic acid derivatives as potential antichagas agents.

Structure-activity relationship analysis showed that carbonyl or sulfonyl derivatives in position R1 on a 4-amino group decreases compounds activity. Alternatively, only a 4-amino group on aromatic ring enhanced the biological activity. Additionally, a nitro group in position R2 increases the activity compounds. On the other hand, Neres and cols reported benzoic acid derivatives as TcTS (1), thus we included novel substitutions in position R3 to determine their influence in biological activity. The most active compound P-057 showed a 97 percentage of trypomastigotes lysis and both reference drugs, nifurtimox and benznidazole showing a percentage lysis of 46 and 47, respectively, tested at same concentration. In conclusion, these novel benzoic acid derivatives could be used as lead compounds for design and synthesis of new series of promising potential antichagas agents.

REFERENCES

1. Neres J., Bonnet P., Edwards PN., Kotian PL., Buschiazio A., Alzari PM., Bryce RA., Douglas KT. *Bioorganic & Medicinal Chemistry*. 2007, 15(5), 2106-2119.
2. Neres J., Brewster ML., Ratier L., Botti H., Buschiazio A., Edwards PN., Mortenson PN., Charlton MH., Alzari PM., Frasch AC., Bryce RA., Douglas KT. *Bioorganic & Medicinal Chemistry*. 2009, 19(3), 589-596.

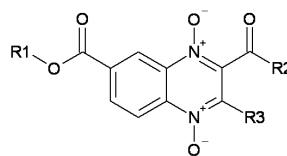
POSTER COMMUNICATIONS – PC.261

SYNTHESIS OF NOVEL QUINOXALINE DERIVATIVES AS POTENTIAL ANTITUBERCULOSIS AGENTS

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The importance of heterocyclic compounds has been long time recognized in the field of synthetic organic chemistry. Quinoxalines, including their fused-ring derivatives, showed various pharmacological activities and particularly, their 1,4-di-*N*-oxides derivatives have demonstrated excellent efficacy as antiviral, anticancer, antibacterial, and antiparasitic agents (1-2). As a part of our ongoing research in the design and synthesis of new antituberculosis drug candidates, two new series of quinoxaline 1,4 di-*N*-oxide derivatives with carboxylate substituent in position R1 have been synthesized (Scheme 1.) and tested *in vitro* against *Mycobacterium tuberculosis*. Biological evaluation was carried out within the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program (3). Benzofuroxane 5-substituted and quinoxaline 1,4 di-*N*-oxide derivatives were obtained according to the synthetic methods previously described (4).



Scheme 1. General structure of novel quinoxaline derivatives.

Results of these compounds showed a good antituberculosis activity in the first level screening with IC₉₀ values of ≤10 µg/mL. Structure-activity relationship analysis showed that compounds with electron withdrawing substituent such as trifluoromethyl group in position R3 had better activity than compounds with methyl group substituent. Also, compounds activity is significantly affected when trifluoromethyl substituent on the quinoxaline nucleus is replaced by a rigid structure or electron donating group such as phenyl group and methyl group in position R2 and/or R3, respectively, showed low or not activity. Additionally, when a substitution at *para* position on phenyl ring with a chlorine atom was carried out, resulted in an enhanced activity compounds. Statistical significant differences were not found between the anti-mycobacterial activities in the two series obtained. In conclusion, these quinoxaline derivatives could be used as lead compounds for design and synthesis of new series of new potentially antituberculosis agents.

REFERENCES

1. Gonzalez M, Cerecetto H, Monge A. *Topics in Heterocyclic Chemistry*. 2007, 11, 179-211.
2. Khan SA, Saleem K, Khan Z. *European Journal of Medicinal Chemistry*. 2008, 43, 2257-2261.
3. <http://taacf.org/Process-text.htm#IC90>
4. Jaso A, Zarranz B, Aldana I, Monge A. *European Journal of Medicinal Chemistry*. 2003, 38, 791-800.

POSTER COMMUNICATIONS – PC.262

IDENTIFICATION OF BINDING MODES OF BENZODIAZEPINE BINDING SITE LIGANDS BY A COMBINED DOCKING-PHARMACOPHORE MODELING APPROACH

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Ligand/protein docking uses energetic scoring functions for evaluating and ranking poses. However, if the precise induced fit structure is not known, as it is the case in the benzodiazepine binding site (bz-site) of the GABAA receptor, docking results are often unsatisfying and lead to ambiguous binding mode hypotheses of the ligands. In contrast, ligand-based pharmacophore models are protein and binding mode independent models which represent the mutual alignment of structurally different, biologically active ligands. Thus, the mutual orientation of different ligands which is provided by the pharmacophore model should be recovered in the poses produced by ligand-protein docking as the pharmacophore-supported binding mode (1). We developed a methodology within MOE (Chemical computing Group) using the SVL programming language which automatically screens for this pharmacophore-supported binding mode in the bz-site of the GABAA receptor.

In the first step, a set of rigid ligands is selected for docking from the widely accepted pharmacophore model of the benzodiazepine binding site (2). We prefer ligands which are biologically characterized by mutagenesis or labeling data. Subsequently, the selected ligands are docked and the energetically plausible poses of each ligand are retained and stored within a database. The search for the pharmacophore-supported binding mode starts with selecting an index ligand (in our case diazepam) from the pharmacophore model. At the beginning each diazepam pose represents a candidate binding mode. Each proposed binding mode of diazepam serves as template for superposition of other compounds (reference ligands like β -Carbolines or Imidazopyridines) based on the alignment in the common pharmacophore model. In the next step the docking poses of each reference ligand are compared with their respective orientation in the pharmacophore-based alignment by calculating the RMSD-value of the atoms characterizing the pharmacophoric features. The binding mode which performs best is considered as the pharmacophore-supported binding mode and further validated using experimental data.

The introduced methodology enabled us to identify the binding mode of benzodiazepine ligands within the bz-site of the GABAA receptor.

ACKNOWLEDGMENT

Lars Richter is grateful to the Austrian Academy of Sciences for financial support under the framework of the DOC programme (grant 2021)

REFERENCES

1. Zhang W, et al. Development of a comprehensive pharmacophore model for the benzodiazepine receptor. *Drug Des Discov* 1995;12:193-248
2. Wallach I, Lilien R. Predicting Multiple Ligand Binding Modes Using Self-Consistent Pharmacophore Hypotheses. *J Chem Inf Model* 2009;49:2116-28

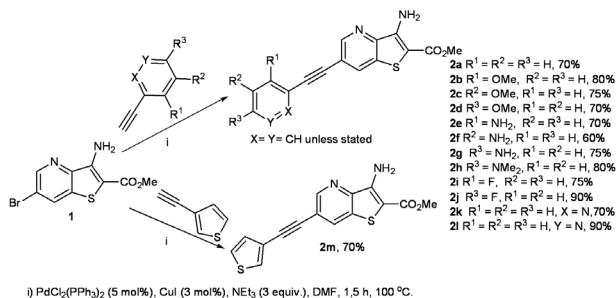
POSTER COMMUNICATIONS – PC.263

SYNTHESIS AND TUMOR CELL GROWTH INHIBITION OF NOVEL METHYL 3-AMINO-6-[(HETERO)ARYLETHYNYL]THIENO [3,2-*b*]PYRIDINE-2-CARBOXYLATES. SARs AND EFFECTS ON THE CELL CYCLE AND APOPTOSIS

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Several novel methyl 3-amino-6-[(hetero)arylethynyl]thieno[3,2-*b*]pyridine-2-carboxylates were prepared by Sonogashira coupling of the methyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate **1**, recently reported by some of us,¹ with (hetero)aryl-acetylenes in high yields (Scheme).



The growth inhibition of all the compounds was evaluated on three human tumour cell lines (MCF-7, NCI-H460, A375-C5) and some SARs were established. Compounds **2d**, **2e** and **2g** are the most promising compounds presenting the lowest GI_{50} values.

Their effects on the normal cell cycle distribution and induction of apoptosis were studied in the NCI-H460 cell line.² All three compounds altered cell cycle distribution and compound **2e** was further shown to induce apoptosis in the same cell line.

ACKNOWLEDGMENTS

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REFERENCES

1. Calhella, R. C.; Queiroz, M.-J. R. P. *Tetrahedron Lett.* 2010, 51, 281-283.
2. Almeida G. M.; Duarte T. L.; Farmer P. B.; Steward W. P.; Jones G.D.D. *Int. J. Cancer* 2008, 122, 1810-1819.

POSTER COMMUNICATIONS – PC.264

PASS BASED VIRTUAL SCREENING OF DRUG-LIKE COMPOUNDS

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The computer program PASS (Prediction of Activity Spectra for Substances) is the product of ideas that originated about forty years ago from the development of a National Registration System for New Chemical Compounds in the former USSR. To select the most promising hits among the newly synthesized compounds submitted for registration, their structural formulae must be analyzed using computer-assisted methods. The current version of PASS 10.1 predicts 4130 kinds of biological activity with average accuracy about 95% based on the analysis of the training set included 266697 drugs, drug-candidates, leads and toxic compounds (in 1993 the appropriate numbers were 114, 9314 and 75%, respectively). Detailed description of PASS approach is presented in the paper [1]. PASS predictions have been obtained for about 250000 molecules from Open NCI database [2] and presented at the NCI web-site (<http://cactus.nci.nih.gov/ncidb2/>). It was shown that, using PASS for virtual screening, it is possible to increase significantly the number of “actives” in the selected sub-set of compounds (85 from 250000 compounds selected as hits, 7 were tested and 4 appeared to be the angiogenesis inhibitors) [2]. In another project medicinal chemists from six countries jointly prepared a virtual library of about 5500 compounds belonging to different chemical classes. 30 hits have been selected for synthesis and experimental testing, and all of them appeared to be anxiolytics, anticonvulsants and cognition enhancers that confirmed the PASS predictions [3,4]. Since 2000 PASS INet service is freely available via Internet (<http://www.ibmc.msk.ru/PASS>), and over 6000 reg-

istered users from 60 countries obtained PASS predictions for more than 120000 compounds. Results of PASS predictions confirmed by the experiment have been published in more than forty papers (for review see [5]). It was demonstrated that some adverse and toxic effects can be also predicted by PASS [6]. Based on the PASS predictions, multitargeted antihypertensive [7] and anti-inflammatory [8] agents have been identified in virtual libraries of chemical compounds. PASS applicability to the analysis of biological activities of phytoconstituents from medicinal plants has been recently shown [9]. We will present an overview of PASS applications in medicinal chemistry for both virtual screening and design of pharmaceutical agents with the required properties.

ACKNOWLEDGMENT

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REFERENCES

1. Filimonov D.A. and Poroikov V.V. (2008). In: *Chemoinformatics Approaches to Virtual Screening*. Eds. Alexandre Varnek and Alexander Tropsha. Cambridge (UK): RSC, p.182-216.
2. Poroikov V.V. et al. *J. Chem. Inform. Comput. Sci.*, 2003, **43**; 228-236.
3. Geronikaki A. et al. *J. Med. Chem.* 2004, **47**: 2870-2876.
4. Geronikaki A. et al. *Bioorg. Med. Chem.*, 2004, **12**: 6559-6568.
5. Geronikaki A. et al. *SAR & QSAR Environ. Res.*, 2008, **19**: 27-38.
6. Lagunin A. et al. *QSAR and Combinatorial Science*, 2009, **28**: 806-810.
7. Lagunin A.A. et al. *J. Med. Chem.*, 2003, **46**: 3326-3332.
8. Geronikaki A.A. et al. *J. Med. Chem.*, 2008, **51**: 1601-1609.
9. Lagunin A. et al. *Curr. Pharm. Des.*, 2010, in press.

POSTER COMMUNICATIONS – PC.265

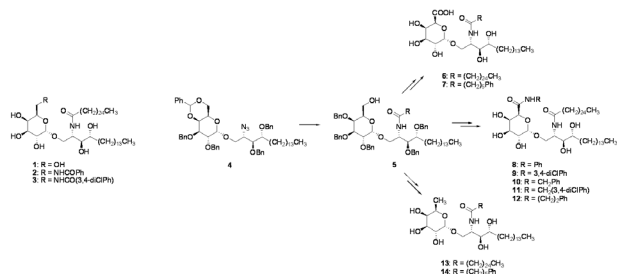
VERSATILE SYNTHESIS OF C-5'-MODIFIED α -GALCER ANALOGUES AS NEW NKT-CELL LIGANDS

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α -Galactosylceramide (**1**), a synthetic glycosphingolipid structurally based on substances extracted from the marine sponge *Agelas mauritanus*, exhibits antitumor activity in mice. Following complexation with the antigen presenting glycoprotein CD1d, α -GalCer is recognized by NKT-cells, which, after activation, release both Th1 and Th2 cytokines. Th1 cytokines are thought to be responsible for the antitumor, antiviral and antibacterial effects of α -GalCer while Th2 cytokines can ameliorate autoimmune diseases. Unfortunately, both responses tend to antagonize each other's biological effect. Hence analogues capable of inducing a Th1/Th2 biased response are highly awaited.

Towards this end, numerous analogues of α -GalCer have been synthesized. Alterations of the galactopyranose ring indicate that the hydroxyls at the 2' and 4' positions of the sugar are critical for biological activity, while modifications of the 3' and 6' positions are more permissive.¹ Modifications at C-6' are possible without loss of antigenic activity,^{2,3} but usually require a laborious synthetic route.⁴ Inspired by the Th1 biased response of certain C-6'-derivatised analogues (**2-3**), designed earlier in our laboratory,³ we decided to further explore modifications at this position. A selective opening of the benzylidene ring of **4** was the key step for a more divergent synthetic approach, in which the C-6'-OH group was modified after the glycosylation. The key intermediate **5** gave access to galacturonic acid derivatives **6-7**, the corresponding uronamides **8-12**, as well as two 6'-deoxygenated analogues (**13-14**), all featuring substituents known to induce Th1 polarisation.



REFERENCES

1. Wu, D. et al. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 3972-3977.
2. Liu, Y. et al. *J. Immunol. Methods* **2006**, 34-39.
3. Trappeniers, M. et al. *J. Am. Chem. Soc.* **2008**, 16468-16469.
4. Stallforth, P.; Adibekian, A.; Seeberger, P.H. *Org. Lett.* **2008**, 1573-1576.

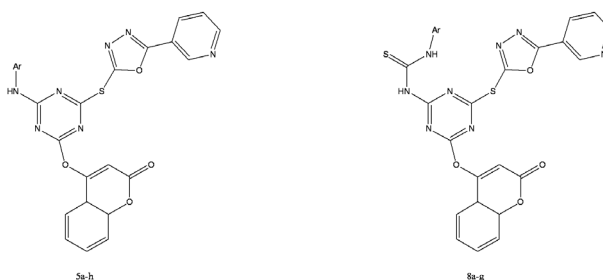
POSTER COMMUNICATIONS – PC.266

A SMALL LIBRARY OF TRI-SUBSTITUTED S-TRIAZINES AS ANTIMICROBIAL AND ANTITUBERCULAR AGENTS

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A small library of tri-substituted *s*-triazines were synthesized by sequential selective substitution at controlled temperature. All the synthesized compounds were evaluated for their *in vitro* antimicrobial and antitubercular activity. Out of total screened compounds seven compounds have shown activity against gram positive bacteria in the range of 3.12-6.25 μ g/mL and seven compounds have shown antitubercular activity against *Mycobacterium tuberculosis* H₃₇Rv, in the range of 1.76-6.25 μ g/mL.



REFERENCES

1. Design, synthesis and antimicrobial evaluation of *s*-triazinyl urea and thiourea derivatives. *Arkivoc*, 2009 (xi), 326-335
2. Synthesis and antimicrobial studies of *s*-triazine based heterocycles. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2010, 25 (1), 121-125

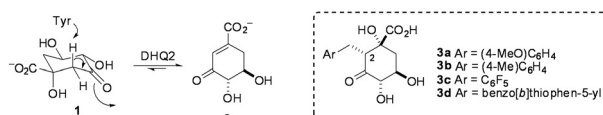
POSTER COMMUNICATIONS – PC.267

STEADY-STATE FLUORESCENCE AND MOLECULAR DYNAMIC STUDIES ON THE INHIBITION OF TYPE II DEHYDROQUINASE BY (2R)-3-BENZYL-3-DEHYDROQUINIC ACIDS

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In recent years, we have focused on the inhibition of type 2 dehydroquinase (DHQ2) from *Mycobacterium tuberculosis* (DHQ2-Mt) and *Helicobacter pylori* (DHQ2-Hp), bacteria in which this enzyme are essential,¹ for the development of new antimicrobial agents against the diseases caused by them.² We have shown recently that the substitution of the equatorial hydrogen of the 3-dehydroquinic acid (**1**), the natural substrate of the DHQ2, by benzyl groups provide potent reversible competitive inhibitors of these enzymes.³ *p*-Methoxybenzyl derivative **3a** and benzothiophene **3d** proved to be the most potent competitive inhibitors against both enzymes, with *K_i* values of 26 nM and 28 nM against *M. tuberculosis* and 170 nM and 160 nM against *H. pylori*, respectively. The structure-activity relationship of these substrate analogues has been studied by steady-state fluorescence techniques and molecular dynamics and it will be presented.



REFERENCES

1. www.essentialgene.org
2. González-Bello, C.; Castedo, L. *Med. Res. Rev.* **2007**, *27*, 177.
3. Hatakeyama, M. *J. Gastroenterol* **2009**, *44*, 239.
4. González-Bello, C. *et al. ChemMedChem* **2009**, *4*, 1980.

POSTER COMMUNICATIONS – PC.268

VIRTUAL SCREENING WORKFLOW FOR GLYCOGEN SYNTHASE KINASE 3 β INHIBITORS

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Glycogen synthase kinase 3 β (GSK-3 β) is a serine/threonine protein kinase participating in important insulin and Wnt pathways. Inhibitors of this kinase show antidiabetic properties, may be used for the treatment of Alzheimer's disease and inflammation, have memory-stabilizing effect and so on. Unique combination of various effects in the same target makes the kinase very attractive for design of new perspective drugs.

We have performed computational search for ATP-competitive GSK-3 β inhibitors in ZINC database with the use of two opposite but complementary methods: structure-based screening (docking with FRED) and ligand-based screening (combination of similarity search with ROCS and electrostatic similarity scoring with EON). ZINC database was filtered according to rules of lead-likeness; additionally, it was required for potential inhibitors to have at least one ring structure with the aim to restrict flexibility of a molecule and make it more synthetically accessible.

Comparative analysis of usability of various scoring functions applied to structure-based computational search for GSK-3 β inhibitors was carried out. The best performance was observed for functions based on the complementarity between the ATP-binding cavity of the kinase and inhibitor molecule (Shapegauss, Chemgauss3, Chemscore). The structure-based screening system was evaluated with the help of ROC analysis to assess overall enrichment of database and BEDROC metrics for early recognition of the hits. After visual inspection of docking results, 2500 reasonable hits were identified, some of them belonging to previously unexplored structure classes.

Ligand-based search was performed using the structure of hymenialdisine as a template. This molecule is a very potent conformationally restricted inhibitor of GSK-3 β possessing rather unusual structure. Consequently, similar molecules may be interesting leads for the design of GSK-3 β inhibitors; similarity search was performed for the same ZINC subset as docking studies. Six thousand hits were identified, and docking was performed to compare them with the hits found during the structure-based search. Despite the fact that hits identified with ligand-based screening were smaller than ones found based on

the structure of the kinase, certain scaffolds appear in both hitlists. We may conclude that the sequential use of ROCS, EON and FRED may be considered as a quick way to identify hits, whereas docking of large databases can be used when chemical diversity is needed to be explored.

POSTER COMMUNICATIONS – PC.269

NOVEL AZULENE-OXINDOLE CLASS KINASE INHIBITORS THAT INDUCE COMPLETE TUMOR REGRESSION IN AML MICE

On Lee, Ching-Huai Ko, Yuan-Jang Tsai, Chih-Peng Liu, Yen-Chun Chen, Hsiang-Wen Tseng, Ying-Chu Shih, Ting-Shou Chen, Chih-Hung Chen, Shu-Jiau Chiou, Ling-Mei Wang, Chrong-Shiong Hwang

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Approximately 30% of AML patients harbor activating internal tandem duplication (ITD) of the FLT3 receptor are associated with poor prognosis. This result suggests FLT3 might be a valid target for the development of AML medication. In the development of potential kinases inhibitors, we focused on utilizing azulene as pharmacophor for constructing novel drug-like compounds to overcome the issues of crowded chemical spaces and intellectual property. Among one of the strategies to bring azulene into drug molecules, we linked azulene to another well-recognized pharmacophor (aza)oxindole to generate a series of novel azulene-oxindoles. These compounds showed potential inhibitory activity toward various kinases such as FLT3-ITD, PDGFR and c-Kit. Among the candidate compounds, ITRI-227 inhibited FLT3-ITD dependent MV4-11 cells growth with IC₅₀ of 307 nM. Western blot analysis confirmed a direct effect of ITRI-227 on phosphorylation of FLT3 in RS4-11 cells upon drug treatment for 1 hr. ITRI-227 inhibited autophosphorylation of FLT3 and phosphorylation of downstream effectors, STAT5 and ERK, in a pattern similar to that seen for cellular proliferation. In *in vivo* PK study, ITRI-227 showed medium volume of distribution (Vss 1.9 L/kg), moderate clearance (1.4 L/hr/kg) and half-life about 1.2 hr after IV dosing. Its oral BA is around 10%. In PO dose escalation studies, ITRI-227 does not reach saturation in Cmax and AUC from 10 to 100 mg/kg. From normalized result, we predicted the drug exposure of ITRI-227 could still push upward to 150 mg/kg dosing level. Following the 50 and 150 mg/kg/bid oral dosing regimen, the therapeutic efficacy of ITRI-227 towards human leukemia MV4-11 subcutaneous xenograft model in female BALB/c nude mice appeared at day 22 and 30 respectively, with complete tumor regression. No specific clinical signs or body weight loss were attributed to the test article during the treatment period. In conclusion, the *in vitro/in vivo* PK/PD/toxicology studies have provided the basis for the clinical developmental potential of ITRI-227 in AML. The bridge studies from discovery to development such as CMC, GLP toxicology, safety and pre-formulation are now in progress.

POSTER COMMUNICATIONS – PC.270

DEVELOPMENT OF NOVEL 1-AZAAZULENE CLASS KINASE INHIBITORS THAT INDUCE COMPLETE TUMOR REGRESSION AND PROLONGS SURVIVAL IN AML MICE

On Lee¹, Hsingjin Eugene Liu², Ching-Huai Ko¹, Yuan-Jang Tsai¹, Chih-Peng Liu¹, Yen-Chun Chen¹, Hsiang-Wen Tseng¹, Ying-Chu Shih¹, Ting-Shou Chen¹, Chih-Hung Chen¹, Shu-Jiau Chiou¹, Ling-Mei Wang¹, Chrong-Shiong Hwang¹

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In continuing development of new drugs, we found 1-azaazulene is a potential pharmacophor with suitable drug-like properties and exhibits potency toward many targets like enzymes, receptors and kinases. In one of our projects, we linked 1-azaazulene to another well-recognized pharmacophor benzimidazole to generate a series of novel azaazulene-benzimidazole compounds. These compounds showed potential inhibitory activity toward a variety of kinases such as FLT3-ITD, RET, TrkA, TrkB and FGFR. Further studies on structure-activity relationship (SAR) provided several compounds meet the criteria for further development. Among these candidate compounds, ITRI-260 inhibited FLT3-ITD dependent MV4-11 cell growth with IC₅₀ of 21 nM and cellular phosphorylation with IC₅₀ of 17 nM. In *in vivo* PK study, ITRI-260 showed favorable oral PK profiles. The maximum concentration of ITRI-260 was 700 nM after a single 100 mg/kg oral dosing in mice, with dose proportional exposure from 10 to 150 mg/kg. ITRI-260 also showed therapeutic efficacy in a human AML orthotopic SCID mouse model following a 100 mg/kg/qd oral dosing regimen. The treatment group showed 100% survival of animals at day 90 after tumor cell inoculation, while the vehicle control group showed 100% mortality. In the other study, ITRI-260 also increased survival in primary tumor cell engraftment model inoculated with cancer cells isolated from sunitinib- and sorafenib-resistant AML patients. Through hERG assay with patch clamp technology, we found higher IC₅₀ of ITRI-260 compared with sunitinib and MLN-518, which indicates that ITRI-260 might arise less cardiotoxic concern. In a 14-day sub-chronic toxicology study in mice, ITRI-260 was generally tolerated at 300 mg/kg/qd. The most prominent adverse effect appeared at 600 mg/kg/qd group is starry sky appearance in spleen, which is moderate and reversible. No specific clinical signs or body weight loss were attributed to the test article. In conclusion, the *in vitro/in vivo* PK/PD/toxicology studies have provided the basis for the clinical developmental potential of ITRI-260 in AML. The bridge studies from discovery to development such as CMC, GLP toxicology, safety and pre-formulation are now in progress.

POSTER COMMUNICATIONS – PC.271

DESIGN AND SYNTHESIS OF GUANOSINE DERIVATIVES FOR THE CHARACTERIZATION OF THE PUTATIVE GUANOSINE RECEPTOR

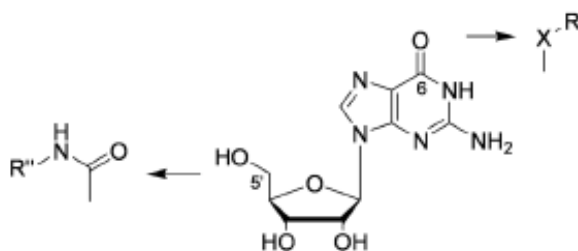
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Guanosine and its nucleotides are released from a variety of cells under physiological and pathological conditions, and in particular astrocytes release fivefold time guanine-based purines than adenine-based counterparts (1). Guanosine has many trophic effects in the CNS, including the stimulation of neurotrophic factor synthesis and release by astrocytes, which protect neurons against excitotoxic death (1).

Rat brain membrane preparations showed specific binding sites for [³H]-guanosine, which are not recognized by other purinergic receptor ligands (2). These findings supported the hypothesis of the existence of a specific G protein-coupled receptor (GPCR) for guanosine.

On this basis, various ligands were designed and synthesized for the further characterization of this elusive binding site. In particular, new guanosine analogues modified at the 6- and 5-positions have been designed and evaluated through an innovative Eu-GTP binding assay. This time-resolved fluorescence technique allows to assess the activation of a receptor through the coupling coupling to a G protein (3).



Biological results confirmed the existence, on rat brain, of a GPCR, which is activated by guanosine and 6-thioguanosine, but not by other reference purinergic receptor ligands. Furthermore, the newly synthesized compounds showed high potencies and resulted, in some cases, more active than guanosine itself.

REFERENCES

1. Ciccarelli, R.; Di Iorio, P.; Giuliani, P.; D'alimonte, I.; Ballerini, P.; Caciagli, F.; Rathbone, M. P. Rat Cultured Astrocytes Release Guanine-Based Purines in Basal Conditions and After Hypoxia/Hypoglycemia. *Glia*, **1999**, *25*, 1, 93-8.
2. Traversa, U.; Bombi, G.; Camaioni, E.; Macchiarulo, A.; Costantino, G.; Palmieri, C.; Caciagli, F.; Pellicciari, R. Rat Brain Guanosine

Binding Site: Biological Studies and Pseudo-Receptor Construction.
Bioorg. Med. Chem., **2003**, *11*, 5417-25.

3. Volpini, R.; Buccioni, M.; Dal Ben, D.; Lambertucci, C.; Lammi, C.; Marucci, G.; Ramadori, A.T.; Klotz, K.-N.; Cristalli, G. Synthesis and Biological Evaluation of 2-Alkynyl-*N*⁶-methyl-5'-*N*-methylcarboxamidoadenosine Derivatives as Potent and Highly Selective Agonists for the Human Adenosine A₃ Receptor. *J. Med. Chem.*, **2009**, *52*, 7897–900.

POSTER COMMUNICATIONS – PC.272

STRUCTURAL INSIGHT INTO MONOACYLGLYCEROL LIPASE, THE ENZYME DEGRADATING 2-ARACHIDONOYLGLYCEROL

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Together with anandamide, 2-arachidonoylglycerol (2-AG) constitutes one of the main representatives of a family of endogenous lipids known as endocannabinoids¹. These act by binding to CB₁ and CB₂ cannabinoid receptors, the molecular targets of the psychoactive compound Δ^9 -THC, both in the periphery and in the central nervous system, where they behave, among others, as retrograde messengers to modulate synaptic transmission. These last years, evidence has accumulated to demonstrate the lead role played by the fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL) in the regulation of anandamide and 2-arachidonoylglycerol (2-AG) levels, respectively. Considering the numerous physiological functions played by endocannabinoid, MAGL is now considered as promising targets for therapeutics. Indeed, it is now well known that inhibitors of this enzyme could reveal useful for the treatment of pain and inflammatory disorders, as well as for cancer therapy.

Therefore, as a first step toward the rational design of such compounds, we report the elucidation of the tridimensional structure of the human MAGL², which was solved by X ray diffraction at the resolution of 2.2 Å.

The overall fold of the enzyme is that of the α/β hydrolase superfamily, with a central core that is similar to that of the other proteins of this family. However, the enzyme distinguishes itself by the particular architecture of its “cap”, a flexible device covering the central core²⁻⁴, and by adopting subtle structural features that adapted the enzyme for the degradation of its natural substrates.

Besides giving us a structural insight into the process that regulates 2-AG signalling, we have now high expectations of developing new MAGL inhibitors on a rational basis. Indeed, there is now an urgent need for the design of valuable inhibitors of 2-AG degradation for both pharmacological and therapeutic purposes, with a special emphasis on improving potency and selectivity versus other serine hydrolases (FAAH and other carboxylesterases). Along this line, the exploitation of a so far unexploited pocket could provide new opportunities for the development of selective drugs that modulate the endocannabinoid signalling.

REFERENCES

1. Di Marzo V. *Nat Rev Drug Discov.* 2008; 7: 438-55.
2. Labar G, Bauvois C, Borel F, Ferrer JL, Wouters J, Lambert DM. *Chembiochem.* 2010; 11: 218-27.
3. Bertrand T, Augé F, Houtmann J, Rak A, Vallée F *et al.* *J Mol Biol.* 2010; 396: 663-73.
4. Schubert C, Grasberger B, Schalk-Hihi C, Maguire D, Lewandowski F *et al.* *WO/2009/132267*

POSTER COMMUNICATIONS – PC.274

HIT-TO-LEAD OPTIMIZATION OF PYRROLO[1,2-A]QUINOXALINES AS NOVEL CANNABINOID TYPE 1 RECEPTOR ANTAGONISTS

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Hit-to-lead optimization of a novel series of N-alkyl-N-[2-oxo-2-(4-aryl-4H-pyrrolo[1,2-a]quinoxaline-5-yl)-ethyl]-carboxylic acid amides are described. High throughput screening (HTS) campaign yielded several hits featuring a pyrrolo[1,2-a]quinoxaline core, as represented by (Figure 1).

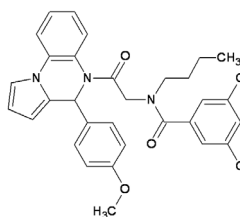


Figure 1

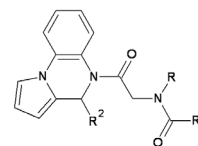
$$K_i = 831 \text{ nM}$$


Figure 2

We aimed at reducing the molecular weight and improving the potency of compound. In order to that, three areas were addressed (Figure 2): (R) the effect of the alkyl side chain, (R1) the effect of acyl groups, and (R2) the effect of groups of the pyrrolo-quinoxaline ring. This optimization process led to iden-

tification of in vitro potent cannabinoid 1 receptor (CB₁R) antagonists representing a new class of compounds in this area.

POSTER COMMUNICATIONS – PC.275

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL SERIES OF MONOACYLGLYCEROL LIPASE INHIBITORS

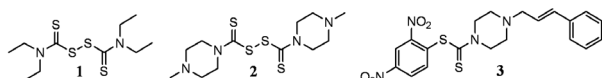
Coco N. Kapanda, Geoffray Labar, Jacques H. Poupaert, and Didier M. Lambert

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The monoacylglycerol lipase (MAGL) is an enzyme of 33 kDa belonging to the superfamily of serine hydrolase. It is involved in the hydrolysis of the 2-arachidonoylglycerol (2-AG) in glycerol and arachidonic acid. 2-AG is an endogenous ligand of cannabinoid receptors CB₁ and CB₂ and acts as retrograde lipid messenger regulating the release of other neurotransmitters. 2-AG is involved in many pathophysiological processes, including regulation of appetite, pain control and cell proliferation.¹ Therefore, inhibition of monoacylglycerol lipase appears as a promising approach for the treatment of disease states in which increased levels of 2-AG may be beneficial.

Our group discovered the MAGL inhibitory properties of disulfiram (**1**), a bis(dialkylaminethiocarbonyl)disulfide derivative,² and showed that arylthioamide scaffold represents a useful template for designing new MAGL inhibitors.³ In this connection, we report here results from pharmacomodulations around these structures.

Among disulfiram analogues, bis(4-methyl-1-piperazinylthiocarbonyl)disulfide (**2**) was found to inhibit pure human MAGL with a pI₅₀ of 6.97±0.05, along with a selectivity ratio of 1825 compared to FAAH inhibition, another endocannabinoids hydrolyzing enzyme. MAGL inhibition within this series was highly dependent on the presence of the disulfide group, as similar compounds lacking this functionality were less potent or unable to inhibit MAGL. Additionally, these derivatives constitute irreversible MAGL inhibitors that probably interact with the sulfhydryl moiety of Cys208 and/or Cys242, located in the vicinity of MAGL active site.



Regarding dithiocarbamate derivatives, 2,4-dinitrophenyl-4-cinnamylpiperazine-1-carbodithioate (**3**) inhibits human MAGL with a pI₅₀ of 6.03 ± 0.09, along with a good selectivity compared to FAAH inhibition (40% maximal inhibition at 10⁻³M). MAGL inhi-

bition within this series was also found to be dependent on the presence of the lipophilic group, as similar compounds lacking, for instance, the cinnamylpiperazine moiety were found less potent inhibitors.

These compounds therefore constitute useful pharmacological tools to explore the endocannabinoid signalling.

REFERENCES

1. Lambert D.M et al. J.Med Chem 2005, 48, 5059-87.
2. Labar et al.ChemBioChem 2007, 8, 1293 – 1297.
3. Kapanda et al. Med. Chem. Res.2009, 18: 243-254.

POSTER COMMUNICATIONS – PC.276

INDEXING CAVITIES IN PROTEIN STRUCTURES

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The identification and characterisation of cavities in protein structures is an aspect of key relevance in molecular biology and drug discovery. Usually, multiple cavities are identified in each protein structure and there is currently no means for indexing those cavities in a unique meaningful manner. To this aim, we introduce an algorithm that allows for generating a unique identifier for each protein cavity based on the pharmacophoric features exposed.

The process leading to the generation of a unique protein environment identifier (PEI) for each existing cavity in a protein structure starts by detecting all cavities present in the protein structure. Once detected, the surface exposed is fragmented on the basis of six pharmacophoric features (hydrophobic, aromatic, donor, acceptor, negatively charged and positively charged) according to the nature of the contributing atoms. Each pharmacophoric patch is represented by a centroid. The ensemble of pharmacophoric centroids of a cavity constitute a cavity graph. This cavity graph is ultimately taken to generate a unique haschcode (PEI) using a modified and further extended adaptation of the Morgan algorithm.

The indexing approach introduced here will be used i) to analyse the dynamics of cavities found in multiple structures for a given protein and ii) to locate at which point of a molecular dynamics run cavities with an optimal druggability profile are created.

POSTER COMMUNICATIONS – PC.277

SOME NEW 3H-PYRROLO[3,2-f]QUINOLIN-9-ONE DERIVATIVES SHOW POTENT AND SELECTIVE ANTILEUKEMIC ACTIVITY

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In the context of pharmacomodulating the properties of 7-phenyl-3H-pyrrolo[3,2-f]quinolin-9-ones we focused on making molecules more hydrophilic in comparison with previous analogues^[1] by binding suitable functional groups onto 1, 3 and 7 positions (figure 1). Such compounds should be of value for *in vivo* studies on pharmacokinetics, metabolism and toxicity. The novel substituted angular 3H-pyrrolo[3,2-f]quinolinones were evaluated for their antiproliferative activity. The most active derivatives showed high selectivity against human leukemia cell lines and potently inhibited their growth, with nM IC₅₀ values. The active compounds strongly inhibited tubulin assembly and the colchicine binding to tubulin. Their activities were equal to or greater than that of the reference compound CA-4.

Flow cytometry study showed that Jurkat cells treated with the most active compounds **11** and **12** (nanomolar IC₅₀s), having an ethyl propanoate and a trimethoxybenzyl group in the 3 position respectively, were arrested in the G₂/M phase of cell cycle in a concentration-dependent manner. This effect was associated with apoptosis of cells, mitochondrial depolarization, generation of reactive oxygen species, activation of caspase-3 and cleavage of the enzyme polyADP-ribose cleavage polymerase. Very interesting structure-activity relationships regarding the position and the nature of substitutions for obtaining potent and selective anti-leukaemias drugs will be discussed.

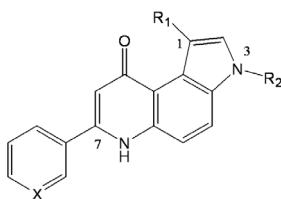


Figure 1. 3H-pyrrolo[3,2-f]quinolin-9(6H)-ones

REFERENCES

1. V. Gasparotto, I. Castagliuolo, M. G. Ferlin *J. Med. Chem.* 2007, 50, 5509-5513.

POSTER COMMUNICATIONS – PC.279

SYNTHESIS OF VLA-4 INTEGRIN LIGANDS AND THEIR GRAFTING TO BLOOD FILTER SURFACES

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The reduction of the number of leukocytes in blood products is essential to avoid harmful reactions "donor-receiver" occurring after blood transfusions. Different methods for leukodepletion exist but the filtration is nowadays the most efficient and practical.

The retention of leukocytes by a specific adhesion effect is considered. The integrins expressed on leukocytes could react with synthetic ligands, which mimic the natural ligands of the integrins and are covalently fixed on the surface of the filters. These strong "ligand-receptor" interactions combined with the physico-chemical effects of the filters should improve their performance.

Different peptidomimetics, related to the active sequence "LDV" (Leu-Asp-Val), recognized by the integrin $\alpha_4\beta_1$, have been studied. The synthesis of a first peptidomimetic ligand will be presented¹ (Figure 1).

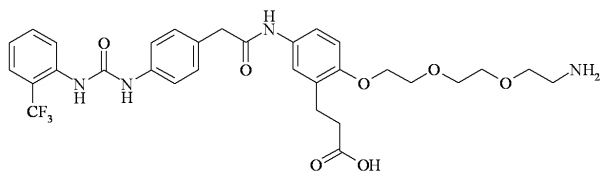


Figure 1

The ligands are grafted on the surface of polypropylene membranes, which are currently used as part of filtration devices of blood products. These oxygen-plasma treated membranes are activated by "wet chemistry" treatments using cyanuric fluoride² as reagent.

Modified materials are characterized by physico-chemical and morphological analyses and then evaluated towards their ability of selectively retaining leukocytes from blood products during filtration experiments.

REFERENCES

1. Momtaz, M.; Rerat, V.; Gharbi, S.; Gérard, E.; Pourcelle, V.; Marchand-Brynaert, J., *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1084-1090.
2. Momtaz, M.; Dewez, J.-L.; Marchand-Brynaert, J., *J. Membr. Sci.*, **2005**, *250*, 29-37.

POSTER COMMUNICATIONS – PC.280

A NEW FRAGMENT BASED LIGAND DESIGN TOOL TO BOOST COMMUNICATION BETWEEN MEDCHEMS AND COMPCHEMS

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From our experience some facts emerge concerning the relationship between MedChems and CompChems today:

- Effective and fast communication between these disciplines is of crucial importance.
- Management is well advised to support both groups by exploiting their own, respective strengths instead of letting MedChems do partial CompChem tasks and vice versa.
- When it comes to computer interaction, MedChems think 2D rather than 3D; CompChems think more 3D.
- Learning curves for computational tools to be used by MedChems must be avoided entirely.

We present a novel piece of software, LeadIT, which has been co-developed with Hoffmann-LaRoche; its user interaction model has been designed from scratch to support MC/CC team building following the principles above. One of the core computation pieces is an interactive *fragment based ligand design* engine which needs 3 mouse clicks to be triggered by MedChems, but has a totally open and configurable chemistry model behind so that the computational specialists can adapt it to corporate needs if requested. Because result delivery is within seconds, the tool has proven to be highly usable in a slideshow-like fashion in teams of Medicinal and Computational chemists at various big pharma world-wide. Results can be refined iteratively and within teams so that time consuming communication loops are avoided.



Basic FBLD and SBDD tasks to be accomplished using LeadIT are:

- a) Growing from seed fragment binders into other parts of protein binding pockets,
- b) Fragment linking of two or more fragment binders, or
- c) Merging multiple overlapping binders into a single potent lead,
- d) Protein-ligand docking using the built-in industry standard FlexX.

For a)-c), based on a vast 3D fragment library, the tool finds fragments which provide an optimal fit with the 'dangling bonds' and which comply with optional filters and pharmacophore features. Synthetic access of results – a major weakness in the early days of de novo design – is taken care of in LeadIT at three levels: during fragment creation, within the query definition, and when creating the results.

Our basic design and computation dogmas will be discussed; examples which map onto experimental data and evolve into novel lead ideas will be presented.

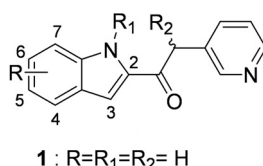
POSTER COMMUNICATIONS – PC.281

DISCOVERY AND OPTIMIZATION OF INDOLE-PYRIDINYL-ETHANONES AS NOVEL INHIBITORS OF INDOLEAMINE-2,3-DIOXYGENASE (IDO), A PROMISING TARGET FOR ANTI-CANCER IMMUNOTHERAPY

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Immunotherapy is a promising novel and validated strategy for cancer therapy. It consists of the therapeutic vaccination of patients to stimulate their (natural) immune system against cancer cells. However, this approach showed a limited efficacy *in vivo* because cancer cells develop enzymatic mechanisms allowing tumours to resist or escape immune rejection. Among the enzymes involved, indoleamine 2,3-dioxygenase (IDO) was identified as a potential actor. IDO catalyses the rapid degradation of tryptophan (Trp) into *N*-formylkynurenine. This results in a local Trp depletion that severely affects T-cells proliferation and is thereby deeply immunosuppressive. Recently, the team of Prof. Van den Eynde demonstrated that many human tumours express IDO in a constitutive manner and that this expression allows cancer cells to escape immune rejection.¹ IDO was thus clearly identified as an attractive target for the development of inhibitors.



The recent elucidation of the three-dimensional structures of IDO,² in complex with phenylimidazole and the cyanide ion (CN⁻), provide important results for the structure-based drug discovery and design of novel IDO inhibitors. In the present work, we applied virtual screening for the discovery of new IDO inhibitors. As a result, five novel scaffolds with inhibitory potencies in the micromolar range were identified. Among these, the most promising candidate (**1**: IC₅₀ = 65 μM) was selected and its inhibitory potency improved by chemical modifications. This led to a 7-fold improvement of the inhibitory potency of the hit selected.

In this communication, the identification of **1**, the synthesis and biological evaluation of analogues as well as a modeling study explaining the SAR's will be presented.³

REFERENCES

1. Uyttenhove, C.; Pilotte, L.; Theate, I.; Stroobant, V.; Colau, D.; Parmentier, N.; Boon, T.; Van den Eynde, B. J. *Nat Med* **2003**, 9, 1269-74.
2. Sugimoto H., Oda S., Otsuki T., Hino T., Yoshida T., Shiro Y. *PNAS* **2006**, 103(8), 2611-6
3. This work is supported in part by the FNRS and Biowin (CANTOL : Convention n° 5678)

POSTER COMMUNICATIONS – **PC.282**

RESTRICTED DOCKING OF THE DHFR INHIBITORS TO THE DHFR RECEPTOR

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"Tuberculostatic drug – DHFR" complexes were modeled with algorithm Infant. The dataset of the drugs includes dihydropyrimidine derivatives, their annealed analogs and the analogs with a podand chain. For the docking procedure, we used the X-ray crystallography data of dihydrofolate reductase with well-known drugs (methotrexate, trimethoprim, 4-bromo-WR99210 [13]) and the data of the molecular orientation obtained with the CiS algorithm. The drug is orientated to the cavity bottom by oxidic radical and methyl group, pyrimidine ring is turned to the protein outlet. Aminoacid residues responsible for binding with drugs were determined using algorithm CoCon [1]. These are: Gln28, Ser49, Thr46, Trp6, Leu50, Arg23, Ile20 and three water molecules (see Fig.).

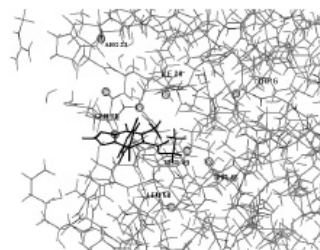


Fig. "Drug – DHFR" complex (atoms of aminoacid residues responsible for binding with the drug are marked)

Low-active molecules have the following shorter contacts then high-active molecules: 1) of Leu50 with the oxyacyclic oxygen, 2) Ile20 with the hydrogen of the aromatic substituent and dihydropyrimidine cycle, 3) Ser49 with the hydrogen of methyl. The following regression model was obtained:

$$\text{pMIC} = 4.47 - 5.6x_1 - 0.015x_2$$

is energy of "bond – angle" stretch-bending interactions (MM3 force field), x_2 is electrostatic energy computed by MERA force field [1]. The cross validation quality is 0.9779 and Fisher's criterion is 43. So, the steric stresses of the complexes lead to decrease of the activity. Moreover, tuberculostatic activity depends on the total energy of the complex. It has been shown that more active drug molecules form more stable complexes.

ACKNOWLEDGMENTS

SKIF–GRID Supercomputer Initiative.

REFERENCES

1. Potemkin V.A., Pogrebnoy A.A., Grishina M.A. Technique for Energy Decomposition in the Study of " Receptor-Ligand" Complexes// J. Chem. Inf. Model., 49: 1389-1406

POSTER COMMUNICATIONS – **PC.284**

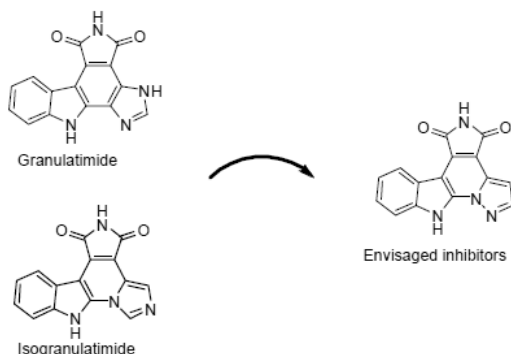
SYNTHESIS AND ANTICANCER EVALUATION OF PYRAZOLIC ANALOGUES OF THE MARINE CHK1 INHIBITORS, GRANULATIMIDE AND ISOGRANULATIMIDE

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Therapeutic intervention targeting the cell cycle has emerged as an important strategy for the development of new anticancer drugs. Chk1 is a major kinase that regulates the G2 checkpoint. As almost 50 to 70 % of tumor cells are defective at G1 due to mutations in tumor suppressor genes, they rely heavily on the G2

checkpoint and thus Chk1 to correct otherwise fatal genetic errors¹. As such, Chk1 inhibitors should potentialise the effect of a genotoxic agent². Granulatimide and isogranulatimide are alkaloids issued from marine source which have been shown to be effective inhibitors of Chk1³. Pyrazolic analogues of these natural compounds have been investigated in order to define new effective and specific inhibitors of Chk1. Both synthetic and biological results will be discussed.



REFERENCES

1. Kaufmann, W. K.; Paules, R. S. *FASEB J.* **1996**, *10*, 238.
2. Weinert, T.; Lydall, D. *Semin. Cancer Biol.* **1993**, *4*, 129.
3. Jiang, X.; Piers, E.; Andersen, R. J.; Roberge, M. *Mol. Cancer Ther.* **2004**, *3*, 1221.

POSTER COMMUNICATIONS – PC.285

CONFORMATIONAL CHANGES INDUCED BY FRAGMENT GROWING: A STRUCTURAL AND THERMODYNAMIC ANALYSIS

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Neuronal nicotinic acetylcholine receptors (nAChRs) are potential therapeutic targets for the treatment of pain, epilepsy and a wide variety of neurodegenerative and psychiatric disorders e.g. Alzheimer's disease, Parkinson's disease, schizophrenia and depression. Several important physiological processes appear to be regulated by a single or a few nAChR subtypes.¹ Subtype selective ligands are needed to aid target validation for the development of safe and selective drugs. However, due to high homology between the binding sites of the different subtypes, the design of selective ligands poses an interesting challenge.

Recently, high-quality X-ray structures of several acetylcholine-binding proteins (AChBPs) co-crystallized with ligands, have become available.^{2,3} These proteins are water soluble homologs of the ligand-binding domain of nAChRs.⁴ Fragment library screening using a variety of orthogonal biochemical and biophysical approaches has resulted in the identification of novel hit fragments. Several of these fragments have been co-crystallized with AChBP. We here describe the growing of one of these fragments into a sub-pocket of the binding site that becomes accessible upon a ligand-induced change in rotameric state of a gate-keeper tyrosine residue. These studies were guided by pharmacological, structural and thermodynamic data and illustrate the detailed insights and molecular understanding that can be obtained by fragment-based design approaches.

REFERENCES

1. Jensen et al. *J. Med. Chem.* **2005**, *48*, 4705-4745
2. Celie et al., *Neuron* **2004**, *41*, 907-14
3. Hansen et al. *EMBO journal* **2005**, *24*, 3635-46
4. Sixma et al. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 311-34

POSTER COMMUNICATIONS – PC.286

CHEMOGENOMIC LIBRARY DESIGN PRODUCTION AND USE

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To date most screening within the pharmaceutical industry has relied on chemical diversity for example High Through-put screening.¹ There has been limited effort to generate screening libraries using the vast amount of published pharmacological data, to pick the mode of action and then ensure the compound is selective by comparison with all known screening data.² The ideal situation is a screening set with potent, selective compounds for every mechanism. Such a collection in combination with a functional disease model would allow phenotypic screening for the identification of new mechanisms of interest.³ This poster will describe our efforts to automatically trawl internal and external databases to identify compounds with these properties. Compounds were selected that had sub 500nM potency, solid availability for multiple screening cycles and potency data for five or more secondary mechanisms. Multi-parameter optimisation was used to select up to five compounds for each target with the best possible combination of potency and selectivity. The resulting Chemogenomics set contains 3155 compounds with coverage of 722 mechanisms split into 11 Gene Family groups as shown in Figure 1, coverage is uneven and is a reflection of areas of traditional interest at Pfizer.

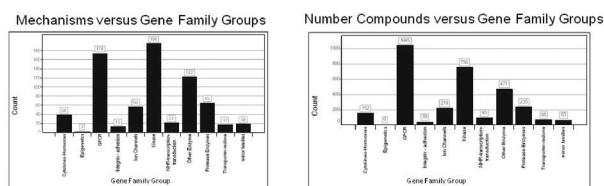


Figure 1

The libraries are available for researchers to use as initial tools in a phenotypic screen where the introduction of selectivity data increases the chance that a particular mechanism has been accurately identified, especially if all five chemogenomic tools for a given target are active. Use of this library within Pfizer has already led to the discovery of a new approach for the conversion of Pancreatic Progenitor Cells to Beta-cells, potentially useful for the treatment of diabetes.

REFERENCES

1. K Bleicher. (2003) Hit and Lead generation: Beyond high-throughput screening. *Nature Rev. Drug Discov.* 2: 369-378.
2. Lee Harland Anna Gaulton. (2009) Drug Target Central. *Expert Opin. Drug Discov.* 4(8):857-872.
3. Charles P.Hart. (2005) Finding the target after screening the phenotype *Drug Discovery Today.* 10: 513-519.

POSTER COMMUNICATIONS – PC.287

NEW SUGAR-DERIVED COMPOUNDS TARGETING SELECTIVELY CD14 AND MD-2 RECEPTORS AS ANTI SEPSIS AND ANTI NEUROPATHIC PAIN AGENTS

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In this poster we present the discovery and the biological characterization of new chemical entities derived from natural sugars targeting selectively the extracellular Toll-like Receptor 4 (TLR4) complex. The main focus of an active collaboration between the Universities of Milano (Italy) and Iowa (USA) is to gain information on the molecular basis of the TLR4 pathway using small molecules capable to interact selectively with the extracellular receptors LBP, CD14, MD-2 and TLR4^[1]. Positively charged monosaccharides with lipid chains have been developed by our group, that inhibit lipopolysaccharide (LPS) and lipid A-induced cytokine production in innate immunity cells^[2-5]. These molecules are also active *in vivo* in contrasting septic shock and other syndromes,

such as neuropathic pain, caused by TLR4 activation^[6]. Biochemical studies indicated that these monosaccharides inhibit the TLR4 pathway by selectively antagonizing the endotoxin binding to the CD14 receptor. The discovery of new lipodisaccharides containing negatively charged sulfate groups, active as mild agonists of TLR4, is also described. These molecules selectively target the MD-2 receptor and are promising leads for the development of non-toxic vaccine adjuvant. All molecules presented, besides being hit or lead compounds for the development of anti-sepsis and anti-inflammatory agents, and immunotherapeutics, are also unique tools for investigating the structural and functional biology of the TLR4 pathway with the chemical genetic approach.

REFERENCES

1. 5-years NIH grant, Award No 2R01 AI059372-06A1 "Regulation of MD-2 Function and Expression".
2. F. Peri, F. Granucci, B. Costa, I. Zanoni, C. Marinzi, F. Nicotra Amino and Hydroxylamino Monosaccharides Inhibit Lipid A Stimulated Activation of Human Dendritic Cells and Macrophages, *Angew. Chem. Int. Ed.* 2007, **46**, 3308-3312.
3. M. Piazza, C. Rossini, S. Della Fiorentina, C. Pozzi, F. Comelli, Bettoni, P. Fusi, B. Costa, F. Peri Glycolipids and benzylammonium lipids as novel anti-sepsis agents: synthesis and biological characterization, *J. Med. Chem.* 2009, **52** (4), 1209-1213.
4. M. Piazza, L. Yu, A. Teghanemt, T. Giannini, J. Weiss, F. Peri, "Evidence of a specific interaction between new synthetic antiseptic agents and CD14", *Biochemistry* 2009, **48**, 12337-12344.
5. F. Peri, F. Nicotra, F. Granucci, B. Costa PCT/EP2007/002279 published the 15/03/2007, LIPID A ANTAGONISTS WITH ANTI-SEPTIC SHOCK, ANTIINFLAMMATORY, ANTI-ISCHEMIA AND ANALGESIC ACTIVITY.
6. I. Bettoni, F. Comelli, C. Rossini, F. Granucci, G. Giagnoni, F. Peri, B. Costa Glial TLR4 Receptor as New Target to Treat Neuropathic Pain: Efficacy of a New Receptor Antagonist in A Model Peripheral Nerve Injury in Mice, *Glia* 2008, **56**, 1312-1319.

POSTER COMMUNICATIONS – PC.288

COMPUTATIONAL STUDIES AND SYNTHETIC APPROACHES FOR THE IDENTIFICATION OF NEW PROTEIN-PROTEIN INTERACTION INHIBITORS (PPI)

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Human Immunodeficiency Virus (HIV) infection is one of the leading causes of death in the world. An essential step in the virus-retroviral life cycle is the integration of the proviral DNA into the cellular genome of the host cell to generate a provirus that remains present as long as the cell survives.

Highly Active Antiretroviral Therapy (HAART) is the standard therapy of infection based on potent cocktails of drugs targeting viral proteins.

Considering that this treatment is associated with severe side effects and emergence of drug-resistant strains, the discovery and development of new anti-HIV agents with novel mechanisms, high potency, less toxicity and broad-spectrum activity against wild-type and mutant HIV-1 strains remain continuously warranted.

In this context the search of small molecules as protein-protein interaction inhibitors (SMPPIIs) represents a new attractive strategy to identify new anti-HIV-1 agents blocking HIV replication, through a different mechanism of action than all other drugs so far used in therapy.

Several cofactors were recently identified as important proteins involved in the HIV-1 life cycle and among them lens epithelium-derived growth factor LEDGF/p75 plays a key role during the integration process.

Taking into account these findings and employing different computational approaches we performed molecular modeling studies with the aim to explore the structural requirements necessary to disrupt the protein-protein interaction.^{1,2}

Our studies resulted in the discovery of small molecules able to prevent PPIs between IN and its cofactor LEDGF/p75 at micro-molar concentration.

The most innovative eco-friendly methods and technologies have been used for the synthesis of the new compounds. In particular the application of microwave-assisted organic synthesis was adopted in the steps requiring heating in order to shorten reaction times and increase the yields.

Research supported by THINC project (HEALTH-F3-2008-201032)

REFERENCES

1. L. De Luca, M.L. Barreca, S. Ferro, F. Christ, N. Iraci, R. Gitto, A.M. Monforte, Z. Debyser, A. Chimirri. *ChemMedChem* **2009**, 4, 1311-1316
2. Submitted for publication

POSTER COMMUNICATIONS – PC.289

NOVEL PYRAZOLE DERIVATIVES AS INHIBITORS OF CELLULAR LTB₄ BIOSYNTHESIS

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Leukotrienes (LTs) are pro-inflammatory lipid mediators derived from arachidonic acid which play pivotal roles in various biological

and pathological processes [1]. Recent studies suggest a crucial role of 5-LO products in cell proliferation and survival particularly of prostate and pancreatic cancer cells. Additionally, the cardiovascular actions of LTs have also been well recognized for many years, especially in atherosclerosis and in myocardial infarction in which each disease is characterized by an inflammatory component at some stage of the associated vascular pathology. Therefore, the inhibition of LT formation became an attracting pharmacological strategy to intervene with these diseases [2]. In present study, we show that certain derivatives of 1,5-darylpyrazole-3-propanoic acid inhibit formation of LTB₄ biosynthesis in intact human neutrophils when stimulated with ionophore A23187. Our efforts for structural optimization resulted compounds with IC₅₀ values between 1.2 and 4.8 µM. Interestingly, the compounds showed no considerable inhibition of 5-LO product formation in a cell-free system suggesting that the compounds may interfere with a regulatory mechanism of 5-LO in intact cells resulting in inhibition of LT formation rather than directly acting on the 5-LO enzyme. We are currently studying the identification of the exact mode of action underlying inhibition of the cellular LT formation. Based on their high selectivity and efficacy, these novel structures might represent promising candidates for further development as suitable inhibitors of 5-LO product synthesis (This work is partly supported by a TUBÝTAK Research Grant 108S210).

REFERENCES

1. Werz, O. 5-lipoxygenase: cellular biology and molecular pharmacology. *Curr. Drug Targets Inflamm. Allergy*, 1(1), 23-44 (2002).
2. Werz, O., Steinhilber, D. Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacol. Ther.* 112(3), 701-18 (2006).

POSTER COMMUNICATIONS – PC.290

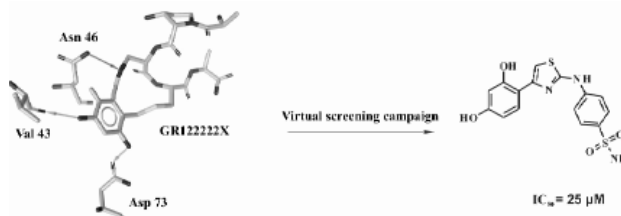
DISCOVERY OF 2-AMINO-4-(2,4-DIHYDROXYPHENYL)THIAZOLES AS NOVEL INHIBITORS OF DNA GYRASE B BY STRUCTURE-BASED VIRTUAL SCREENING APPROACH

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The emergence of bacterial resistance to most of the clinically used antibiotics is driving an urgent need for the development of novel and effective antibacterial agents.¹ The main challenge remains the discovery of highly potent antibacterials with broad spectrum of efficacy and improved safety profile.^{2,3} One of the well established targets of the antibacterial agents is the DNA gyrase, a unique bacterial type II topoisomerase originating from

the gyrase, HSP90, histidine kinase, MutL (GHKL) enzyme super-family that catalyzes the introduction of negative supercoils into the DNA using the concurrent ATP hydrolysis.



Cyclothalidines⁴ are a class of bacterial DNA gyrase B (GyrB) sub-unit inhibitors, targeting its ATP-binding site. Starting from the available structural information on cyclothalidine GR122222X,⁵ an *in silico* virtual screening campaign was designed combining molecular docking calculations with three-dimensional structure-based pharmacophore information. A novel class of 2-amino-4-(2,4-dihydroxyphenyl)thiazole based inhibitors with low micromolar antigyrase activity was discovered.⁶

REFERENCES

1. Brown, E.D.; Wright, G.D. *Chem. Rev.* 2005, 105, 759.
2. Silver, L.L. *Biochem. Pharm.* 2006, 71, 996.
3. Perdihi, A.; Kovač, A.; Wolber, G.; Blanot, D.; Gobec, S.; Solmajer, T. *Bioorg. Med. Chem. Lett.* 2009, 19, 2668.
4. Anghern, P.; Buchmann, S.; Funk, C.; Goetschi, E.; Gmuender, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbbers, T.; Masciadri, R.; Nielsen, J.; Reindl, P.; Ricklin, F.; Schmitt-Hoffmann, A.; Theil, F.-P. *J. Med. Chem.* 2004, 47, 1487.
5. Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* 1996, 15, 1412.
6. Brvar, M.; Perdihi, A.; Oblak, M.; Peterlin Mašič, L.; Solmajer, T. *Bioorg. Med. Chem. Lett.* 2010, 20, 958.

POSTER COMMUNICATIONS – PC.291

OPTIMIZATION OF THE FIRST LEAD COMPOUND TARGETING THE *TRYPANOSOMA BRUCEI* METACASPASE 2

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Metacaspases (MCAs) are important cysteine proteases in *Trypanosoma brucei* and are considered as a new attractive drug target for antiparasitic chemotherapeutics. A first series of inhibitors has been prepared and were found to possess modest antiparasitic activity and excellent selectivity when compared to mammalian caspases.¹ Further optimization efforts include P₁', P₁ and P₂ modifications of the lead compound UAMC-00599 in order to obtain more potent and selective inhibitors (Fig. 1). Different warheads at the P₁' position will be investigated. Then, various functional groups will be introduced in the P₁ side chain to lower basicity and thus to enhance membrane permeability. Furthermore, hydrophobic uncharged moieties at the P₂ position will be extensively explored to enhance the selectivity of these compounds.

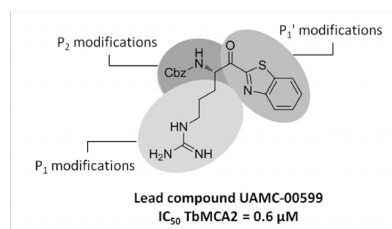


Figure 1: P₁', P₁ and P₂ modifications of the lead compound UAMC-00599 targeting TbMCA2.

REFERENCES

1. Berg, M., Van der Veken, P., Joossens J., et al. *Bioorg. Med. Chem. Lett.* **2010**, 20, 2001-2006.

POSTER COMMUNICATIONS – PC.292

FIRST SULFONIC ACID ANALOGUE OF IDRAPARINUX, THE ANTICOAGULANT PENTASACCHARIDE

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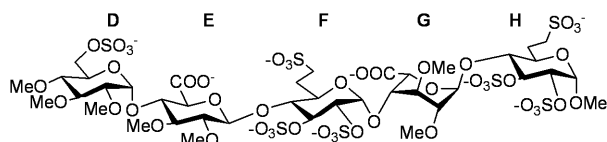
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Heparin, a highly sulfated linear polysaccharide of animal origin is widely used as an anticoagulant drug. Heparin binds to the protease inhibitor antithrombin III (AT-III) through a unique pentasaccharide sequence; upon binding, a conformational change occurs in AT-III, allowing inhibition of coagulation factors Xa and thrombin. A synthetic pentasaccharide analogue of the antithrombin-binding domain of heparin [1] possessing higher anti-Xa activity and longer half-life than heparin was developed by Sanofi-Synthelabo and Organon, and it was marketed as a new antithrombotic drug under the name Arixtra. The lead compound of the second-generation, "non-glycosamino" glycan Xa-inhibitors is Idraparinux, a fully O-sulfated, O-methylated pen-

tasaccharide, which is not only much easier to synthesize, but also displays higher activity and a longer duration of action than analogues synthesized before. [2]

The aim of our Research Group is the preparation of sulfonic acid analogues of Idraparinux in which the sulfate esters are replaced systematically with isosteric sulfonic acid moieties. These negatively charged sulfonic acid analogues can be resistant towards esterases, accordingly the desired biological effect in the organism can be sustained for a longer period of time. Last year we reported the synthesis of three sulfonic acid analogues of the GH fragments, and three analogues of the EF fragments. [3]

Here, we present the synthesis of the first sulfonic acid analogue of the anticoagulant Idraparinux containing methylsulfonic acid moieties at the F and H units. Preliminary results of anti-Xa assay of the pentasaccharide also will be discussed.



Structure of the 6,6''-disulfonic acid analogue of Idraparinux

REFERENCES

1. M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinäy, J. C. Jacquinet, G. Torri *Carbohydr. Res.* **1986**, *147*, 221-236.
2. M. Petitou, C. A. A. van Boeckel *Angewandte Chemie Int. Ed.* **2004**, *43*, 3118-3133.
3. M. Herczeg, L. Lázár, A. Borbás, A. Lipták, S. Antus: *Org. Lett.* **2009**, *11*, 2619-2622

POSTER COMMUNICATIONS – PC.293

EVALUATION OF NUCLEOSIDE HYDROLASE INHIBITORS FOR THE TREATMENT OF AFRICAN TRYPANOSOMIASIS

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In the search for potential chemotherapeutic agents against trypanosomiasis, a library of 52 *N*-arylmethyl-substituted iminoribitol derivatives was designed and synthesized as potent and selective inhibitors of *Trypanosoma vivax* inosine-adenosine-guanosine nucleoside hydrolase (TviAG-NH). However, when tested against bloodstream form *Trypanosoma brucei brucei*, only one inhibitor, *N*-(9-deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-D-ribitol (UAMC-00363 Fig. 1), displayed a significant activity ($IC_{50} = 0.49 \pm 0.31 \mu M$). A validation in an *in vivo* model of African trypanosomiasis showed promising results for this compound.¹

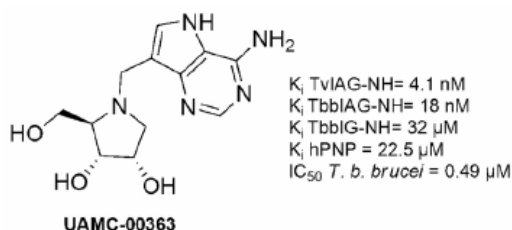


Fig. 1. Structure of UAMC-00363. Inhibition constants (K_i) are provided against IAG-NH from *T. vivax* (TviAG-NH) and *T. b. brucei* (TbbiAG-NH), IG-NH from *T. b. brucei* (TbbiG-NH) and human purine nucleoside phosphorylase (hPNP). IC_{50} value (μM) is provided for *T. b. brucei*.

Several experiments were performed to investigate why only UAMC-00363 showed antiparasitic activity.¹ First, the compound library was screened against *T. b. brucei* IAG-NH and IG-NH to confirm the inhibitory effect of the compounds previously demonstrated on *T. vivax* IAG-NH. Second, to verify the uptake of these compounds by *T. b. brucei*, their affinity for the nucleoside P1 and nucleoside/nucleobase P2 transporter of *T. b. brucei* was tested. Only UAMC-00363 displayed a significant affinity towards the P2 transporter. It was also shown that UAMC-00363 is concentrated into the cell via at least one additional transporter, since it displayed no resistance against P2 knockout mutants of *T. b. brucei*. Consequently, no cross-resistance with the diamidine or the melaminophenyl arsenical classes of trypanocides is expected. Third, three enzymes of the purine salvage pathway of procyclic *T. b. brucei* (IAG-NH, IG-NH and MTAP) were investigated using RNAi knockdown. All these studies showed that it is probably not sufficient to target only the nucleoside hydrolase activity to block the purine salvage pathway of *T. b. brucei* and therefore it is possible that UAMC-00363 acts on an additional target.

REFERENCES

1. Berg, M., Kohl, L., Van der Veken, P. et al. *Antimicrob. Agents Chemother.* **2010**, doi: 10.1128/AAC.01787-09

POSTER COMMUNICATIONS – PC.294

INVESTIGATIONS ON NEW DIARYLPYRRAZOLE DERIVATIVES FOR THE SYNTHESIS OF POTENT ANTIPLATELET AND ANTI-INFLAMMATORY COMPOUNDS WITH COX-2/TXA₂ INHIBITION

Sultan Nacak Baytas¹, Serdar Unlu¹, M. Fethi Sahin¹

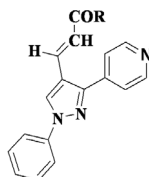
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It is well known that inhibition of the enzyme cyclooxygenase (COX) is the principal mechanism for the efficacy of NSAIDs. Although COX-2 inhibitors are better tolerated than classical NSAIDs with improved gastric safety profile, their prolonged use causes cardiovascular side effects which limit their clinical use [1].

Thromboxane A₂ (TXA₂) is a potent platelet activator and constrictor of vascular and bronchial smooth muscles, while prostacyclin (PGI₂), potent endogenous inhibitor of platelet function, possesses a strong vasodilator effect. Furthermore, PGI₂ is currently presented as the physiologic antagonist of TXA₂. So, balance between TXA₂ and PGI₂ is crucial for the cardiovascular system [2].

The recent doubts about cardiovascular toxicity of COX-2 inhibitors prompted us to synthesize dual inhibitor of COX-2 and thromboxane synthase enzymes. We have aimed to have safer COX-2 inhibitor by suppressing formation of TXA₂ by COX-1 at platelets to protect balance between TXA₂ and PGI₂.

In our previous study, we synthesized diarylpyrrazole compounds possessing 3-methyl-2(3H)-benzoxazolinone moieties and evaluated their antiinflammatory and antiplatelet activities. To overcome solubility problems and achieve better biological results, we have designated and synthesized novel compounds which bear a central pyrrazole ring whose 1,3,4-positions are substituted with phenyl, pyridyl and substituted cinnamic acid residues, respectively. The preparation of 1,5-diarylpyrrazole derivatives to screen their COX-2 and thromboxan synthase inhibitory activities is currently under investigation in our laboratory.



REFERENCES

1. Menozzi G, Merello L, Fossa P, Mosti L, Piana A, Mattioli F, Il Farmaco, 58, 795-808, 2003.
2. De Leval X, Hanson J, David JL, Masereel B, Pirotte B, Dogne JM., Curr. Med. Chem. 11, 1243-52, 2004.

POSTER COMMUNICATIONS – PC.295

MANNANOSE-BASED DC-SIGN INHIBITORS; THE DISCOVERY OF ALTERNATIVE HYDROPHOBIC BINDING POCKETS

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DC-SIGN is a C-type lectin on dendritic cells that binds invading pathogens and mediates an adaptive immune response from T cells.¹ Some pathogens (as HIV-1) bind to DC-SIGN and use it as entering point to DCs. Accordingly, inhibition of pathogen interaction with DC-SIGN specific inhibitors is considered as a plausible concept for new anti-infectives. DC-SIGN specifically binds branched mannose and fucose-containing ligands on the pathogen surface with high affinity and their oligosaccharides with modest affinity.² We have recently reported a pseudo-1,2-mannobioside with moderate antiviral activity in the Ebola infection model (IC₅₀ = 0.62 mM), while its azide derivative, inhibited DC-SIGN adhesion with IC₅₀ = 1.1 mM (measured by surface-plasmon resonance).³ To improve binding affinities of monovalent glycoconjugates, we have designed glycoconjugates based on pseudo-1,2-mannobioside that could bind into hydrophobic binding pockets on DC-SIGN CRD unoccupied by native ligands. These binding pockets were identified by careful examination of crystal structure of DC-SIGN CRD in complex with tetramannoside Man₄ (PDB code: 1SL4, Figure 1). We have synthesized promising candidates and determined their affinities to DC-SIGN by an in vitro assay that measures inhibition of DC-SIGN-mediated immature dendritic cell adhesion. Additionally, we have performed docking studies to rationalize the results and to suggest further optimization. The assay data demonstrate that our efforts to design and synthesize mannose-based DC-SIGN inhibitors resulted in compounds, which inhibit DC-SIGN-mediated adhesion in low micromolar range.

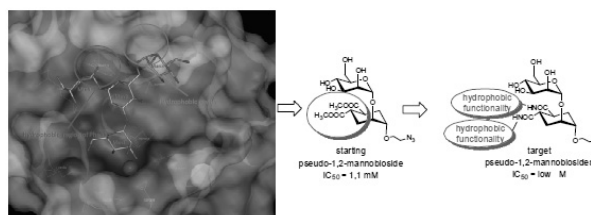


Figure 1.

REFERENCES

1. U Švajger, M Anderluh, M Jeras, N Obermajer. Cell. Signal., 2010, accepted for publication.

2. Y Guo, H Feinberg, E Conroy et al. *Nat. Struct. Mol. Biol.*, **2004**, 11(7), 591-8.
3. JJ Reina, S Sattin, D Invernizzi et al. *ChemMedChem*, **2007**, 2 (7), 1030-1036.

POSTER COMMUNICATIONS – PC.296

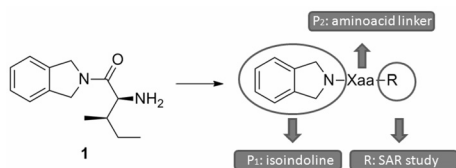
STRUCTURE-ACTIVITY RELATIONSHIP STUDY ON ISOINDOLINE INHIBITORS OF DIPEPTIDYL PEPTIDASE 8 AND 9

Van Goethem S.¹, Van der Veken P.¹, Joossens J.¹, Lambeir A. M.², Chen X.³, De Meester I.², Haemers A.¹, Augustyns K.¹

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Dipeptidyl peptidase 8 (DPP8) and DPP9 are members of the prolyl peptidase family, which also includes DPPIV (EC 3.4.14.5), fibroblast activation protein (FAP), prolyl oligopeptidase, and others. Compared with well-studied DPPIV, the functions of cytoplasmic enzymes DPP8 and DPP9 are yet to be elucidated. Nevertheless DPP8 and DPP9 have garnered much attention following research results from Merck suggesting that the use of the DPP8/9 inhibitor *allo*-Ile-isoindoline **1** is associated with severe toxicity in animal models.¹ Contrasting with these findings, two recent studies suggest that DPP8/9 inhibition alone cannot account for these toxic symptoms.² Another study claims the observation that DPP8 and DPP9 are up regulated in experimentally induced asthma and that these peptidases specifically respond to the inflammatory stimulus.³ In addition DPP9 appears to be a rate-limiting enzyme for degradation of proline-containing peptides.⁴ These intriguing data call for further investigation.

In order to verify whether the reported toxicity observations are related to DPP8/9 inhibition, or solely due to compound mediated off-target effects and to further study the clinical relevance of potential enzyme involvement in pathologies, there is an urgent need for new, preferably structurally distinct inhibitors of these enzymes. The goal of the present project was to conceive the structure-activity relationship on a chemically diverse set of inhibitors containing an isoindoline moiety. The inhibitors' affinity toward the different DPPs will be discussed, including their potential to discriminate between DPP8 and DPP9.



REFERENCES

1. Lankas, G. R., et al. Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes – Potential importance of selectivity over dipeptidyl peptidase 8 and 9. *Diabetes* **2005**, 54, 2988-2944
2. Roseblum J. S., et al. The case against GI toxicity from acute DPP8/9 inhibition. *Diabetes*, **2007**, 56, A138, Poster presented at the American Diabetes Association congress 2007; Burkey B. F., et al Adverse effects of dipeptidyl peptidases 8 and 9 inhibition in rodents revisited. *Diabetes, obesity and metabolism* **2008**, 10, 1057-1061
3. Schade J., et al. Regulation of expression and function of dipeptidyl peptidase 4 (DP4), DP8/9, and DP10 in allergic responses of the lung in rats. *J. Histochem. Cytochem.*, **2007**, 56, 147-155
4. Geiss-Friedlander R. et al The cytoplasmic peptidase DPP9 is rate-limiting for degradation of proline-containing peptides **2009**, 284, 27211-27219

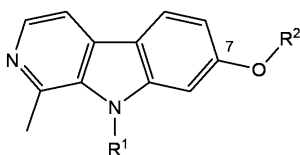
POSTER COMMUNICATIONS – PC.297

INHIBITION STUDY OF MONOAMINE OXIDASES BY β -CARBOLINE DERIVATIVES

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Harmine is a reversible inhibitor of human monoamine oxidase A (MAO-A) (1) and the crystal structure of human MAO-A in complex with harmine has been recently published. (2) This crystal structure shows that close to the methoxy group of the harmine moiety, a lipophilic pocket is left vacant within the binding site of hMAO-A. Our objective was to optimize the β -carboline series against MAO-A in order to explore this pocket. Therefore, a series of β -carboline derivatives has been synthesized. The compounds were evaluated for their human monoamine oxidase A and B inhibitory potency and their K_i values were calculated. The results show that O-alkylated compounds with lipophilic groups like cyclohexyl, phenyl and aliphatic chains increase the inhibition of MAO-A compared to harmine. Compound with the trifluorobutyloxy group, was the most active of this series, with a K_i against MAO-A of 3.6 nM. Molecular docking studies show that the trifluorobutyloxy chain occupies the hydrophobic pocket vacant with harmine. The O-alkylated compounds are less active on MAO-B than on MAO-A. However, several compounds show a better inhibition on MAO-B compared to harmine.



R¹ = H, benzyl

R² = Alkyl, Allyl, (CH₂)₃CF₃, (CH₂)₂OCH₃, (CH₂)₂OH, CH₂-c-Hx, CO-Ar, (CH₂)_n-Ar (n = 1,2), CH₂-pyridyl, (CH₂)_n-N(CH₃)₂ (n = 2,3), (CH₂)₂-morpholine

Keywords: β -carboline; MAO inhibition; Docking; X-ray.

REFERENCES

1. Kim, H.; Sablin, S. O.; Ramsay, R. R. *Arch Biochem Biophys* 1997, 337, 137-42.
2. Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. *Proc Natl Acad Sci U S A* 2008, 105, 5739-44.

POSTER COMMUNICATIONS – PC.298

ENOL MIMICS WITH A FLEXIBLE SIDE CHAIN AS INHIBITORS OF MYCOBACTERIUM TUBERCULOSIS DEHYDROQUINASE

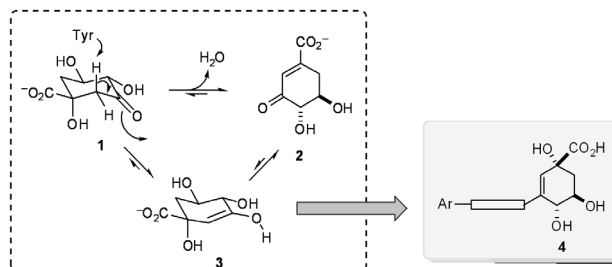
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For years, antibiotics have been fundamental in combating infectious diseases caused by bacteria. Despite the enormous therapeutic arsenal available today, we face a serious problem because the infections caused by pathogenic bacteria have become resistant to the actual antibiotic therapies. For example, tuberculosis, pneumonia or gonorrhea are increasingly more difficult to treat. This phenomenon is especially significant in important diseases such as tuberculosis, where the commonly used therapy has become less efficient. It is therefore necessary to develop updated therapies targeting infectious diseases, in particular, drugs with new mechanisms of action to combat the growth of antibiotic-resistant bacteria.

One of the strategies for the development of new antibiotics is to design compounds capable of interfering with essential processes for bacterial survival. This possibility has been studied in our research group in the last few years.¹ It consists in the design and the synthesis of compounds whose mode of action is based on the selective and the effective inhibition of an essential enzyme, dehydroquinase. Based on the enzymatic mechanism, we have designed and synthesized several analogues of the reaction intermediate **3**, compounds **4**, that have a flexible arm which blocks the action of two key residues in catalysis. Our recent progress made on the design and the synthesis of *M. tuberculosis*

sis type II dehydroquinase inhibitors will be presented in this communication.



REFERENCES

1. González-Bello, C.; Castedo, L. *Med. Res. Rev.* **2007**, 27, 177

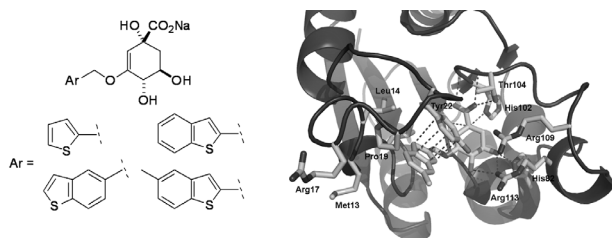
POSTER COMMUNICATIONS – PC.299

STEADY-STATE FLUORESCENCE AND MOLECULAR DYNAMIC STUDIES ON THE INHIBITION OF TYPE II DEHYDROQUINASE BY (2R)-3-BENZYL-3-DEHYDROQUINIC ACIDS

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The ulcer-causing *Helicobacter pylori* bacterium with strains resistant to clarithromycin is becoming more and more prevalent.^{1,2} Specifically, it has been estimated that the widespread of *H. pylori* resistance to clarithromycin is <5% for adults in northern Europe, but as high as 20% in southern Europe. It is present in approximately 13% of patients in USA, but in selected regions such as Alaska has been reported to have rates as high as 30%.² In addition, *H. pylori* infected persons are at increased risk for mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma.³ Therefore, the development of new agents that are able to overcome existing resistance mechanisms or that have novel mechanisms of action is much needed. In the last few years, our research group has been studying the possible development of new antibiotics whose action is based on the selective and effective inhibition of an essential route in bacteria, the shikimic acid pathway. Herein, we present the synthesis of novel inhibitors with high affinity for *Helicobacter pylori* type II dehydroquinase, the third enzyme of this route, and efficient inhibition characteristics. The structure of *Helicobacter pylori* type II dehydroquinase in complex with the most potent inhibitor shows the aromatic functional group interacts with the catalytic Tyr22 by π -stacking, expelling the Arg17 side-chain, which is essential for catalysis, from the active site. The structure therefore explains the favorable properties of the inhibitor and will aid in design of improved antibiotics.⁴



REFERENCES

1. Duck, W. M. et al. *Emerg. Infect. Diseases* **2004**, *10*, 1088.
2. Megraud, F. *Gut* **2004**, *53*, 1374.
3. Hatakeyama, M. *J. Gastroenterol* **2009**, *44*, 239.
4. González-Bello, C. et al. *J. Med. Chem.* **2010**, *53*, 191.

POSTER COMMUNICATIONS – PC.300

SYNTHESIS AND BIOLOGICAL EVALUATION OF OPTIMIZED THIAZOLIDIN-4-ONE-BASED MURD LIGASE INHIBITORS

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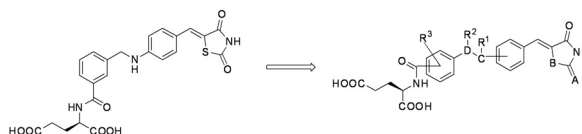
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The search for novel antibacterial agents with new mechanisms of action is one of the key strategies emerging for combating drug-resistant bacteria.¹ Recently, there has been an increased interest in exploiting the enzymes involved in the early cytoplasmic steps of peptidoglycan biosynthesis for antibacterial drug discovery. Among them are the ATP-dependent Mur ligases (MurC to MurF) that catalyze a series of reactions leading to UDP-MurNAc-pentapeptide by sequentially adding L-Ala (MurC), D-Glu (MurD), L-Lys or *meso*-diaminopimelic acid (MurE) and D-Ala-D-Ala dipeptide (MurF) to the starting MurC substrate UDP-MurNAc.²

We synthesized and biologically evaluated a series of 5-benzylidenerhodanine- and 5-benzylidenethiazolidine-2,4-dione-based inhibitors of MurD ligase from *E. coli*.³ The most potent compounds inhibited the enzyme with IC₅₀ values in the low micromolar range. The binding mode of this structural type of MurD inhibitors was established by X-ray crystallography and protein NMR. The crystal structures of MurD in complex with thiazolidine-4-one inhibitors provide useful information for structure-based design of novel inhibitors with improved inhibitory activity against MurD. D-Glutamic acid moiety of the inhibitor binds into the D-Glu binding pocket of MurD active site, where it forms several hydrogen bonds with the protein. Rhodanine or thiazolidine-2,4-dione ring occupies the uracil-binding pocket of natural MurD substrate UDP-MurNAc-L-Ala and interacts with the side chain hydroxyl group of Thr36. Other hydrogen bond

interactions observed between inhibitors and the active site residues are mediated through crystal water molecules.

To further explore the structure-activity relationship of thiazolidine-4-one-based compounds for MurD inhibition, rhodanine and thiazolidine-2,4-dione rings were replaced by pseudothiohydantoin, hydantoin and succinimide, which can also form observed hydrogen bonds with Thr36 in the uracil-binding pocket. Furthermore, the middle part of the known inhibitors was also a subject of extensive structural modification in order to investigate additional interactions in the MurD active site.



REFERENCES

1. Chopra, I.; Schofield, C.; Everett, M.; O'Neill, A.; Miller, K.; Wilcox, M.; Frère, J.-M.; Dawson, M.; Czaplewski, L.; Urleb, U.; Courvalin, P. *Lancet Infect. Dis.* **2008**, *8*, 133-139.
2. Barreateau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* **2008**, *32*, 168-207.
3. Tomašič, T.; Zidar, N.; Rupnik, V.; Kovač, A.; Blanot, D.; Gobec, S.; Kikelj, D.; Mašič, L.P.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 153-157.

POSTER COMMUNICATIONS – PC.301

THE SYNTHESIS, MOLECULAR MODELLING AND PHARMACOLOGICAL TESTING OF NOVEL ANALOGUES OF OLANZAPINE

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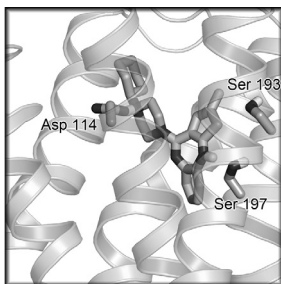
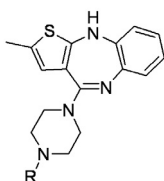
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Atypical antipsychotic drugs offer several notable benefits over typical antipsychotics, including greater improvement in the treatment of negative symptoms, cognitive function, prevention of the deterioration of the quality of life, and fewer extrapyramidal symptoms (EPS). Olanzapine is one such drug; however concerns about EPS have been replaced by concerns about other side effects such as weight gain, hyperlipidemia, type II diabetes mellitus and neutropenia. Olanzapine was introduced to the market in 1996 by Eli Lilly, and exhibits affinity towards a multitude of receptors, predominantly D₂ and 5-HT_{2A} as well as H₁ and M₁ receptors. This multi-acting receptor profile is believed to be unique to olanzapine's therapeutic mechanism of action, but also contributes to an array of undesirable effects.

The presented work examines potential new drug candidates as novel analogues of olanzapine by the incorporation of varied

aliphatic groups to the distal nitrogen, including structures with variations in chain length and branching (acyclic), carbocyclic, heterocyclic (isosteric replacement), saturation and ionization.

The presented work also incorporates rational drug design with the assistance of molecular modelling techniques using the recently published β_2 -adrenoceptor as a template for homology models of the dopamine D₂ and serotonin 5-HT_{2A} receptors to analyse *in silico* interactions between the ligands and the receptor. Furthermore, preliminary *in vitro* analyses were undertaken to assess the affinities of these novel analogues towards D₂ and 5-HT_{2A} receptors, thus aiding validation of molecular modelling, and shedding light on potentially improved antipsychotic therapies for the future.



POSTER COMMUNICATIONS – PC.302

EFFICIENT ACCESS TO ORTHOGONALLY PROTECTED DISULFIDE BRIDGE MIMETICS

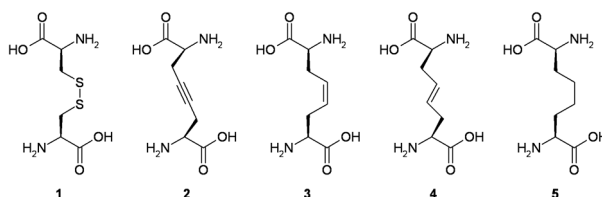
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Many naturally occurring and engineered biologically active peptides are cyclized via disulfide bridges. However, the disulfide bond is not as strong as a carbon-carbon bond, it can also be easily reduced *in vivo* to the open chain peptide. Interest in peptidomimetics and *in vivo* stabilization of peptides has led us to examine disulfide bridge analogues where the disulfide bridge is replaced by different carbon chain linkers. Our primary interest is the bicyclic peptide C-4 that stimulates the activity of human tissue kallikrein 3 (KLK3, also known as prostate specific antigen, PSA).¹ The KLK3 stimulating peptide has the potential to provide a new non-invasive therapy for prostate cancer.² The peptide consists of 13 amino acids, with one terminal disulfide bridge between cysteines 1 and 13 and one internal between cysteines 5 and 10.

We have successfully developed efficient, high-yielding, enantiopure synthetic procedures of four disulfide bridge mimetics **2–5**, possessing an alkyne, a *Z*-alkene, a *E*-alkene and an fully saturated linker instead of the disulfide bridge. These linkers have different geometries and should be able to mimic any conformation of the disulfide bridge **1**. The mimetics are protected

by completely orthogonal protecting groups (Fmoc and Boc as *N*-terminal protecting groups and *tert*-butyl and methyl esters as *C*-terminal protecting groups). We have utilised alkyl copper-zinc/haloalkyne chemistry (previously developed by Knochel) to synthesise a disulfide bridge mimetic containing an alkyne moiety (**2**). Selective and full reduction of the triple-bond using Pd/BaSO₄ and Pd/C respectively, enabled access to the *Z*-alkene and the fully saturated mimetics (**3** and **5**). For the enantiopure synthesis of the corresponding mimetic possessing an *E*-alkene (**4**), we successfully employed a Grubbs cross-metathesis reaction.



REFERENCES

1. Wu P., Leinonen J., Koivunen E., Lankinen H., Stenman U.-H. *Eur. J. Biochem.* **2000**, 267, 6212-6220.
2. Koistinen H., Närvänen A., Pakkala M., Hekim C., Mattsson J.M., Zhu L., Laakkonen P., Stenman U.-H. *Biol. Chem.* **2008**, 389, 633-642.

POSTER COMMUNICATIONS – PC.303

S-NITROSYLATION AND ATTENUATION OF CALCIUM FLUX BY PENTACYCLOUNDECANE DERIVATIVES

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Glutamate-related excitotoxic neuronal cell injury and death has been reported to contribute to almost every major neurodegenerative disorder.^{1,2} Excitotoxicity can occur by over activation of NMDA-sensitive glutamate receptors and the subsequent excessive calcium influx into the associated cells.^{3,4} There are numerous insults that can lead to excessive glutamate release and stimulation of glutamate receptors, with subsequent excitotoxicity and cell death. All of them share the same pathway in that large amounts of glutamate is released from the injured cells which then reach the thousands of nearby cells, causing them to depolarise, swell, lyse and die by necrosis. In chronic diseases, it is proposed that modest amounts of glutamate over an extended period are to blame for the excitotoxic damage.^{3,5,6} To attenuate the effects of excitotoxicity, three mechanisms of action are proposed for this study. The first is S-nitrosylation of crucial cysteine residues in the NMDA receptor channel, the second is the

blockage of excessively stimulated voltage gated Ca^{2+} channels and the third is through NMDA receptor channel antagonism by the polycyclic amines.

A novel series of polycyclic amines, containing nitrogen monoxide donating moieties, were synthesised and tested for calcium channel and N-methyl-D-aspartate (NMDA) receptor modulating activity. These included unsaturated nitro compounds and nitro esters, or nitrates. The nitrates were obtained via the reaction of hydroxyl functionalities with thionylchloride nitrate. All of the compounds synthesised exhibited significant ($p < 0.01$) S-nitrosylation capacity. The calcium channel activity of the polycyclic amines was evaluated using a KCl mediated fluorescent calcium flux assay. All the compounds exhibited better calcium channel antagonism than the lead structure, NGP1-01, with 8-aminoethyl-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-2-(4-nitrobenzoate) exhibiting calcium channel blockade comparable to that of nimodipine at 10 μM and 1 μM . All the compounds exhibited NMDA/glycine mediated N-methyl-D-aspartate receptor (NMDAR) calcium influx inhibition at 100 μM , with 8-aminoethyl-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-2-[4-(2-nitroethenyl)benzoate] showing the most significant NMDAR inhibitory activity.

No clear correlation was observed between the S-nitrosylation capabilities of the compounds and their calcium channel activity or NMDAR channel antagonism. All the compounds synthesised exhibited promising calcium channel and NMDAR channel inhibitory activity and thus show promise as potential agents against neurodegeneration.

REFERENCES

1. R.P. Simon, J.H. Swan, and B.S. Meldrum, *Science*. 1984, 244, 798-800.
2. D.W. Choi, *Neurosci Lett*. 1985, 58, 293-297.
3. S.A. Lipton, *Nat. Rev. Drug Discovery*. 2006, 5, 160-170.
4. M. Arundine and M. Tymianski, *Cell. Mol. Life Sci*. 2004, 61, 657-668.
5. P. Lipton, 1999. *Physiol. Rev*. 1999, 79, 1431-1568.
6. J.A. Kemp and R.M. McKernan, *Nature Neurosci*. 2002, 5, 1039-1042.

POSTER COMMUNICATIONS – PC.304

NOVEL CCR9 ANTAGONISTS SYNTHESIS, OPTIMIZATION AND BIOLOGICAL ACTIVITY

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Crohn's disease is an autoimmune disease affecting mainly the small intestine in which large numbers of antigen-experienced T

cells accumulate within the intestinal lamina propria. The chemokine receptor CCR9 is expressed by most T cells trafficking to the small intestine. Blockade of the interaction of CCR9 on gut-homing T cells with its only ligand TECK, also expressed in the small intestine, is believed to specifically inhibit the migration of pro-inflammatory T cells into the small intestine and will result in a therapeutic benefit. As shown for other chemokine receptor programs high *in vitro* potency is a necessary prerequisite however successful drug candidates will have also to fulfil other parameters like adequate physico-chemical and ADMET properties.¹⁾

Here we report on the synthesis and biological profiling of a novel type of potent CCR9 antagonists. The first generation of compounds is featuring an aromatic *tert*.butyl group which contributes to very high logP values and secondly is prone to metabolic attack. Therefore, our derivation program focussed on replacing this *tert*.butyl group by metabolically more stable groups with similar space filling. We investigated two compound series and identified a SF5-substituted derivative which is combining improved stability and adjusted logP values with high potency in a CCR9-dependent Ca^{2+} mobilization assay.

In summary, we show here that in our case the *tert*.butyl group can be successfully replaced by a SF5 group² leading to a candidate with favourable overall properties. Furthermore, this protocol of replacing a *tert*.butyl by a SF5 group might also be useful in other Medchem programs where an aromatic *tert*.butyl group is creating an issue of high lipophilicity and metabolic instability.

REFERENCES

1. Amanda El Proudfoot; *Expert Opin. Investig. Drugs* 19(3), 345-355 [2010]
2. John T. Welch; *Bioorg. Med. Chem.* 15(21), 6659-6666 [2007]

POSTER COMMUNICATIONS – PC.305

BIOLOGICAL EVALUATION OF THE ANTITUMOUR ACTIVITY OF IMIDOSELENOCARBAMATES DPR235 AND DPR380

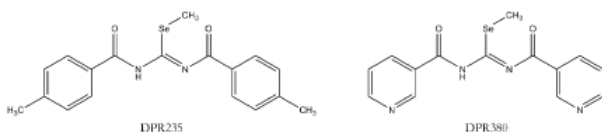
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Cancer is becoming an increasingly significant disease worldwide¹. It's well known that the trace element selenium (Se) appears to have cancer preventive properties based on a con-

verging body of evidence from epidemiologic, clinical and experimental studies^{2,3}. Though mechanisms of action for seleno-compounds as anticancer agents are not fully understood, it has been reported that the dose and chemical form of selenium are determinant factors on its antitumour activity⁴.

Among the growing list of seleno-compounds with desirable anticancer activity, we previously reported the synthesis of various imidoselenocarbamates with significant *in vitro* antiproliferative activity against human prostate cancer cells PC-3⁵. To further characterize the antitumour activity of these compounds, here we extend the evaluation of the antiproliferative action of two of them, DPR235 and DPR380, to a panel of four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and two non-malignant cell lines (184B5 and BEAS). We also analyze the ability of DPR235 and DPR380 to induce ROS overproduction and apoptosis, as well as their effect on cell cycle distribution in MCF-7 cells.



REFERENCES

1. Rizky Abdulah, Kaori Miyazaki, Minato Nakazawa, Hiroshi Koyama; Journal of Trace Elements in Medicine and Biology, Volume 19, Issues 2-3, 2 December 2005, Pages 141-150.
2. Gurusamy, K. Biol. Trace Elem. Res, 2007, 118, 191-206.
3. Ceung, E.; Wadhwa, P.; Dorff, T.; Pinski, J. Expert Rev. Anticancer Ther. 2008, 8, 43-50.
4. Rikiishi, H. J. Bioenerg. Biomembr. 2007, 39, 91-98.
5. Plano, D.; Sanmartin, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. Bioorg. Med. Chem. Lett., 2007, 17, 6853-6859.

POSTER COMMUNICATIONS – **PC.306**

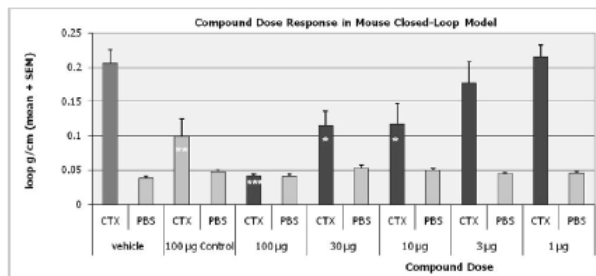
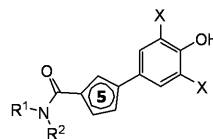
INHIBITORS OF THE CFTR CHLORIDE ION CHANNEL AS POTENTIAL TREATMENT FOR ACUTE SECRETORY DIARRHEA: DEVELOPMENT OF 5-MEMBERED HETEROCYCLES SUITABLE FOR PRE-CLINICAL EVALUATION

Kevin Doyle¹, Graham Jones¹, Michael Russell¹, Joanne Peach¹, Amanda Van de Poël¹, Carrie-Anne Molyneaux¹, Jackie Macritchie¹, Angus MacLeod¹, Joanne Shearer¹, David Cronk¹, Gary Clark¹, Alan Beresford¹, Eugenio de Hostos², Tue Nguyen², David Brown²

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Acute secretory diarrhea is responsible for the deaths of up to 2 million children a year in the developing world. The current standard of care is a glucose/salt solution, known as oral rehydration therapy (ORT), which is a palliative treatment for the disease. However, the labor intensive regimen alongside the lack of symptomatic relief leads to a high level of noncompliance. The identification of an adjunct therapy to work alongside ORT and improve time to symptomatic relief is paramount.

Acute secretory diarrhea is caused by an intestinal infection with pathogens that include *vibrio cholera* resulting in the output of large volumes of watery stools. The major mechanism for this infection is the over-activation of the chloride ion channel, CFTR (cystic fibrosis transmembrane conductance regulator), present in the epithelial cells within the intestinal wall. We demonstrate that inhibition of this ion channel with 5-membered heterocyclic compounds gives blockage of chloride transport in a variety of *in vitro* systems. In addition, we show that a selection of these compounds is efficacious in two animal models for diarrheal disease. The identification and optimization of a series of 5-membered heterocyclic inhibitors suitable for pre-clinical evaluation will be discussed.



POSTER COMMUNICATIONS – **PC.307**

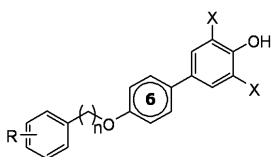
INHIBITORS OF THE CFTR CHLORIDE ION CHANNEL AS POTENTIAL TREATMENT FOR ACUTE SECRETORY DIARRHEA: DEVELOPMENT OF 6-MEMBERED HETEROCYCLES

Kevin Doyle¹, Michael Russell¹, Joanne Peach¹, Stephen Penrose¹, Amanda Van de Poël¹, Graham Jones¹, Jackie Macritchie¹, Angus MacLeod¹, Sebastian Brückner¹, Joanne Shearer¹, David Cronk¹, Gary Clark¹, Alan Beresford¹, Eugenio de Hostos², Tue Nguyen², David Brown²

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POSTER COMMUNICATIONS – PC.308

N-SUBSTITUTED DERIVATIVES OF DEXTROMETHORPHAN ARE INHIBITORS OF $\alpha 3\beta 4$ NICOTINIC ACETYLCHOLINE RECEPTOR

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Dextromethorphan (DM) is widely used ingredient of antitussive OTC medicaments. Due to its complex pharmacology scientists suggested several other potential therapeutic uses (e.g., in Huntington's and Parkinson's diseases or cocaine dependence). Our previous studies indicated DM as potent inhibitor of neuronal nicotinic acetylcholine receptor (nAChR). Molecular modeling studies allowed us to identify a binding site within the nAChR ion channel and to design a series of N-substituted DM derivatives with optimized interactions with $\alpha 3\beta 4$ subtype of nAChR. Some of these molecules were synthesized and characterized structurally [Fig.1].

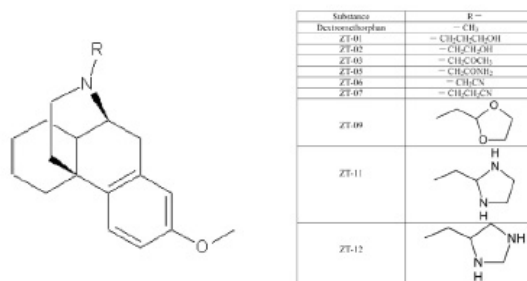


Fig. 1 The structure of dextromethorphan and selected synthesized derivatives.

Currently new derivatives are tested for functional activity against $\alpha 3\beta 4$ nAChR in electrophysiological assay. *In vitro* experiments are performed on HEK-293 cells stably transfected with rat $\alpha 3$ -nAChR and $\beta 4$ -nAChR genes. DM and its new derivatives could play a pivotal role in developing new more efficient therapy for nicotine addiction. The latest scientific evidence demonstrates that $\alpha 3\beta 4$ nAChRs are densely expressed in habenular - interpeduncular (Hb – IPN) system regulating the dopaminergic pathway responsible for addiction development. In next step of the project the most promising compounds will be investigated in animal tests for blocking of nicotine dependence.

POSTER COMMUNICATIONS – PC.309

NOVEL ORGANOSELENIUM DERIVATIVES: A NEW CLASS OF POTENT ANTILEISHMANIAL AGENTS

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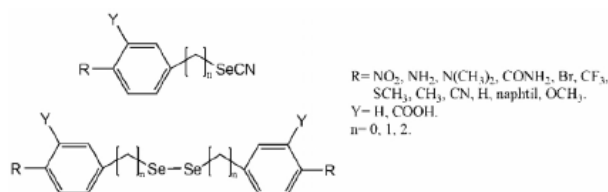
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The leishmaniasis are a group of tropical diseases. World Health Organization (WHO) estimates that the disease results in 2 million new cases a year and that there are 12 million people currently infected worldwide (1).

Different studies recognised the trace element selenium as a new defense strategy against *Leishmania* infection (2), and also the importance of possible potential interference of selenium derivatives with the redox system of the parasites (3).

Due to the urgent need for innovative drugs and previous work of our research group in organoselenium compounds (4-5), we carried out the synthesis and biological evaluation of selenocyanate and diselenide derivatives, according with these general structures:



All the synthesized compounds were subjected to *in vitro* screening against *L. infantum* promastigote and the most active were tested in amastigote model. In order to establish the selectivity index (SI) their cytotoxic effect was carried out against Jurkat and THP-1 cell lines. Nine derivatives exhibited IC_{50} values better than miltefosine and six of them better than edelfosine than were used as standard drugs. Among all, some compounds combine high potency and low cytotoxicity with selectivity index better than reference drugs.

REFERENCES

- Kedzierski, L.; Zhu, Y.; Handman, E. *Parasitology* **2006**, *133*, S87-S112.
- Culha, G.; Yalin, E.; Sanguen, K. *Asian J. Chem.* **2008**, *20*, 3104-3108.
- Sculaccio, S.A.; Rodríguez, E.M.; Cordeiro, A.T.; Magalhães, A.; Braga, A.L.; Alberto, E.E.; Thiemann, O.H. *Mol. Biochem. Parasitol.* **2008**, *162*, 165-171.
- Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6853-6859.
- Sanmartín, C.; Plano, D.; Domínguez, E.; Font, M.; Calvo, A.; Prior, C.; Encío, I.; Palop, J.A. *Molecules* **2009**, *14*, 3313-38.

POSTER COMMUNICATIONS – PC.310

NOVEL INDOLYL ACRYLIC ACID DERIVATIVES AS POTENT INHIBITORS OF DIACYLGLYCEROL ACYLTRANSFERASE

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Triglyceride (TG) is a major form of stored energy in eukaryotes, and it is synthesized primarily through acyl CoA: diacylglycerol acyltransferase (DGAT) reaction that catalyzes the final and the only committed step in the glycerol phosphate pathway involving in the biosynthesis pathways of TG within the insulin-sensitive tissues including the adipose tissue, skeletal muscles, liver, and the β -cells of the pancreas. TG synthesis is important in mammals for energy storage in the adipose tissue and muscle, fat absorption in the intestine, and the assembly of lipoprotein in the liver and in the small intestine as well. As TG is also essential for the accumulation of adipose tissue in the body, inhibition

of TG synthesis may reduce the incidence of obesity and its related diseases. Recently, it has been reported that diet-induced obesity caused by high fat diet could be effectively inhibited in DGAT-1 knock-down mice, and abnormal glucose metabolism was improved by increase of the susceptibility of insulin and leptin, the dietary hormones. As inhibition of DAGT has emerged as a therapeutic target for inhibiting TG synthesis, a potent DGAT inhibitors would be useful for the treatment of metabolic diseases. In the search of novel DGAT inhibitors, it was found that novel indolyl acrylic acid derivatives showed potent DGAT inhibitory effect. Herein, the structure-activity relationships of the indolyl acrylic acid derivatives will be presented.

POSTER COMMUNICATIONS – PC.311

PEROXIREDOXIN INHIBITORS TO SELECTIVELY KILL CANCER CELLS THROUGH A ROS-MEDIATED MECHANISM

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Most cancer cells exhibit an increase in reactive oxygen species (ROS) generation, which enable to maintain their malignant phenotypes. Cancer cells, under oxidative stress associated with increased metabolic activity and production of ROS, are highly dependent on antioxidant system for survival and thus more susceptible to oxidative insults by ROS-stressing agents than normal cells. Peroxiredoxin (Prx), the novel H_2O_2 eliminating enzyme, is known to be elevated in a variety of human cancers. Recent studies have suggested that this biochemical property of Prxs in cancer cells can be exploited for therapeutic benefits. In our search for novel anticancer agents to inhibit Prx I, it was found that N-(3-acetylphenyl)-4-(biphenyl-4-ylmethyl)piperidine-1-carboxamide effectively inhibits the reduction of Prx I from cysteine sulfenic acid to cysteine sulfhydryl acid through interruption of electron transfer from reducing system and causes severe ROS accumulation in cancer cells. Further biochemical study suggests that the Prx-mediated H_2O_2 elimination may serve as a novel therapeutic target for cancer intervention. Herein, the structure-activity relationships and biochemical study of the piperidine-1-carboxamide derivatives will be presented.

POSTER COMMUNICATIONS – PC.312

DESIGN AND SYNTHESIS OF QUINOLINE DERIVATIVES AS A NOVEL CLASS OF ACYL COA: CHOLESTROL ACYL TRANSFERASE INHIBITORS

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Acyl-CoA: Cholesterol acyltransferase (ACAT, E.C.2.3.1.26) is the primary enzyme responsible for the intracellular esterification of free cholesterol with fatty acyl-CoA to produce cholesterol esters in various tissues. Accumulation of cholesteryl ester leads to the formation of foam cells and a hallmark of atherosclerosis lesions. Inhibition of ACAT is expected to reduce plasma lipid levels by inhibiting intestinal cholesterol absorption and to prevent progression of atherosclerotic lesions by inhibiting the accumulation of cholesteryl esters in macrophage. Thus, the inhibitor of ACAT activity could be the competitive drug in the prevention and treatments of hypercholesterolemia and atherosclerosis by inhibiting a formation of cholesteryl ester within the cell. Recently, it was reported that shikonin derivatives with naphthoquinone template possessed ACAT inhibitory activity in vitro as well as cell-based assay system. Based on the activity of the naphthoquinone template, a series of novel quinoline derivatives were synthesized. Herein, the structure-activity relationships of the quinoline derivatives will be presented as potential ACAT inhibitors.

POSTER COMMUNICATIONS – PC.314

A NOVEL CHEMOATTRACTANT PROTEIN, KARATOXIN, FROM THE DORSAL SPINES OF THE REDFIN VELVETFISH, *HYPODYTES RUBRIPINNIS*Ohura K^{1*}, Shinohara M¹, Nagasaka K², Nakagawa H³, Edo K³¹ Department of Pharmacology, Osaka Dental University, Osaka, ² Fukae Nagasaka Clinic, Hiroshima, ³ University of Tokushima, Tokushima, Japan.

A chemoattractant protein from the dorsal spines of the redfin velvetfish, *Hypodytes rubripinnis* was isolated by a combination of affinity chromatography. The glycoprotein with a molecular mass of 110 kDa is named Karatoxin.

The redfin velvetfish, *Hypodytes rubripinnis* is a small congiopodid fish, that belongs to the Scorpaenidae group known as scor-

pionfish. However, *H. rubripinnis* is dangerous to man. More recently, we have observed that Karatoxin, the glycoprotein purified from the dorsal venom have cytotoxic activity on murine P388 leukemia cells (1).

In our search for bioactive compounds we have been investigating for chemoattractant activity from the dorsal venom of *H. rubripinnis*. Here we report biological activities of Karatoxin as a novel chemoattractant lectin from the dorsal venom of *H. rubripinnis*.

Hypodytes rubripinnis were collected by the local fishermen from the coast of Hiroshima Prefecture, Japan. The collected fish were transported alive or frozen to our laboratory. The dorsal spines (total 14) from *H. rubripinnis* were cut from their base.

Mitogenic activity by the murine splenocytes was determined by the cell culture assay using the MTT tetrazolium salt.

Polymorphonuclear leukocytes (neutrophils) and macrophages were induced by intraperitoneal injection of 1 % glycogen solution into male guinea-pigs, and collected by centrifugation in PBS. Chemotaxis were measured by membrane filter method using 48-well chemotaxis chamber.

We had reported that the dorsal venom protein from *H. rubripinnis* showed weak agglutination with rabbit erythrocytes and degranulation of the rat mesenteric mast cells. The agglutination caused by the venom was inhibited with the treatment of D-mannose. The venom also induced mitogenic activity on the murine splenocytes in the dose range of 10 to 100 µg/ml. These results suggested that the dorsal venom protein contain lectin components.

In this study, we report some biological activities of Karatoxin (110 kDa), a dorsal mannose-containing glycoprotein from *H. rubripinnis*. Karatoxin exhibited not only cytotoxic activity on the murine P388 leukemia cells, but also mitogenic activity on the murine splenocytes. In addition, Karatoxin had chemotactic activity of guinea-pig neutrophils and macrophages in a dose of low ranges (0.625 µg/ml - 5.0 µg/ml). Thus, Karatoxin appears to be a unique lectin.

Therefore, we have suspected that Karatoxin might affect inflammatory and immunomodulatory processes. Karatoxin appears to be a D-mannose-binding lectin. Our data suggest that *H. rubripinnis* venom may be a resource for pharmacologically active substances, such as lead compounds and research tools.

REFERENCES

1. Nagasaka K, Nakagawa H, Satoh F, Hosotani T, Yokoigawa K, et al. A novel cytotoxic protein, Karatoxin, from the dorsal spines of the redfin velvetfish, *Hypodytes rubripinnis*. Toxin Rev. 2009;28:260-265.

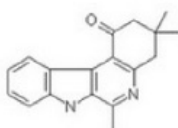
POSTER COMMUNICATIONS – PC.315

NEW HIGH-EFFECTIVE NEUROPROTECTOR – CARBACETAM

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As a result of systematic searches of the substances with influence on CNS our laboratory together with Donetsk Medical Institute worked out new full original high-efficient neuroprotector Carbacetam relating to the class of β -carbolines



Carbacetam is endogenous modulator of GABA_A- benzodiazepine receptor complex (diazepin binding inhibitor). This is powerful nootropic agent with very low tranquilizer activity, anti-amnesic action is 100 times and antihypoxic action 29-35 times as higher by piracetam, has any hypnotic and muscle relaxation activity and also any irritating, allergic, gonado- and embriotoxic or mutagenic action. It has IV toxicity class with LD₅₀ for mouse 346 and 1250mg/kg for rats. The drug was successfully passed following trials: full pre clinic and I and II clinical stages and was patented as stress protector, anti-amnesic, antihypoxic, antianxiotic and antishocking agent.

Friendly technology of this API was considerably improved by exclusion of dangerous materials: perchloric acid and diethyl ether from synthesis scheme.

POSTER COMMUNICATIONS – PC.316

ANTIOXIDANT PROPERTIES OF 4-QUINOLONES COMPARED TO STRUCTURALLY RELATED FLAVONES

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Oxidative stress, the result of overproduction of free radicals such as the hydroxyl radical, is a common occurrence in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. It is suggested that oxidative stress exists before the onset of these neurodegenerative diseases, leading to, or exacerbating deterioration.¹

Flavones are naturally occurring plant compounds with hydrogen donating substituents responsible for well established antioxidant activity.² Hydroxy-substituted flavones were selected as lead compounds, compared to similarly substituted 2-phenylquinolin-4(1H)-ones which were synthesised using the Conrad-Limpach method.³ The compounds, 2-phenylquinolin-4(1H)-one, 6-hydroxy-2-phenylquinolin-4(1H)-one, 7-hydroxy-2-phenylquinolin-4(1H)-one and 8-hydroxy-2-phenylquinolin-4(1H)-one were characterised by NMR, IR and MS. Quinoline, 4-hydroxyquinoline and 8-hydroxyquinoline were also included as reference compounds.

Antioxidant activity was evaluated using different *in vitro* assays to evaluate the potential value of the synthesised 2-phenylquinolin-4(1H)-ones compared to the existing structurally related flavones. The oxygen radical absorbance capacity⁴ was performed to establish the ability of the synthesised compounds to scavenge peroxy radicals. The 2-phenylquinolin-4(1H)-ones demonstrated better activity than the related flavones with 7-hydroxy-2-phenylquinolin-4(1H)-one observed as the best scavenger in the group.

The ferric reduction/antioxidant power assay⁵ assesses the theoretical inhibition of the Haber-Weiss reaction, leading to reduced hydroxyl radical production. In this assay the 2-phenylquinolin-4(1H)-ones also demonstrated better activity than the related flavones with 8-hydroxy-2-phenylquinolin-4(1H)-one having activity comparable to Trolox, a water soluble derivative of vitamin E.

Results from the nitroblue tetrazolium assay⁶ which evaluates the ability of a compound to scavenge superoxide anions, indicated moderate activity for the 2-phenylquinolin-4(1H)-ones, with 7-hydroxy-2-phenylquinolin-4(1H)-one demonstrating the best activity.

Results from the lipid peroxidation assay⁶ showed 6-hydroxy-2-phenylquinolin-4(1H)-one to be the best scavenger of hydroxyl radicals in the 2-phenylquinolin-4(1H)-ones series.

The results from the different assays indicated that the 2-phenylquinolin-4(1H)-ones acted as chain breaking antioxidants, with a postulated hydrogen donor mechanism of action and inhibiting radical mediated damage better than the related flavones, showing promise as possible neuroprotective agents

REFERENCES

1. W.R. Markesbery, T.M. Montine and M.A. Lovell, Pathogenesis of neurodegenerative diseases. 2001, 21-52
2. J. Robak and J. Gryglewski, Biochemical Pharmacology. 1988, 37, 837-841
3. R. Somanathan and K.M. Smith. Journal of Heterocyclic Chemistry. 1981, 18, 1077-1079
4. B. Ou, M. Hampsch-Woodill, R.L. Prior. Journal of Agriculture and Food Chemistry, 2001. 49, 4619-4626
5. I.F.F. Benzie and J.J. Strain. Analytical Biochemistry, 1996. 239, 70-76
6. P. Ottino and J.R. Duncan. Free Radical Biology and Medicine. 1997, 22, 1145-1151

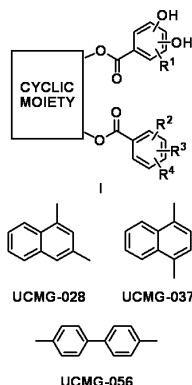
POSTER COMMUNICATIONS – PC.317

NOVEL SYNTHETIC INHIBITORS OF FATTY ACID SYNTHASE (FASN) WITH *IN VITRO* AND *IN VIVO* ANTICANCER ACTIVITY

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Fatty acid synthase (FASN) is highly expressed in breast carcinoma and other human cancers.¹ However, its therapeutic exploitation has been limited by the lack of pharmacological inhibitors that are potent, specific and stable enough.



Targeting FASN inhibition, in the present work we have synthesized a new series of compounds of general formula **I**, that were evaluated for their cytotoxic capacity in a panel of human breast cancer cell lines (SK-Br3, MCF-7, and MDA-MB-231) and in two types of non-malignant cells (fibroblasts and adipocytes), as well as for their effect in FASN activity.^{2,3}

We have identified three potent FASN inhibitors: UCMG-028, UCMG-037 and UCMG-056 (69-90% inhibition of FASN activity) that are highly cytotoxic against SK-Br3 (IC₅₀ < 30 μM) cells. Importantly, these compounds do not stimulate carnitine palmitoyltransferase-1 (CPT-1) activity *in vitro* and they do not decrease body weight *in vivo*, in contrast to previously reported FASN inhibitors such as C75. In addition, UCMG-028 blocked the growth of BT474 breast cancer xenografts.

Taken together, our results show that synthesized compounds **I** represent a novel class of synthetic FASN inhibitors endowed

with *in vitro* and *in vivo* anticancer activity, that do not exhibit adverse effects on body weight *in vivo*. Therefore, they hold promise for further target directed anticancer drug studies either alone or co-administered with other antitumoral drugs. We are currently exploring their full potential with the synthesis of a larger structural field to carry out an in-depth structure-activity relationship (SAR) study, as well as assessing their *in vivo* anticancer efficacy in breast cancer animal models. These results will enable the definitive validation of FASN as a therapeutically useful option for cancer treatment.

This work was supported by grants from Instituto de Salud Carlos III (FIS PI04/1417, FIS PI082031, and RD06-0020-0028; ISCIII-RETIC RD06; and FIS PI082031), the Susan G. Komen Breast Cancer Foundation (PDF-0504073), the Ministerio de Ciencia e Innovación (MICINN, CIT-090000-2008-10 and SAF-2007-67008-CO2-01), Comunidad Autónoma de Madrid (S-SAL-249-2006), and the Spanish Society of Medical Oncology (SEOM08), Ramón y Cajal (SO-G), and FPU (CT) programs from MICINN.

REFERENCES

1. Kuhajda, F. P. *Cancer Res.* **2006**, 66, 5977.
2. López-Rodríguez, M. L.; Benhamú, B.; Turrado, C.; Ortega-Gutiérrez, S.; Puig, T.; Brunet, J.; Colomer, R. Patent WO/2009/000864, **2008**.
3. Puig, T.; Turrado, C.; Benhamú, B.; Aguilar, H.; Relat, J.; Ortega-Gutiérrez, S.; Casals, G.; Marrero, P. F.; Urruticoechea, A.; Haro, D.; López-Rodríguez, M. L.; Colomer, R., *Clin. Cancer Res.* **2009**, 15, 7608.

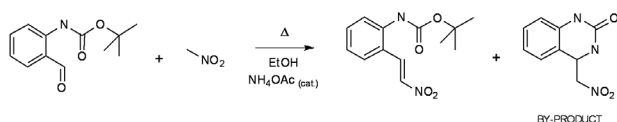
POSTER COMMUNICATIONS – PC.318

MICROWAVE-ASSISTED ONE-POT SYNTHESIS OF SUBSTITUTED 3,4-DIHYDRO-1H-QUINAZOLIN-2-ONES

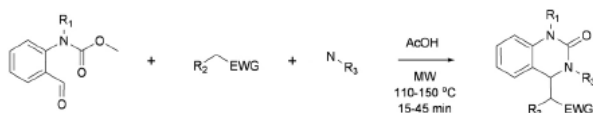
Krzysztof Więckowski, Luke Odell

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Quinazoline- and quinazolinone-derivatives constitute an important group of pharmacologically-active compounds. As inhibitors of enzymes and receptor ligands they have been investigated for decades and found application in treatment of various prevalent diseases (e.g. doxazosin, methaqualone, erlotinib) [1-4]. This study presents a new, facile and environmentally-friendly method for the synthesis of functionalized 3,4-dihydro-1H-quinazolin-2-ones. The procedure described herein was developed in the course of an optimisation of side process accompanying the Henry reaction between *N*-alkoxycarbonyl-2-aminobenzaldehydes and nitromethane.



The method worked out in the optimization allows total conversion of the benzaldehyde to the product (LCMS) with good isolated yields and short reaction time. Further studies revealed a broad scope of the reaction. Tolerance of different types of amines and nucleophiles makes this reaction a facile way to obtain various 1-, 3- and 4-substituted 3,4-dihydro-1H-quinazolin-2-ones.



Considering the simplicity of the method and the functional group compatibility (e.g. EWG, carbonyl) this transformation has significant potential, especially in medicinal chemistry applications - as a source of easily-accessible building blocks or final structures.

REFERENCES

1. Smits R. A., Adami M., Istyastono E. P., Zuiderveld O. P., van Dam C. M., de Kanter F. J., Jongejan A., Coruzzi G., Leurs R., de Esch I. J.; *J Med Chem.* 2010 Mar 25;53(6):2390-400.
2. Garofalo A., Goossens L., Lemoine A., Farce A., Arlot Y., Depreux P. J.; *Enzyme Inhib. Med. Chem.* 2010, 25 (2), 158-71.
3. Alagarsamy V., Raja Solomon V., Dhanabal K.; *Bioorg. Med. Chem.*, 2007, 15 (1), 235-241
4. Rzasa R. M., Kaller M. R., Liu G., Magal E., Nguyen T. T., Osslund T. D., Powers D., (...), Norman M.H.; *Bioorg. Med. Chem.* 2007, 15 (20), 6574-6595.

POSTER COMMUNICATIONS – PC.319

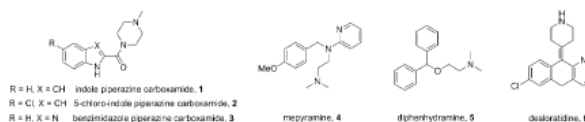
MOLECULAR MODELLING AND SYNTHESIS OF DUAL HISTAMINE H₁/H₄R-ANTAGONISTS

Wagner Eva, Gobleder Susanne, Straßer Andrea

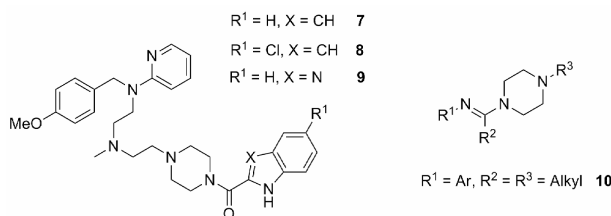
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Indole and benzimidazole piperazine carboxamides **1**, **2**, **3** are known to be potent histamine H₄-receptor antagonists [1]. The histamine H₄-receptor is discussed to be involved in inflammation and regulation of the immune system [2]. Histamine H₁-receptor antagonists like mepyramine **4**, diphenhydramine **5** and desloratadine **6** are used for treatment of allergic diseases. New studies show that combined application of **2** and **4** in the acute murine asthma model exhibits synergistic inhibitory effects on eosinophil accumulation in the bronchoalveolar lavage fluid [3]. Thus, the design of dual H₁/H₄R-antagonists may result in a new class of drugs for therapy of type-I allergic diseases, like rhinitis and con-

junctivitis. Therefore, we pursued two different strategies: 1) Development of ligands with one H₁- and one H₄-pharmacophore, coupled by an alkyl spacer; 2) Development of ligands with one common H₁/H₄-pharmacophore.



Based on the molecular modelling results, the most promising compounds were synthesized. Compounds **7**, **8** and **9** represent examples with regard to strategy 1, whereas **10** represents a general structure for strategy 2.



All compounds will be analyzed in GTPase and binding assays at hH₁R and hH₄R with regard to potency, efficacy and affinity.

REFERENCES

1. Venable et al. *J. Med. Chem.* **2005**, 48, 8289-8298.
2. Thurmond et al. *Nature Reviews Drug Discovery*, **2008**, 7, 41-53.
3. Deml et al. *Mol. Pharmacol.* **2009**, 76, 1019-1030.

POSTER COMMUNICATIONS – PC.320

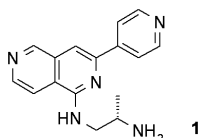
2,6-NAPHTHYRIDINES AS POTENT AND SELECTIVE INHIBITORS OF THE NOVEL PROTEIN KINASE C ISOTYPES

Maurice J. van Eis, Jean-Pierre Evenou, Philipp Floersheim, Christoph Gaul, Sandra W. Cowan-Jacob, Gabriele Rummel, Walter Schuler, Andre Strauss, Anette von Matt, Eric Vangrevelinghe, Juergen Wagner and Nicolas Soldermann

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Multiple members of the protein kinase C (PKC) family play a central role in the signalling cascades of the immune system, the key players being the classical PKC isotypes α/β and the novel PKC isotype θ .¹ In particular for PKC θ a crucial role in T cell receptor (TCR) – mediated T-cell activation and proliferation has been established.² The different PKC isotypes and in particular PKC θ , are therefore considered to be attractive therapeutic targets for the treatment of autoimmune diseases and the prevention of allograft rejection.³

Here we describe the discovery of a novel series of protein kinase C inhibitors with drug-like properties based on the 2,6-naphthyridine template. Example compounds potently inhibit the novel PKC isotypes δ , ϵ , η , θ and display 10-100 fold selectivity over the classical PKC isotypes α/β . Prototype compound **1** was found to be strongly immunosuppressive *in vitro* and *in vivo*. *In vitro*, anti-CD3 / anti-CD28-induced lymphocyte proliferation could be effectively blocked in 10 % rat whole blood. In mice, **1** dose-dependently inhibited *Staphylococcus aureus* enterotoxin B-triggered increase in IL-2 serum levels after oral dosing.



REFERENCES

1. Roffey, J.; Rosse, C.; Linch, M.; Hibbert, A.; McDonald, N. C.; Parker, P. J. *Curr. Opin. Cell Biol.* **2009**, *21*, 268.
2. (a) Kingeter, L. M.; Schaefer, B. C. *J. Immunol.* **2008**, *181*, 6244. (b) Sun, Z.; Arendt, C. W.; Ellmeier, W.; Schaeffer, E. M.; Sunshine, M. J.; Ghandi, L.; Annes, J.; Petrzilka D.; Kupfer, A.; Schwartzberg, P. L.; Littman, D. R. *Nature*, **2000**, *404*, 402.
3. (a) Baier, G.; Wagner, J. *Curr. Opin. Cell Biol.* **2009**, *21*, 262. (b) Lee, M. R.; Duan, W.; Tan, S. *Expert Opin. Ther. Targets*, **2008**, *12*, 535.

POSTER COMMUNICATIONS – PC.321

EVALUATION OF PAMPA TECHNIQUE FOR THE EARLY PREDICTION OF PASSIVE PERMEATION OF NANOCARRIERS THROUGH THE BLOOD-BRAIN BARRIER

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Blood-brain barrier (BBB) is a complex organization separating the brain from the systemic circulation and regulating cerebral homeostasis. It represents the main route followed by chemical entities to reach the central nervous system (CNS). This barrier is located at the endothelial cells of cerebral micro vessels which are sealed by tight-junctions, preventing paracellular diffusion of most compounds. Transcellular route is therefore the predominant pathway to reach the CNS either by passive diffusion or active transport. Actually, efflux pumps such as P-glycoproteins (P-gp) or multi-drug resistance associated proteins (MRP), expressed at the BBB, prevent xenobiotics to enter in cerebral parenchyma. The development of strategies to enable the permeation of low-permeant drugs and/or substrates of these efflux transporters is therefore of prime importance. In this field, nanocarriers loaded with drugs are a promising alternative to

influence the permeability of active compounds. Prediction of the ability of these loaded-nanoparticles to increase the permeation of low-permeant active compounds through BBB as early as possible in research and development thus remains an important challenge.

Parallel Artificial Membrane Permeability Assay (PAMPA) has been developed to fast predict passive permeation through biological membranes. This technique lies on a liquid artificial membrane separating a donor and an acceptor compartments. In this way, different biological barriers can be modelled depending on the nature of the liquid membrane immobilized [1] [2] [3]. BBB permeability has already been predicted with PAMPA using a phospholipidic membrane, allowing the ranking of the compounds into classes [2]. Recently we developed a new full-organic artificial membrane to predict passive cerebral penetration avoiding the well-known limitations of biological material [4]. In this study, permeability of nanoparticles loaded with a reference-set of compounds has been tested with PAMPA in order to evaluate the ability of the technique to provide information on passive diffusion of such complex combination.

REFERENCES

1. Wohnsland F.; Faller B. *J. Med. Chem.* 2001, *44*, 923-930
2. Di L.; Kerns E. H.; Fan K.; McConnell O. J.; Carter G. T. *Eur. J. Med. Chem.* 2003, *38*, 223-232
3. Ottaviani G.; Martel S.; Carrupt P. A. *J. Med. Chem.* 2006, *49*, 3948-3954
4. Passeleu Le Bourdonnec C., Boccard J., Rudaz S., Carrupt P.A., Martel S. In preparation, 2010.

POSTER COMMUNICATIONS – PC.322

RATIONAL DEVELOPMENT OF SELECTIVE ALLOSTERIC INHIBITORS OF BCR-ABL FOR TREATMENT OF PHILADELPHIA POSITIVE CML

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Philadelphia chromosome (Ph) is recognized as the cytogenetic hallmark of most chronic myelogenous leukemia (CML) and some of acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) patients. Philadelphia chromosome originated in

the reciprocal t(9;22) chromosomal translocation. The fusion of the breakpoint cluster region (Bcr) gene on chromosome 22 with the Abelson kinase (Abl) gene on chromosome 9 creates the Bcr-Abl oncoprotein. The constitutively active fused Bcr-Abl tyrosine kinase was recognized as the initial inducer of the leukemogenesis in blood cells. There is an increased interest in Academia and Industry to focus on developing selective Bcr-Abl inhibitors. Most of the Bcr-Abl inhibitors developed *ad hoc* (Imatinib, Nilotinib, and Dasatinib) are ATP competitors that bind either to the ATP-binding cleft or nearby. However, approximately one third of Imatinib-treated patients suffer cardiotoxicity or irresponsiveness due to resistance associated with Abl kinase domain mutations. To overcome those drawbacks, we aim at targeting leukemia cancer cells with novel molecular entities that bind selectively to alternative binding sites that are different than the ATP-cleft. The discovery that N-terminal myristated Abl is docked in a capping mechanism that inserts the myristate moiety into a hydrophobic "myristoyl binding pocket" (MBP) in an autoregulatory manner of Abl sparked the idea of developing myristate analogues as allosteric inhibitors of Bcr-Abl. Benefitted by advanced techniques of computational chemistry, sets of potential myristate analogues were examined and proposed for further examination. Those were synthesized, purified and characterized, and screened for their activity in a cell-based auto-phosphorylation and colonogenicity potential in a Bcr-Abl positive leukemia cells. Initial *in vitro* results demonstrate the usefulness of this approach, most probably due to the MPB being a binding site for allosteric inhibitors of Bcr-Abl. Additionally; some of the new derivatives are shown to override the "gatekeeper" mutation T315I. Further optimization studies are being performed.

REFERENCES

1. Quintás-Cardama A, Kantarjian H & Cortes J *Flying under the radar: the new wave of BCR-ABL inhibitors* Nature Reviews Drug Discovery October 2007 (6) 834-848.
2. Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD. *Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia*. Nature Reviews Cancer May 2007, 7(5) 345-56.

POSTER COMMUNICATIONS – PC.323

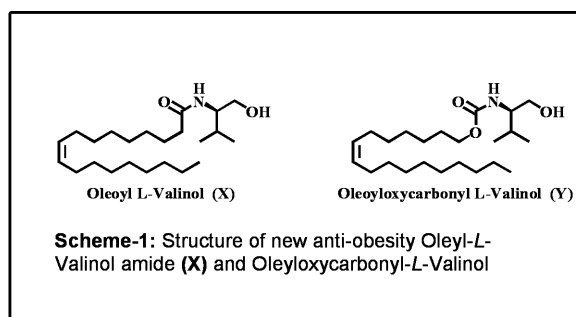
DEVELOPMENT OF NOVEL ANTI-OBESITY AGENTS THAT MODULATE COGNITIVE FUNCTIONS

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A series of novel compounds encompassing natural fatty acid and their corresponding amino and alcohols derivatives were conjugated with substituted amino ethanol. The compounds were purified and characterized. The fatty acid residue included saturated alkyl chains as well as *mono*-, *di*-, *tri*- and *tetra*- conjugated double bonds. Fatty acids and their corresponding amino and alcohols alkyl chain were in the ranges of 16 to 20 carbon atoms. The linkage to different substituted amino ethanol was implemented via -C(O)-NH-; -NHC(O)-; -O-C(O)-NH-; and -NH-C(O)-NH-linkers. The biological activity of these compounds was evaluated *in vivo*. Sabra mice were assigned at random to different groups of 10 mice. The mice were fed ad libitum, body weight and food consumption were monitored during the experiment. In the second week mice were evaluated for their cognitive function using the eight arm maze 15 min following compounds administration during 5 days.



Two leading compounds [Oleyl-L-Valinol amide (**X**) and Oleyloxycarbonyl-L-Valinol amide (**Y**; **Scheme-1**) showed significant inhibition of weight gain (about 10%) short time after administration of 1mg/kg ($p < 0.001$, 0.01 respectively). The effect of both compounds lasted for 15 days and was associated with decreased food consumption ($p < 0.05$) increased activity ($p < 0.001$ $p < 0.01$) and improvement in cognitive function ($p < 0.05$) with no change in neurological score. This was associated in the hypothalamus with decreased 2-Arachidonoyl Glycerol (2-AG) levels ($p < 0.05$) and increased NE and 5-HT levels ($p < 0.05$). Both compounds improved cognitive function which was related to increase in NE levels ($p < 0.05$) in the hippocampus. Compound-X enhanced POMC expression in the hypothalamus with no change in CB1, TRPV1 or 5HT1A expression. In contrast to oleylethanolamide (OEA), MDS revealed that (**X**) does not bind in higher affinity to either of CB-1, -2, FAAH, TRPV-1 nor PPAR α . Substantial evidence indicates that (**X**) exerts its anti-obese and cognitive effects through a distinct mechanism that is assumed to involve activating of pro-opiomelanocortin (POMC). MDS revealed that (**Y**) does not bind in higher affinity to either of CB-1, -2, TRPV-1 or PPAR α but exert its activity on FAAH. Compound-X and Y resembles the effect of moderate caloric restriction (to 60%) which is beneficial and they produce weight reduction by affecting both energy intake and expenditure.

The results presented in this study underline the importance of the region-isomers (*Z*- vs. *E*) of the lipophilic tail and the stereo-center (*L* vs. *D*) in the amino alcohol segment.

REFERENCES

1. Oveisi F, Gaetani S, Eng KT, Piomelli D. *Oleylethanolamide inhibits food intake in free-feeding rats after oral administration* Pharmacol Res. 2004 May; 49(5):461-6.
2. Lo Verme J, Gaetani S, Fu J, Oveisi F, Burton K, Piomelli D. *Regulation of food intake by oleylethanolamide*. Cell Mol Life Sci. 2005 Mar; 62(6):708-16.

POSTER COMMUNICATIONS – PC.324

NEW PIPERIDINE-BASED RENIN INHIBITORS

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The Renin-Angiotensin-Aldosterone System (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.

Starting from known diazabicyclononene¹ and tetrahydropyridine² derivatives, the development of a new class of piperidines as renin inhibitors is reported. Using substituents previously optimized to fill the renin S3 sub-pocket in a different series,³ highly potent piperidine based inhibitors were obtained. Further introduction of polar groups at the 4-position of the piperidine moiety allowed to discover new inhibitors with good pharmacological activities in a double transgenic rat model.

REFERENCES

1. Bezençon et al. *J. Med. Chem.* **2009**, 52, 3689.
2. Remeň et al. *Bioorg. Med. Chem. Letters*, **2009**, 19, 6762.
3. Chen et al. *Bioorg. Med. Chem. Lett*, manuscript submitted.

POSTER COMMUNICATIONS – PC.325

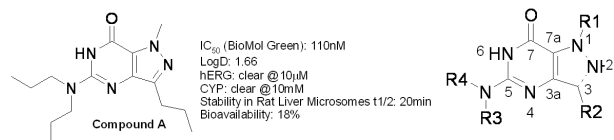
A NOVEL SERIES OF POTENT PDE1A INHIBITORS

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Cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) are key second messenger molecules in cells, synthesized by guanylyl and adenylyl cyclases. By playing a role as 'relays' in signal transduction pathways, these molecules are key to controlling normal and pathological cell responses. Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that hydrolyze cyclic nucleotides and thereby control the cellular levels of these second messengers. To date, 21 mammalian PDE genes have been cloned and classified on the basis of sequence homology and biochemical properties into 11 families (PDE1-PDE11).¹⁻³ Because of their key role in cellular signaling, several PDE family members have been explored for therapeutic potential. Some data point to a role of PDEs in chondrocyte biology and it has been suggested that PDE4 is involved in cartilage catabolic events.⁴ The physiological role of the PDE1 family is less well understood, however, the focus has been primarily on PDE1B and PDE1C.⁵⁻⁶ Our research indicated that PDE1A played a role in chondrocyte biology and a program was initiated to identify selective PDE1A inhibitors to further explore this finding.

In this poster, we describe the design and synthesis of novel PDE1A ligands by a scaffold hopping approach. 5-Amino-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one was decorated with a variety of substituents. SAR studies based on the initial lead compound A (Scheme 1) were performed, which led to potent PDE1A inhibitors. Selectivity against a variety of PDE isoforms will be shown together with activity in a cellular cAMP assay. Finally, results obtained with compounds from the series showing protective effects in an *ex vivo* bovine explant model will be presented.



(Scheme 1)

REFERENCES

1. Soderling, S. H.; Beavo, J. A. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin. Cell Biol.* **2000**, 12, 174-179.
2. Conti, M.; Jin, S.-L. C. The molecular biology of cyclic nucleotide phosphodiesterases. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, 63, 1-38.
3. Mehats, C.; Andersen, C. B.; Filopanti, M.; Jin, S.-L. C.; Conti, M. Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends. Endocrinol. Metab.* **2002**, 13, 29-35.
4. Tenor H et al Phosphodiesterase isoenzyme families in human osteoarthritis chondrocytes-functional importance of Phosphodiesterase 4. *Br J Pharmacol.* (2002) 135:609-18
5. Goraya TA, Cooper DM. (2005) Ca²⁺ calmodulin-dependent phosphodiesterase (PDE1) : current perspectives. *Cell Signal.* 17: 789-97
6. Kakkar R, Raju RV, Sharma RK. (1999) Calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE1). *Cell Mol Life Sci.* 55: 1164-86

POSTER COMMUNICATIONS – PC.326

NOVEL SYMMETRICAL BIS-QUATERNARY CHOLINE KINASE INHIBITORS AS ANTICANCER AGENTS

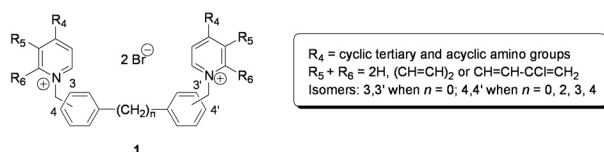
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Choline is an essential anabolic substrate for the synthesis of phospholipids. Choline kinase (ChoK) phosphorylates choline to phosphocholine (PCho) that serves as a precursor for the production of phosphatidylcholine, the major phospholipid constituent of membranes and substrate for the synthesis of lipid signaling molecules. Nuclear magnetic resonance (NMR)-based metabolomic studies of human tumors have identified a marked increase in the intracellular concentration of PCho relative to normal tissues.¹

ChoK is overexpressed in human breast, lung, colorectal, and prostate tumors, a finding that suggests the involvement of this enzyme in carcinogenesis.

With the aim of obtaining new structure-activity relationships and the study in depth of the structural parameters that define the ChoK inhibitory and antiproliferative activities, the synthesis of a new set of compounds (**1**) is proposed (Figure). These compounds are tested in an *ex vivo* system using human ChoK as a target, and the antiproliferative activity is analysed on HT-29 cultured cells by measuring the number of cells after 6 days of incubation with the compound, relative to control, non-treated cells.²



Figure

ChoK inhibitors provide evidence that the blockade of PCho is a valid strategy for the development of new anticancer agents, opening a new avenue for the development of antitumour drugs with a novel mechanism of action. The screening of new derivatives may improve currently available compounds and widen their therapeutic margin to facilitate their entry into clinical studies.

REFERENCES

1. A. Yalcin; B. Clem; S. Makoni; A. Clem; K. Nelson; J. Thornburg; D. Siow; A.N. Lane; S.E. Brock; U. Goswami; J.W. Eaton; S. Telang; J. Chesney. Selective inhibition of choline kinase simultaneously attenuates MAPK and PI3K/AKT signaling. *Oncogene*, **2010**, 29, 139-149.

2. M.C. Núñez, A. Conejo-García, R.M. Sánchez-Martín, M.A. Gallo, A. Espinosa, J.M. Campos. QSAR as a Tool for the Development of Potent Antiproliferative Agents by Inhibition of Choline Kinase. *Current Computer-Aided Drug Design*, **2007**, 3, 297-312.

POSTER COMMUNICATIONS – PC.327

ALZHEIMER'S DISEASE: IDENTIFICATION OF BACE1 INHIBITORS USING FRAGMENT BASED DYNAMIC LIGATION SCREENING (DLS)

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Recently, dynamic ligation screening (DLS) has been introduced as a fragment-based method for drug discovery [1]. The approach combines target-assisted formation of the inhibitory species and detection of reversibly ligated fragments in a functional assay through their competition with a fluorogenic enzyme substrate [2] or via a binding assay employing fluorescence polarization. The approach has been applied to several cysteine protease targets and has demonstrated to provide non peptide inhibitors through iteratively reversed DLS starting using from peptides [3].

Herein, the extension of DLS to aspartic proteases is described. For this purpose, β -secretase (BACE1) (aspartyl protease) was selected as a protein target to develop, optimize and validate this approach. BACE1 plays a key role in Alzheimer's disease (AD) because it cleaves amyloid precursor protein to produce the amyloid β_{1-42} peptide fragments, principal components of senile plaques [4]. The poor ability of the existing BACE1 inhibitors to cross the blood-brain-barrier has made the development of specific BACE1 inhibitors one of the most active areas in drug discovery for AD [5,6].

First, a fluorogenic substrate and directing probe were designed and optimized and their kinetic and inhibitory parameters (V_{max} , K_M , k_{cat}/K_M , IC_{50} , respectively) were determined. On the other hand, assay conditions like buffer, enzyme, concentrations of substrate, enzyme and directing probe or incubation time were optimized. The developed method was adapted to high throughput screening (HTS) format using 384 microtiter plates and diverse amine libraries assembled by employing a maximum common substructure (MCS) concept were screened. Apparent K_i values of the dynamic ligation products were determined. Fragment hits were validated by synthesis and testing of the corresponding irreversible ligation products. One fragment has been identified as BACE1 inhibitor, being a good starting point towards for iteration with the aim to develop higher affinity inhibitors.

* The authors thank the EU for an **Intra-European Fellowship (IEF) Marie Curie** to M. I. Fernández-Bachiller in the **7th Framework** (Call: FP7-PEOPLE-IEF-2008).

REFERENCES

- Schmidt, M. F.; Rademann, J. *Trends Biotechnol* **2009**, 27 (9), 512-521.
- Schmidt, M. F.; El-Dahshan, A.; Keller, S.; Rademann, *Angew. Chem. Int. Ed.* **2009**, 48, 6346-6349.
- Schmidt, M. F.; Isidro-Lobet, A.; Lisurek, M.; El-Dahshan, A.; Tan, J.; Higenfeld, R.; Rademann, J. *"Angew Chem Int Ed Engl.* **2008**, 47 (17), 3275-3278.
- Willem, M.; Lammisch, S.; Haass, C. *Semi. Cell. Dev. Biol.* **2009**, 20 (2), 175-182.
- Silvestri, R. *Med. Res. Rev.* **2009**, 29 (2), 295-333.
- Huang, W. H.; Sheng, R.; Hu, Y. Z. *Curr. Med. Chem.* **2009**, 16 (14), 1806-1820.

POSTER COMMUNICATIONS – PC.328

CHOLESTANE DERIVATIVES AS ANTITUMOR AND ANTIANGIOGENIC DRUGS

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Cholestane derivatives and their possible use for prevention, inhibition and treating of proliferative and angiogenic disease in mammals will be described. The growth of new blood vessels in animals is essential for organism growth¹ as well as for growth of solid tumors and metastasis². Endothelial cells are the main player for angiogenesis³ and could be a target for an antiangiogenic therapy because they are nontransformed and are easily accessible to treating with achievable doses of antiangiogenic agents, and are also unlikely to acquire drug resistance⁴. The vascularization plays a crucial role in cell nutrition and oxygen distribution. Tumor angiogenesis targeting in the sense of discovery of novel potentially drugs includes inhibition of proteolytic enzymes that break down the extracellular matrix surrounding existing capillaries, inhibition of endothelial cell proliferation, migration and enhancement of tumor endothelial cell apoptosis. Potent angiogenic inhibitors capable of blocking tumour growth appear to have potential for development of novel generations of anticancer drugs.

Antiangiogenic activity has been described in some steroids, e.g., medroxyprogesteron, however with only small clinical activity; 2-methoxiestradiol has been found to inhibit proliferation, migration and invasion of endothelial cells *in vitro* as well as having antiangiogenic effects in certain *in vivo* tumor models; another

examples is squalamin etc. However, small attention has been devoted in this respect to cholestane derivatives. We have found that they are suitable for inhibition of different tumor cell lines in micromolar concentrations with minimal effects on normal cells. We shall present that treatment with cholestane derivatives induces cytotoxicity and growth inhibition of breast and prostate carcinoma cells and further that they are capable to inhibit angiogenesis in tumors and therefore that they can be used as antiangiogenic and apoptotic drugs, hence as anticancer drugs.

ACKNOWLEDGMENT

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REFERENCES

- e.g. Folkman et al., 1974, *Adv Cancer Res.*, 19(0):331-358; Folkman et al., 1992, *J Biol Chem.*, 267(16):10931-10934; Carmeliet, 2005, *Nature*, 438 (7070):932-936.
- e.g. Bhat and Singh, 2008, *Food Chem Toxicol*, 46:1334-1345; Kesisis et al., 2007, *Curr Pharm Des.*, 13(27):2795-2809; Risau, 1997, *Nature*, 386(6626):671-674.
- Bagley et al., 2003, *Cancer Res.*, 63(18):5866-5873).
- Bhat and Singh, 2008, *Food Chem Toxicol*, 46:1334-1345.

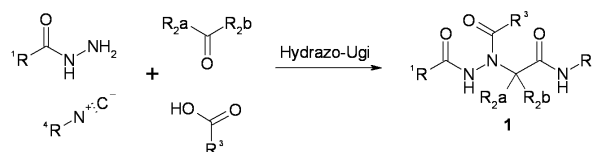
POSTER COMMUNICATIONS – PC.329

NOVEL MOTIFS FOR PEPTIDOMIMETIC DESIGN EMERGING FROM HYDRAZO-UGI REACTION

Mikhail Krasavin,* Ekaterina Lakontseva and Vladislav Parchinsky

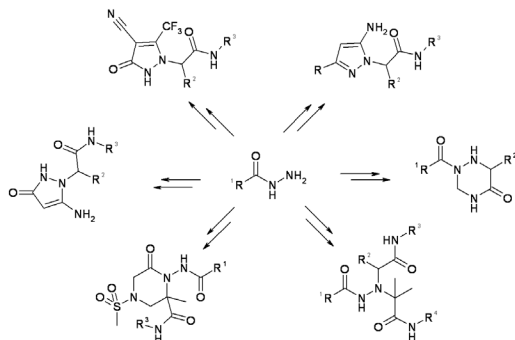
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Isocyanide-based multi-component reactions have served as a rich source of novel peptidomimetics. We have recently revisited a relatively underexplored variant of the Ugi reaction in which the classical amine component is replaced with hydrazine (more specifically, N-acylhydrazine) termed hydrazo-Ugi reaction.



While the resulting hydrazinopeptide-like structures **1** themselves are useful motifs for designing pseudopeptide replacements for bioactive peptides and introducing secondary structure bias known as hydrazino turn, broader applications of the hydrazo-Ugi reaction in elaborating more complex peptidomimetics and heterocycles still remain to be explored.

We have actively investigated the avenues for using the hydrazo-Ugi reaction in accessing new peptidomimetic chemical space and have identified several reliable directions in that respect, as briefly outlined below.



These chemistries will be presented in further detail along with some associated profiles on peptidergic biological targets.

POSTER COMMUNICATIONS – PC.330

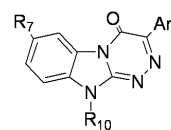
NEUROPROTECTIVE EFFECT OF NOVEL SELECTIVE A_{2A} ADENOSINE RECEPTOR ANTAGONISTS: 3-ARYL-[1,2,4]TRIAZINO[4,3-*a*]BENZIMIDAZOL-4(10H)-ONES (ATBIS)

Pugliesi I.,¹ Taliani S.,¹ Salerno S.,¹ Simorini F.,¹ La Motta C.,¹ Marini A.M.,¹ Da Settimo F.,¹ Cosconati S.,² Marinelli L.,² Cosimelli B.,² Greco G.,² Novellino E.,² Scatena A.,³ Fornai F.,³ Paparelli A.,³ Trincavelli M.L.,⁴ Martini C.⁴

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Adenosine is a physiological nucleoside which plays an important role in many patho-physiological conditions in the central nervous system (CNS), as well as in peripheral organs and tissues through modulation of specific membrane G protein-coupled receptors (ARs), currently classified into A_1 , A_{2A} , A_{2B} , and A_3 subtypes.^[1]

A_{2A} ARs occur mainly on neurons in the striatum and on cholinergic interneurons, but they are also found in the nucleus accumbens, olfactory tubercle, hippocampus and cerebral cortex. Recent evidences showed that A_{2A} ARs may modulate a number of cellular processes affecting neuronal cell death in a broad spectrum of brain injury. A_{2A} ARs resulted up-regulated in noxious brain conditions and their blockade confers robust brain neuroprotection. As this effect is exerted without observable peripheral effects, there is an increasing interest in the development of selective A_{2A} AR antagonists as novel protective agents in neurodegenerative diseases.^[2]



ATBI I

In recent years, we have described a series of 3-aryl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10H)-ones (ATBIs) **I** as potent adenosine receptors antagonists.^[3,4] In this study, using **I** as lead compound, we have synthesized a novel series of ligands featuring modified substituents at the 10 position, combined with an appropriate aromatic ring at the 3-position. Synthesis, biological evaluation and structure-activity relationships for these new derivatives ATBI **I** will be presented.

Finally, the most promising A_{2A} AR ATBI antagonist was evaluated for its neuroprotective effect on PC12 cells treated with dopamine neurotoxins: MPP⁺ or methamphetamine. We found a neuroprotective effect consisting of full prevention of neurotoxin-induced cell loss, as measured by combining a variety of staining techniques. These latter results, which were confirmed by combining neurotoxic models, each owing a different mechanism of action, offer a promising perspective for such a compound as a valuable tool to protect dopamine neurons.

REFERENCES

1. Fredholm et al, *Pharmacological Reviews* 2001, 53 (4), 527–552.
2. Stone, T. W.; Ceruti, S.; Abbracchio, M. P. *Handb. Exp. Pharmacol.* 2009, 193, 535–587.
3. F. Da Settimo, et al. *J. Med. Chem.* 2001, 44, 316–327.
4. F. Da Settimo, et al. *DDR* 2004, 63, 1–7.

POSTER COMMUNICATIONS – PC.331

DEVELOPMENT OF NEW DERIVATIVES OF AMINOGLYCOSIDES WITH ENHANCED SUPPRESSION OF DISEASES-CAUSING NONSENSE MUTATIONS

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Many human genetic diseases, such as cystic fibrosis (CF), Usher syndrome (US), Duchenne muscular dystrophy (DMD), Hurler syndrome (HS) and several types of cancer result from nonsense mutations that cause premature termination of the protein synthesis. For many of those diseases there is presently no effective treatment. Recent studies have shown that some aminoglycoside antibiotics have the ability to impose the mammalian ribosome to read past a false-stop signal and generate full-length functional proteins. However, high toxicity of those drugs in

humans limits their therapeutic use. To date, no efforts have been made to optimize aminoglycosides activity as stop codon readthrough inducers.

The main objective of this research is to develop novel aminoglycoside variants that will have efficient termination suppression activity, and at the same time will have reduced toxicity against mammalian cells.

Towards these ends, series of new derivatives of paromomycin were designed, synthesized and their ability to readthrough stop codon mutations was examined in both *in vitro* and *ex vivo* mammalian systems. Our most recent discovery was the pseudotrisaccharide **3** (Figure 1), which showed several folds greater suppression activity and far lower toxicity than those of gentamicin and paromomycin [1]. This finding placed compound **3** on the top among all the natural and/or synthetic aminoglycosides reported to date for the potential use in translational therapy. In order to further improve readthrough efficacy of **3** here we will discuss on the design, synthesis and evaluation of a series of new derivatives of aminoglycosides that differ from the previous leads by the presence of a chiral C6'-methyl group on the glucosamine ring. Our results indicate that addition of both the (*R*)-C6'-methyl and the AHB group at N-1 position significantly improve the readthrough efficiency. The observed structure-activity relationship in new compounds are discussed on the basis of their binding affinity to human rRNA A-site, inhibition of protein translation in prokaryotic and eukaryotic systems, and their *in vitro* and *ex vivo* ability to readthrough premature stop codon contexts derived from a series of different genes that underline CF, US, DMD and HS genetic diseases [2].

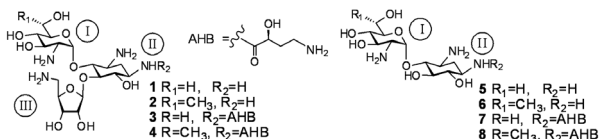


Figure 1. Novel aminoglycoside readthrough inducers.

REFERENCES

1. I.Nudelman, A.Rebibo-Sabah, M.Cherniavsky, V.Belakhov, M.Hainrichson, F.Chen, J.Schacht, D.S.Pilch, T.Ben-Yosef, and T.Baasov. – *J. Med. Chem.*, 52, No. 9, 2836-2845 (2009).
2. I.Nudelman, D.Glikin, B.Smolkin, M.Hainrichson, V.Belakhov, and T.Baasov. – *Bioorg. Med. Chem.*, submitted, 2010.

POSTER COMMUNICATIONS – PC.332

MOLECULAR IMAGING AGENTS FOR EGFR BASED ON THE 4-ANILINOQUINAZOLINE SCAFFOLD

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Patients presenting subclasses of cancers that overexpress EGFR or HER2, two members of the ErbB family of growth factor receptors, have poorer prognoses, with higher risks of disease recurrence and metastatic events. Despite FDA approval of small molecule EGFR/HER2 inhibitors, the widespread use of these therapeutics has been hampered by an inability to identify subpopulations sensitive to anti-EGFR/HER2 therapy. This project addresses this issue through the design and synthesis of PET and SPECT imaging agents specific to these subclasses of cancer, by ligating the characteristic 4-anilinoquinazoline inhibitor scaffold to a linker bearing an appropriate radioisotope. The modular synthetic approach employed produces a more readily optimized series of molecular probes, while strategic placement of the imaging modality in solvent-accessible space minimizes the negative effect of its presence on the binding of the radiotracer in the active sites of the receptors.

Supported by DOE Award ER647823 Susan B Komen Foundation grant BCTR65906.

POSTER COMMUNICATIONS – PC.333

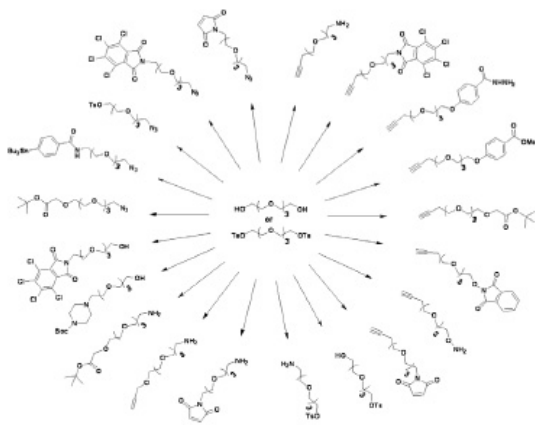
NANOMEDICINAL CHEMISTRY: SYNTHESIS OF HETEROBIFUNCTIONAL LINKERS FOR POLYVALENT MULTIFUNCTIONAL NANOPARTICLES

Robert N. Hanson, Kinh Luan Dao, J. Adam Hendricks, Marshall Brennan, Anton Kozhushnyan, Victoria Ronga, Helen Trinh Pham and Teresa Dunstan

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As part of our program in developing polyvalent multifunctional nanoparticles it was necessary to prepare new linkers for selective ligation of appropriate terminal groups. In this study we describe our approaches for preparing specific heterobifunctional oligoethylene glycol (OEG) linkers. These linkers serve as modular units that allow us to introduce small molecule targeting groups, imaging moieties, chemotherapeutic drugs or combinations thereof onto a nanoparticulate surface by using chemo-orthogonal ligation chemistry. We will describe several

examples of modular functionalized nanoparticle assembly using this approach. Preliminary data suggest that our versatile assortment of heterobifunctional linkers has the potential for developing multiply targeted therapeutic agents and disease selective drug delivery systems.



Supported by DoD PRCP and DOE ER64823

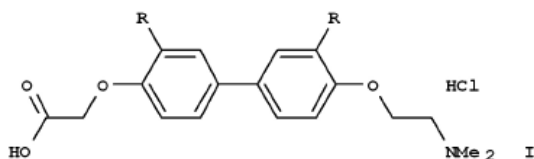
POSTER COMMUNICATIONS – PC.334

SUBSTITUTED 4,4'-BIS(OXY)BIPHENYL PROTEOMIMETICS AS INHIBITORS OF ESTROGEN RECEPTOR-ALPHA CO-ACTIVATOR BINDING

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Significant interest has focused on the development of small molecules that can inhibit the binding of coactivator proteins to nuclear receptors as a means of modulating hormonal function. Proteomimetics, small molecules that mimic peptidyl structures offer one approach to this problem. We have designed and prepared a small series of novel 4,4'-bis(oxy)biphenyl derivatives using a modular, convergent strategy. The compounds offer the potential for exploring chemical diversity within the biphenyl scaffold as well as at the termini. Synthetic methods and preliminary results will be discussed.



Supported by DoD PCRP.

POSTER COMMUNICATIONS – PC.335

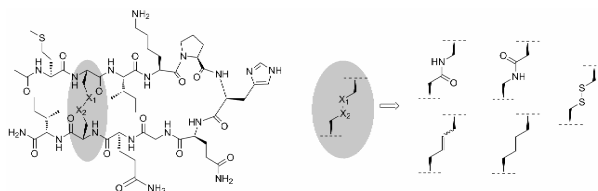
A NEW APPROACH TOWARDS INHIBITION OF ANGIOGENESIS: CYCLIC PEPTIDE ANALOGUES OF VEGF₈₁₋₉₁ FRAGMENT

M. Isabel García-Aranda,¹ Yasmina Mirassou,² Benoit Gautier,³ Nicolas Inguibert,³ Michel Vidal,³ Mercedes Martín Martínez,¹ M. Ángeles Jiménez,² M. Teresa García-López,¹ M. Jesús Pérez de Vega,¹ M. Rosario González-Muñiz.¹

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The inhibition of the interaction between VEGF and its receptors constitutes a valuable approach in the search of new antiangiogenic agents, due to the importance of this interaction in the triggering of the angiogenesis process. Taking into account the key role of VEGF₈₁₋₉₁ fragment for the VEGF interaction with its receptors,^{1,2} and aiming to preserve its β -hairpin conformation, new cyclic peptide derivatives were designed restricting the conformation in order to fix the β -hairpin structure. Compounds with bridges of different nature were prepared by introduction of the adequate modifications in two positions of the sequence, trying to preserve the residues described as key for the interaction. The influence of these modifications, not only in the structure but also in the biological activity, was studied. Additionally, considering that the β -turn of the native VEGF₈₁₋₉₁ fragment is slightly distorted, due to the presence of an extra amino acid residue, two series of decapeptide analogues were also prepared, just by removing the residue Glu⁸⁷ or Gly⁸⁸. On the other hand, incorporation of an α -MePro, which is known to stabilize β -turn conformations, instead of the Pro⁸⁵ residue of the native fragment was also performed. This will avoid *cis/trans* rotamery and would help in the stabilization of the hairpin.

In this communication we will comment on the synthesis, NMR conformational analysis, and binding affinities for the Flt-1 receptor of the proposed VEGF₈₁₋₉₁ peptide derivatives.



REFERENCES

- Keyt, et al. J. Biol. Chem. 1996, 271, 5638-5646.
- Muller, et al. Proc. Natl. Acad. Sci.. 1997, 94, 7192-7197.

POSTER COMMUNICATIONS – PC.336

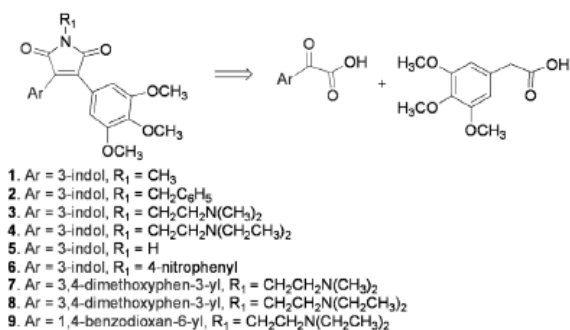
DESIGN, SYNTHESIS AND ANTICANCER PROPERTIES OF NEW CDKS INHIBITORS

Giovanni Casula,¹ Ruben Francisco,¹ Salvatore Plescia,² Maria Grazia Cusimano,² M. Dolors Pujol^{1*}

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Hartwell, L. H., Hunt, R. T. and Nurse, P. M. won the 2001 Nobel Prize in Medicine for their description of cyclin-dependent kinase mechanisms and its role in the regulation of the cell cycle. CDKs are considered a potential target for anti-cancer treatment, these kinases phosphorylated the retinoblastoma (Rb) tumor suppressor protein.¹ The phosphorylated Rb causes the release of members of the transcription factors, E2F proteins. It is noteworthy that inhibition of CDK4 results in hyperphosphorylation of Rb and restricts the passage only to G₁ phase, whereas CDK2 regulates G₁ and also S phases. Malumbres and col. have reported that knockdown of CDK4 in mammary tumor cells prevents tumor formation.² These results suggest that selective inhibition of CDK4 could be a more valuable mechanism to cancer treatment than that of CDK2.3

An efficient approach to the synthesis of unsymmetrical 3,4-diarylmaleimides has been developed including a Perkin condensation between the arylacetic acid and the corresponding arylketoacid followed by reaction with aminoalkylamines (Scheme 1).



Scheme 1

In this report we describe several maleimides designed to explore the pharmacophore and their effect on cytotoxic activity and CDK selectivity. These compounds were found to be interesting such as antitumor possessing good antiproliferative activity against human carcinoma cell lines. Several of these compounds showed inhibition of pRb phosphorylation and arrest tumor cells at the G₁ phase. Structure–activity relationships were also investigated.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Minister of Science and Technology (CTQ2007-60614/BQU) for the financial support.

REFERENCES

1. Sandhu, C.; Slingerland, J. *Cancer Detect. Prev.* **2000**, 24, 107-112.
2. Ortega, S.; Malumbres, M.; Barbacid, M. *Biochim Biophys. Acta. Rev. Cancer* **2002**, 1602, 73-87.
3. Malumbres, M.; Barbacid, M. *Cancer Cell* **2006**, 9, 2-8.

POSTER COMMUNICATIONS – PC.337

PREPARATION OF NEW SELFOTEL STRUCTURAL ANALOGUES TO TREAT TINNITUS

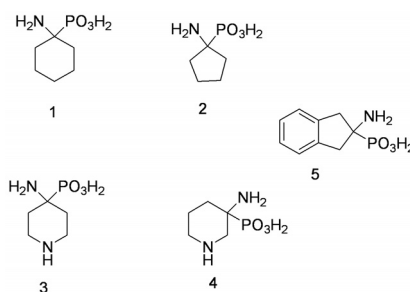
Zofia A. DZIUGANOWSKA¹, Jean-Noël VOLLE², David VIRIEUX², Jean-Luc PIRAT² and Paweł KAFARSKI¹

¹Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland ²Institut Charles Gerhardt – UMR 5253 – ENSCM – 8, Rue de l'Ecole Normale – F-34296 Montpellier Cedex 5 – France

Nowadays, tinnitus - an ailment of the auditory system - starts to be the public health problem. It afflicts about 10 % of Western Europe society [1]. Although some alleviating agents exist, there is no convincing treatment available. Lately, it has been found that tinnitus is connected with activity of N-methyl-D-aspartic acid and glutamate receptors [2].

Organophosphorus compounds are effective pharmacological drugs. Furthermore, it is known that they have also biological activity as NMDA and glutamate antagonists [3].

Herein, we present cyclic aminophosphonates. Their synthesis is based on Oleksyszyn-Soroka reaction [4]. These compounds (Pic. 1) can be potential NMDA and glutamate antagonists.



Pic. 1

REFERENCES

1. Coles R.R.A., *J. Laryngol. Otol.*, **1984**, 9, 195-202.
2. Ruel J., Chabbert C., Nouvian R., Bendris R., Eybalin M., Leger C.L., Bourien J., Mersel M., Puel J.L., *J. Neurosci.*, **2008**, 28, 7313-7323.

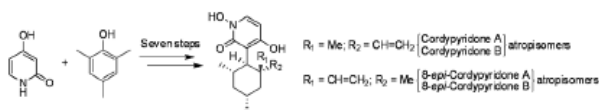
- Lejczak B., Kafarski P., *Top Heterocycl Chem*, **2009**, 20, 31-63, Springer Berlin-Heidelberg.
- Ziora Z., Kafarski P., *Phosphorus, Sulfur and Silicon*, **2009**, 184, 1047-1053.

POSTER COMMUNICATIONS – PC.338

THE TOTAL SYNTHESIS OF (±)-CORDYPYRIDONE AND RELATED COMPOUNDS AS ANTIMALARIAL AND ANTIBACTERIAL AGENTS

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The family of natural products containing the *N*-hydroxypyridone motif are of significant interest for total synthesis due to their compact structure, pronounced biological activities and possible modes of action. Cordypyridone A and B were selected as targets due to their antimalarial activity against *Plasmodium falciparum*,¹ antibacterial activity against *Staphylococcus aureus*,² and the ability of cordypyridone A to induce erythropoietin gene expression in human cells.³ This work details the total synthesis of cordypyridone A and B⁴ and demonstrates the generality of the methodology as applied to the total synthesis of an additional natural product, pyridoxatin, and other *N*-hydroxypyridone analogues as antimalarial and antibacterial agents.

REFERENCES

- Isaka M. *et al. J. Org. Chem.* 2001, 66, 4803.
- Clardy J. *et al. Org. Lett.* 2002, 4, 671.
- Houck D. J. *Nat. Prod.* 1999, 62, 397.
- Jones, I. L.; Moore, F. K.; Chai, C. L. L. *Org. Lett.* **2009**, 11, 5526.

POSTER COMMUNICATIONS – PC.340

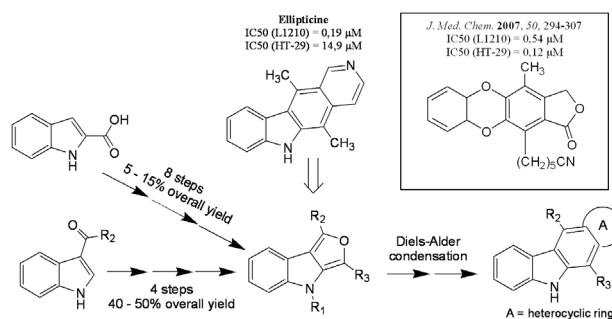
DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF NEW TETRACYCLIC COMPOUNDS RELATED TO ELLIPTICINE

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Ellipticine is a natural product isolated from *Ochrosia elliptica* with significant cytotoxic activity by several mechanisms of action. Its tetracyclic structure exhibits antineoplastic activity against several human cancer cell lines. The initial isolation of ellipticine was closely followed by its first synthesis. Since then, many other syntheses have been reported by Gribble and col. and other authors and its structure has been widely modified in order to obtain more efficacious, selective and less toxic new compounds.

Our group had previously reported a series of ellipticine analogues including 1,4-denzodioxin nucleus. Recently, we designed a new series of condensed indoles with anticancer activity. Herein, we present two new alternative pathways to prepare the intermediate isobenzofuroindole starting from either indole-2-carboxylic acid (8 steps) or 3-indolylalkanones (4 steps) (see scheme below).



Some interesting steps (involving protection of indole, directed *ortho*-metallation and cyclization) had been studied and developed in our laboratory. These methods lead us to the intermediate diene, which reacts with the corresponding dienophiles to obtain the desired compounds. Other attempts starting from indole-3-carboxylic acid or 3-indolylacetone nitrile were unsuccessful. The total synthesis and the biological activity of several compounds will be reported.

ACKNOWLEDGMENTS

to the Ministry of Science and Technology (CTQ 2007-60614) for financial support. One of us (J.B.) acknowledges the Generalitat of Catalunya for predoctoral fellowship.

REFERENCES

- G. Gribble, M. Sautnier, M. Sibi, J. Obaza-Nutaitis et al. *J. Org. Chem.* **1984**, 49, 4518-4523.
- C. Bozzo, N. Mur, P. Constants, M. D. Pujol. *Eur. J. Org. Chem.* **2009**, 13, 2174-78.
- M. Romero, P. Renard, D. Caignard, G. Atassi, J. Solans, M. D. Pujol. *J. Med. Chem.* **2007**, 50, 294-307.

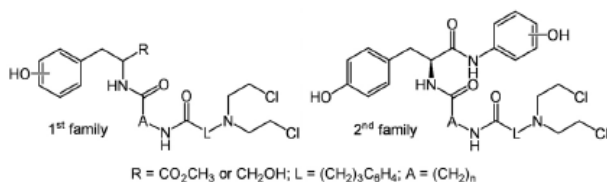
POSTER COMMUNICATIONS – PC.341

DEVELOPMENT OF TYROSINE-CHLORAMBUCIL HYBRIDS SHOWING ACTIVITY AGAINST BREAST AND OVARIAN CANCERS

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In our search for anticancer agents with improved selectivity towards breast and ovarian cancers,¹⁻³ we have synthesized two families of amino acid-linked nitrogen mustard hybrids. The new hybrids are designed to mimic 17 β -estradiol, the female sex hormone, in order to bind to the estrogen receptor. The first family is composed of tyrosine (*ortho*-, *meta*- or *para*-) methyl ester or tyrosinol moiety linked to chlorambucil. For the second family, *para*-tyrosine was transformed into several propionamide derivatives. Afterwards, chlorambucil was linked to the propionamides either directly or via a tether chain leading to various tyrosine-nitrogen mustard hybrids. The phenol group of the tyrosine moiety is used as a potential bioisosteric function of the 17 β -estradiol phenol group. The hybrids were made using two distinct synthetic methodologies which will be described and compared in terms of their efficiency. MTT assays on several breast and ovarian cancer cell lines show that the novel compounds generally possess higher biological activities when compared to chlorambucil. They are up to 9 times more powerful than the parent drug. The cytotoxic activity of these tyrosine-chlorambucil hybrids could advantageously be used to provide compounds with anticancer activity against hormone-dependent female cancers. Molecular modeling shows the possible interactions of the hybrids with the estrogen receptor alpha.



REFERENCES

1. A. Gupta, P. Saha, C. Descôteaux, V. Leblanc, É. Asselin and G. Bérubé. Design, synthesis and biological evaluation of estradiol-chlorambucil hybrids as anticancer agents. *Bioorganic & Medicinal Chemistry Letters*, **20**, 1614-1618 (2010).
2. C. Van Themsche, S. Parent, V. Leblanc, C. Descôteaux, A.-M. Simard, G. Bérubé and É. Asselin. VP-128, a novel estradiol-platinum(II) hybrid with selective antitumour activity towards hormone-dependent breast cancer cells *in vivo*. *Endocrine-Related Cancer*, **16** (4), 1185-1195 (2009).
3. C. Descôteaux, V. Leblanc, G. Bélanger, S. Parent, É. Asselin and G. Bérubé. Improved Synthesis of Unique Estradiol-Linked Platinum(II) Complexes Showing Potent Cytocidal Activity and Affinity for the Estrogen Receptor Alpha and Beta. *Steroids*, **73**, 1077-1089 (2008).

POSTER COMMUNICATIONS – PC.342

ECOSCALE, A METRIC TOOL TO ASSESS THE SCALABILITY OF A REACTION EARLY IN DRUG DEVELOPMENT

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In the pharmaceutical industry, it is important to advance promising drug candidates as soon as possible through the pipeline of drug discovery. One way to achieve this goal is to find out as early as possible when the originally developed medicinal protocol is no longer adequate (mainly for cost and safety issues) and a new protocol needs to be developed for larger quantities. This underlines the importance in early phase drug discovery of the use of synthetic protocols that are easily scalable.

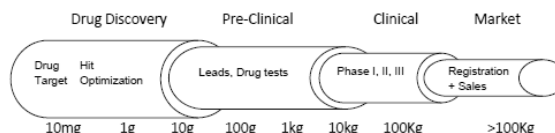


Figure 1: A reaction with a high score on the EcoScale is likely suitable for multi kg synthesis.

To facilitate the selection of adequate protocols, a new transparent and user-friendly metric system, the EcoScale,^{1,2} was developed to assess the scale-up potential of a laboratory scale reaction. For every reaction protocol this EcoScale can be calculated based on 6 basic reaction parameters:

- Yield
- Price of the reaction components
- Safety of the reagents
- Technical Setup
- Temperature and time of the reaction
- Workup and Purification.

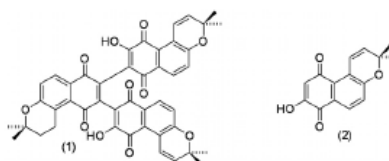
The EcoScale is designed by first defining an ideal reaction which has a maximum value of 100 and then subtracting penalty points of a relative weight for 6 important parameters. The result is a number on a scale from 0 to 100. A value higher than 75 is a good protocol, a value between 75 and 50 is acceptable and below 50 is described as a poor protocol. The flexibility of the system is such that the penalty points can be adapted to the preferences and needs of the chemist or organization.

The power of the EcoScale is that it can be used in a time efficient way in early stage drug development to select reaction pro-

protocols that are more suitable for scale up which finally results in a gain of time and money.

REFERENCES

1. EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters. Koen Van Aken, Lucjan Strekowski, Luc Patiny Beilstein Journal of Organic Chemistry **2006**, 2 No. 3.
2. The EcoScale as a Post-reaction Analysis Tool to Select Reaction Conditions for Scale-up Koen Van Aken, Lucjan Strekowski, Luc Patiny Handbook of Green Chemistry, Oxford University Press, **2010** (in press).
3. For on-line calculation of the EcoScale see <http://www.ecoscale.org>



REFERENCES

1. Jonathon A V Coates, E D Jones, S Cox, Ian T Crosby, David G Bourke, Tyrone P Jeynes. PCT Int. Appl. **2005** WO2005095376
2. Ian T Crosby, Mark L Rose, Maree P Collis, Paula J de Bruyn, Philip L C Keep, Alan D Robertson, Aust J Chem **2008**, 61, 768-784.

POSTER COMMUNICATIONS – PC.343

DISCOVERY OF A NOVEL NON-NUCLEOSIDE INHIBITOR OF HEPATITIS B VIRUS (HBV)

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We will present the discovery and development of a non-nucleoside inhibitor of Hepatitis B Virus.¹

Hepatitis B virus (HBV) is one of the most prevalent viral infections of humans, it attacks the liver and can cause both acute and chronic disease. The WHO estimated 350 million chronic carriers and that about 2 billion people have been infected. An estimated 600,000 person die each year of HBV associated illnesses. HBV causes a potentially life-threatening liver infection and is a major global health problem and the most serious type of hepatitis. Current therapies include; the biological agent Interferon, and the nucleoside analogues Lamivudine, Adefovir dipivoxil, Ribavirin. There is no small molecule non nucleoside inhibitor of HBV.

During our work on conocuvone derived naphthoquinone trimers for the treatment of HIV,² we discovered a potent inhibitor (1) of Hepatitis B Virus in an antiviral selectivity screen. During attempts to resynthesise (1) for proof of concept studies, we altered the synthesis in order reduced side reactions and difficult to remove by-products. As a result we discovered the small molecule (2) that also was a potent inhibitor of HBV. The development of (2) as an agent to treat HBV infections will be presented.

POSTER COMMUNICATIONS – PC.344

NOVEL CHEMICAL APPROACH TO GENERATE DIVERSE DEPSIPEPTIDES DERIVATED FROM THE NATURAL PRODUCT 'A54556 COMPLEX' FOR ANTIBIOTIC SCREENING

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Over the past decades, more and more depsipeptides including acylated depsipeptides were separated and identified from terrestrial microorganisms and marine organisms. Generally, naturally occurring acylated depsipeptides contain unusual amino acids and building blocks. Most of them will offer a new frontier in both synthetic organic chemistry and biological activities. In particular, unique unusual structures will offer the challenge of exploitation of novel synthetic methods, reactions, reagents, catalysts, etc. However, practically, it is rather difficult to isolate a larger amount of naturally occurring acylated depsipeptides because of minute constituents in organisms, difficulty of collection of organisms, and resistance of laboratory culturing. Thus, the limits of the quantity preclude the precise structure determination as well as clarification of detailed biological activities. In fact, the proposed structures of many cyclic peptides have been revised by synthetic works. Thus, the total synthesis is still playing a final means for the structure determination of naturally occurring acylated depsipeptides just like several decades ago. Furthermore, the efficient large-scale production of naturally occurring acylated depsipeptides by synthesis will offer an opportunity to investigate their biological activities in detail. One of the characteristic features of cyclic peptides will be their conformational rigidity and stability in vivo, in contrast to their linear counterparts. In addition, unusual amino acid and non-amino acid moieties of naturally occurring acylated depsipeptides will offer the lead structures of new biologically useful compounds. On the basis of our previous work on total solid-phase synthesis of cyclic peptides, we establish and develop a totally novel chemical approach to synthesize diverse depsipeptides derivat-

ed from the natural product 'A54556 complex' by on-resin head-to-tail cyclization. This powerful and universal total solid-phase synthesis strategy will be applicable to the parallel preparation or one-bead one-peptide (OBOP) library of naturally occurring acylated depsipeptides and the artificial mimic derivatives for systematic research into the structure-activity relationship (SAR) of potent lead antibiotic compounds.

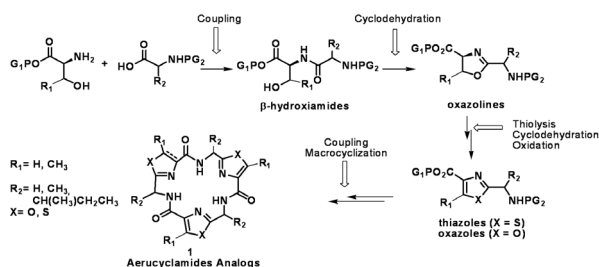
Key words: total solid-phase synthesis; acylated depsipeptides; A54556 complex; lead antibiotic compound; high throughput screening

ACKNOWLEDGMENT

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REFERENCES

1. Michel, K.H. & Kastner, R.E. **US patent** 4492650 (1985).
2. Berthold Hinzen, Siegfried Raddatz, Holger Paulsen, et al. **ChemMedChem** 2006, 1, 689–693.
3. Heike Brotz-Oesterhelt, Dieter Beyer, Hein-Peter Kroll, et al. **Nat. Med.** 2005, 11(10): 1082-1087.



Scheme 1

ACKNOWLEDGMENTS

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REFERENCES

1. a) Portmann, C.; Blom, J. F.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2008**, 71, 1193. b) Portmann, C., Blom, J. F., Kaiser, M., Brun, R., Jüttner, F. Gademann, K. *J. Nat. Prod.* **2008**, 71, 1891.
2. a) Mahler, G.; Serra, G.; Dematteis, S.; Saldaña, J.; Domínguez, L.; Manta, E. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1309. b) Sellanes, D.; Scarone, L.; Mahler, G.; Manta, E.; Baz, A.; Dematteis, S.; Saldaña, J.; Domínguez, L.; Serra, G. *Drug Design Discovery* **2006**, 3, 625. c) Sellanes, D.; Manta, E.; Serra, G. *Tetrahedron Letters*, **2007**, 48, 1827. d) Scarone, L.; Fajardo, J.; Saldaña, J.; Domínguez, L.; Espósito, P.; Dematteis, S.; Wipf, P.; Manta, E.; Serra, G. *Letters in Drug Design and Discovery*, **2009**, 6, 413.

POSTER COMMUNICATIONS – PC.346

APPROACH TO THE SYNTHESIS OF AEURUCYCLAMIDES ANALOGS AS POTENTIAL ANTIPARASITIC DRUGS

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Aerucyclamides A, B, C and D were isolated from the cyanobacteria *Microcystis aeruginosa* PCC 7806. This compound class encompasses hexacyclopeptides alternating in hydrophobic and hydrophilic (Ser, Thr, and Cys) amino acids. The side chains of these polar amino acids are heterocyclized to form oxazole or thiazole rings or their reduced derivatives.

Aerucyclamide B, shows submicromolar (0.7 μM) activity against *Plasmodium falciparum* and Aerucyclamide C is the most active of them against *T. brucei rhodesiense*.¹

As part of our search for candidates for antiparasitic new drugs,² we embarked in the synthesis of Aerucyclamides analogs. In the present work, we present a convergent strategy that involves the coupling between hydrophobic and hydrophilic amino acids, thiolysis, cyclodehydration/ oxidation processes and coupling between different heterocycles fragments. (Scheme 1)

POSTER COMMUNICATIONS – PC.347

DISCOVERY OF NOVEL TIRAZOLONE MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITORS DERIVED FROM MITRATAPIDE USING SOLID- AND SOLUTION-PARALLEL SYNTHESIS

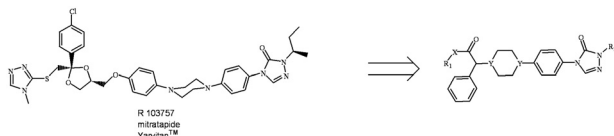
L. Meerpoel*, L. Backx, L. van der Veken, E. Fraiponts, K. de Waepenaert, H. Borghys, G. Mannens and P. Roevens.

Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium.

Microsomal Triglyceride Transfer Protein (MTP) is a lipid transfer protein that is involved in the assembly of very low density lipoproteins (VLDL) in the liver and chylomicrons in the gut wall. Genetic loss of functional MTP is the basis of the human recessive disorder called abetalipoproteinemia. This disease results from an inability of both the liver and intestine to secrete respectively VLDL and chylomicrons leading to extremely low plasma lipid levels and malabsorption of dietary fat. In recent studies it has been shown that MTP inhibitors lower blood lipids with potential use in hyperlipidemia, obesity and atherosclerosis. Previously, we identified R103757 as a novel inhibitor of MTP ($\text{IC}_{50} = 6 \text{ nM}$) and demonstrated that this compound inhibits ApoB secretion of the human hepatoma cell line HepG2 ($\text{IC}_{50} = 26 \text{ nM}$). After oral dosing up to

5 mg/kg in normolipidemic dogs, R103757 decreased plasma cholesterol ($ED_{50} = 1.8$ mg/kg), phospholipid ($ED_{50} = 2.1$ mg/kg) and triglyceride ($ED_{50} = 3$ mg/kg) levels, in a time- and dose-dependent manner, up to -75%, -74% and -52% of baseline respectively. A complete inhibition of PPTG (post-prandial triglyceride) uptake and subsequent weight lowering effects in obese dogs was observed at 0.63 mg/kg. Because of these excellent effects and a safe profile, R103757 was developed and marketed as YervitanTM for treatment of overweighted and obese dogs.

Recently, we elaborated some novel classes of MTP inhibitors derived from mitratapide aiming at an improved developable druglike profile. In this communication we will present the discovery of novel MTP-inhibitors using solid- and solution phase parallel synthesis strategies. Exploring several linkers and different synthetic strategies finally resulted in a four to six steps solid-phase synthesis of focused libraries which revealed several highly potent MTP inhibitors. The synthesis, *in silico* physico-chemical profile schemes and some structure-activity relationship of the compounds obtained will be presented.



POSTER COMMUNICATIONS – PC.348

STRUCTURE-ACTIVITY RELATIONSHIP EXPLORATION OF JNJ 16269110, A NOVEL GUT SPECIFIC MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITOR, USING SOLID-PHASE PARALLEL SYNTHESIS

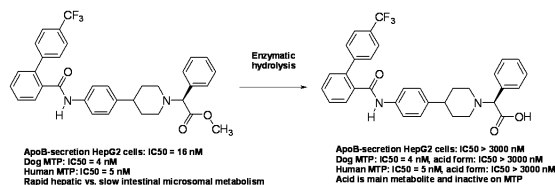
L. Meerpoel*, L. Backx, Louis Van der Veken, Marcel Viellevoe, E. Fraiponts, K. de Waepenaert, G. Mannens, R. Gilissen and P. Roevens.

Johnson and Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium.

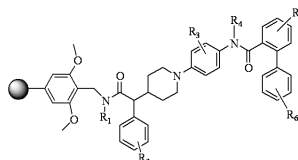
JNJ 16269110 (R256918) is a gut specific MTP (Microsomal Triglyceride Transfer Protein) inhibitor for the treatment of obesity. MTP is a lipid transfer protein essential for the assembly and secretion of the very low density lipoproteins (VLDL) in the liver and chylomicrons in the intestine. JNJ 16269110, is a methylester specially designed to exhibit limited activity in the liver due to rapid degradation by esterases. In agreement with a soft drug concept to limit hepatic exposure, JNJ 16269110 shows high metabolic stability in intestinal S9 fractions but weak metabolic stability in hepatic S9 fractions.

In this communication we will present the SAR exploration of novel JNJ 16269110-derived MTP-inhibitors using solid-phase parallel synthesis strategies. Exploring several linkers and different synthetic strategies finally resulted in a four to six steps

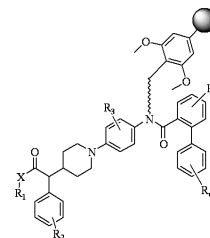
solid-phase synthesis of focused libraries which revealed several highly potent ApoB/MTP inhibitors. The synthesis schemes and structure-activity relationship of the compounds obtained will be presented.



Solid-Phase approach 1



Solid-Phase approach 2



POSTER COMMUNICATIONS – PC.349

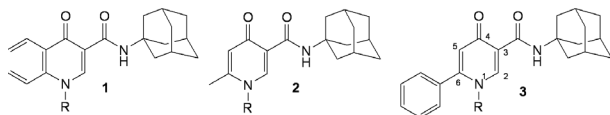
DESIGN OF NOVEL SELECTIVE CB₂ RECEPTOR AGONISTS AND/OR INVERSE AGONISTS BASED ON A 4-OXO-1,4- DIHYDROQUINOLINE-3-CARBOXAMIDE OR ON A 4-OXO-1,4-DIHYDROPYRIDINE SCAFFOLD

Didier M. Lambert¹ Jamal El Bakali,² Giulio G. Muccioli,¹ Nicolas Renault,³ Delphine Pradal,² Mathilde Body-Malapel,⁴ Madjid Djouina,⁴ Virginie Andrzejak,¹ Pierre Desreumaux,⁴ Philippe Chavatte,³ and Régis Millet²

¹ Université catholique de Louvain, Louvain Drug Research Institute, B-1200 Bruxelles, Belgium, ² Université Lille-Nord de France, Institut de Chimie Pharmaceutique Albert Lespagnol, ³ Laboratoire de Chimie thérapeutique & ⁴ Physiopathologie des maladies inflammatoires de l'intestin, Université, 59045 Lille, France.

Growing evidence shows that CB₂ receptor is an attractive therapeutic target. Our groups previously described the synthesis, pharmacological characterization, and structure-activity relationships of selective CB₂ ligands based on a 4-oxo-1,4-dihydroquinoline-3-carboxamide scaffold (**1**)^{1,2}. The structure-activity relationships studies highlighted a significant correlation between affinity and/or selectivity towards the CB₂ receptor and structural features such as an aliphatic moiety, especially an adamantyl substituent, on the C-3 carboxamide group as well as a *n*-pentyl chain in *N*-1 position. Concerning the functionality of these ligands, most of the compounds behaved as selective CB₂ agonists. Starting from this series, we describe here the medi-

nal chemistry approach leading to the development of simplified analogues with a 4-oxo-1,4-dihydropyridine scaffold as CB₂ receptor inverse agonists.



When R = pentyl, **2** is a CB₂ selective agonist and **3** is a CB₂ selective inverse agonist

R-CB₁: K_i > 3000 nM

R-CB₁: K_i = 592 ± 97 nM

R-CB₂: K_i = 20 ± 3 nM

R-CB₂: K_i = 4.0 ± 0.4 nM

The compounds reported here show high affinity and potency at the CB₂ receptor, with only a modest affinity for the centrally expressed CB₁ cannabinoid receptor. Further, we found that the functionality of this series is controlled by its C-6 substituent, since agonists bear a methyl (**2**) or a *tert*-butyl group and inverse agonists a phenyl (**3**) or 4-chlorophenyl group, respectively. Finally, *in silico* receptor-based studies also allow suggesting a putative binding mode for this series of high affinity CB₂ inverse agonists.

REFERENCES

1. Stern et al. J. Med. Chem. 2006 49:70-9;
2. *ibid.* 2007;50:5471-84.

POSTER COMMUNICATIONS – PC.350

SEARCH FOR HISTAMINE H₄ RECEPTOR LIGANDS IN THE GROUP OF 4-(4-METHYLPYPERAZINO) DERIVATIVES OF 1,3,5-TRIAZINE

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The histamine H₄ receptor (H₄R) is the latest identified receptor belonging to the histamine receptor family. This receptor is preferentially expressed on hematopoietic and immune cells (*e.g.* basophils, eosinophils, mast cells, macrophages), suggesting a role in immunological and inflammatory processes [1]. Recently Connelly *et al.* described that the H₄R is also present in the central nervous system (CNS) as a post-synaptic receptor [2].

Although the reference antagonist JNJ 7777120 is an indole derivative more and more lead structures with a 2-aminopyrimidine element as privileged structure are described. Many groups follow this pyrimidine line as it is also a useful element in some other drug targets [3,4]. Analysis of structure-activity relationships allowed creating the general structure of pyrimidines showing in most cases H₄R antagonist potency (Fig 1) [4].

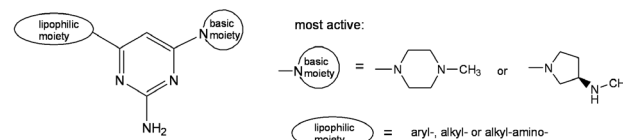


Fig 1. General blueprint of pyrimidine H₄R ligands

The aim of this work was the search for H₄R ligands in the group of structurally related 1,3,5-triazine derivatives in order to check, if introduction of additional nitrogen to pyrimidine core will influence the affinity.

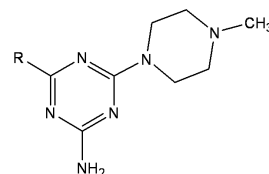


Fig 2.

The series of 4-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine derivatives with different substituents in 6-position was synthesized (Fig 2). X-ray structure analysis was performed for one representative triazine. Compounds were tested for the human H₄R affinity. H₄R binding assay was performed on Sf9 cells expressing human H₄R co-expressed with G-protein Gα₁₂ and Gβ_{1γ2} subunits. Non-specific binding was determined in the presence of 100 μM unlabelled histamine [5]. The compounds have shown H₄R affinities with K_i values in the (sub)micromolar concentration range.

It may be concluded that H₄R slightly tolerates additional nitrogen in the heterocyclic core and the affinity largely depends on the character of substituent R.

ACKNOWLEDGMENTS

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REFERENCES

1. Tiligada E. et al. Expert Opin. Investig. Drugs **2009**, 18, 1519
2. Connelly W.M. et al. Br. J. Pharmacol. **2009**, 157, 55
3. Smits, R. A. et al. DDT **2009**, 14 (15-16), 745
4. Engelhardt, H. et al. Curr. Opin. Drug. Discov. Develop. **2009**, 12, 628
5. Sander K. et al. Bioorg. Med. Chem. **2009**, 17, 7186

POSTER COMMUNICATIONS – PC.351

SCANNING MOLECULAR SHAPE AND MOTIF TO DISCOVER SMALL MOLECULE GPCR MODULATORS

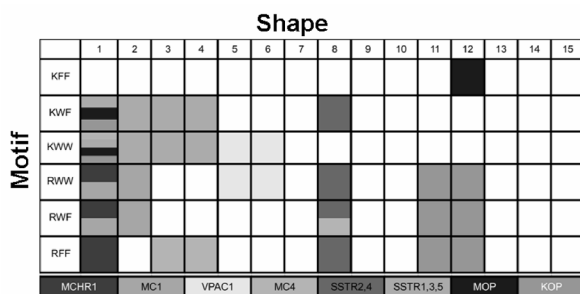
Meutermans Wim

Alchemia Ltd., 3 Hi Tech Court, Eight Mile Plains, Queensland, Australia 4113

Alchemia has established a new approach towards the discovery and optimization of small molecule GPCR modulators (orthosteric and/or allosteric). The approach starts with the screening of a pyranose-based Diversity Scanning Library (DSL), ie a library that is systematically diverse in 3D shape and in amino acid motifs it mimics. The library is designed to evenly and comprehensively cover the conformational space of tri-peptides for a diverse set of tri-peptide motifs.

Screening of the library helps identify receptor selective ligand shapes and motifs, without requiring any prior knowledge of the binding site. Once ligand shapes and motifs are identified, optimization can be carried out directly on the same drug-like scaffold, in a logical modular hit-to-lead process.

The presentation will outline the concept and include proof-of-concept examples on GPCRs for orthosteric (sst₁₋₅ and MCH₁) and allosteric (VPAC1) modulators, including in vitro and in vivo data.



POSTER COMMUNICATIONS – PC.352

IDENTIFICATION OF NOVEL, SMALL MOLECULE BRADYKININ B1 ANTAGONISTS WITH ORAL ACTIVITY IN RODENT MODELS OF PAIN AND INFLAMMATION

Alastair Parkes, David Hallett, James Madden, Xiaolu Wang, Bart Ellenbroek, Frederic Machet, Tom Schwagarus, Christina Platt, Nicole Höschen, Mark Gemkow, Rachel Grigg, Andrea Cesura and John Kemp

Evotec, 114 Milton Park, Abingdon, UK, OX14 4SA. 01235 441340, alastair.parkes@evotec.com

The Bradykinin B1 and B2 receptors are GPCRs that play a role in pain and inflammation. The B2 receptor is constitutively expressed under normal physiological conditions and its activation in response to inflammatory or noxious insult is believed to account for acute pain responses. In contrast, the B1 receptor is not expressed in healthy tissue but is upregulated following tissue injury. Activation of B1 receptors is believed to account for the chronic phase pain responses. Therefore, an orally active, small molecule B1 antagonist has been the goal of a number of drug discovery companies.

Evotec's approach to finding small molecule B1 antagonists was to identify leads which, in contrast to the majority of B1 antagonists, were active at both rodent and human B1 receptors thus enabling them to be profiled in rodent models of pain and inflammation.

This poster will describe how Evotec identified and optimised a novel series of small molecule B1 antagonists which resulted in the identification of potent, selective compounds that are orally active in rodent models of pain and inflammation.

POSTER COMMUNICATIONS – PC.353

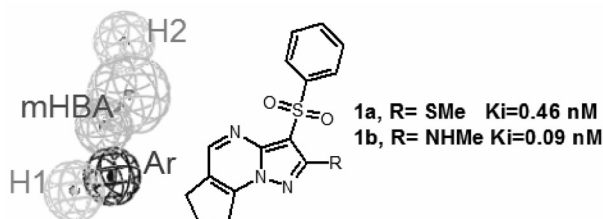
NEW PHARMACOPHORE MODEL OF 5-HT₆ RECEPTOR LIGANDS AND DISCOVERY OF NEW HIGHLY POTENT ANTAGONISTS

Alexander Ivachtchenko, Sergey Tkachenko, Madina Kadiyeva, Alex Khvat, Volodymyr Kysil, Oleg Mitkin, Ilya Okun

ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego CA 92121, USA

Though the role of 5-HT₆ receptor in many CNS diseases is widely anticipated, lack of definite progress in the development of 5-HT₆ receptor-oriented drugs indicates a need for further discoveries of novel chemotypes with high potency and high selectivity to the receptor.

We present the synthesis and biological evaluations of a series of new 'angular' (1) and 'linear' (2) cycloalkane-annelated 3-phenylsulfonylpyrazolo[1,5-a]pyrimidines as novel ligands of the 5-HT₆ receptors. Phenylsulfonylcyclopentapyrazolopyrimidines 1(a, b) were found to be a highly selective 5-HT₆ receptor antagonists with high affinity (low picomolar range) and potency.



Compounds of the series 1 and 2 were synthesized in a few steps starting from benzenesulfonylacetonitrile and subsequent reaction of intermediate 1H-pyrazole-3,5-diamine with corresponding 2-formylcycloalkanone.

Based on the SAR analysis of series 1 and 2, we have suggested a new pharmacophore model of 5-HT₆R ligands comprised of a strong multiple hydrogen bond acceptor group (mHBA), a hydrophobic site(s) (H1, H2), and π -electron donor aromatic or heterocyclic ring (Ar). The new model excludes a positive ionizable atom (PI), considered in previous models as an essential recognition element of the GPCR-ligands.

We have performed a molecular pharmacology profiling of the most active representatives of series 1 and 2 on a panel of 70 therapeutic targets (GPCRs, ion channels, transporters, and enzymes) to characterize a spectrum of their activities. Selected compounds exhibited extremely high selectivity toward 5-HT₆R and did not interact with various histaminergic, other serotonergic, α -, β -adrenergic, dopaminergic receptors, calcium, sodium, and potassium channels, transporters of neuro mediators and enzymes tested. The compounds did not interact with the potential liability hERG channels either.

ADME optimization of the hit-compounds may lead to the development of new drug candidates for the treatment of cognition and mood disorders.

POSTER COMMUNICATIONS – PC.354

NOVEL INHIBITORS OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (mPTP)

Raffaella Amici^{1,5}, *Mario Varasi*^{2,5}, *Gilles Pain*^{1,3}, *Manuela Villa*¹, *Agnese Abate*^{2,5}, *Anna Cappa*^{2,5}, *Eva Milanesi*¹, *Paolo Bernardi*⁶, *Alberto Gambalunga*⁶, *Marco Ballarini*¹, *Saverio Minucci*⁴, *Simon Plyte*¹, *Daniele Fancelli*^{1,5}.

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Mitochondria are central to both accidental (necrotic) and programmed (apoptotic) cell death.

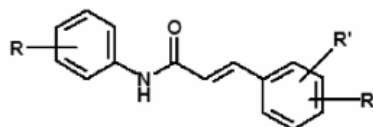
A key mitochondrial mechanism causing cell death is the opening of the permeability transition pore (PTP), a high conductance channel of the inner mitochondrial membrane.

The opening of PTP leads to osmotic swelling, and can cause rupture of the outer mitochondrial membrane followed by release of inter-membrane proteins into the cytosol, finally inducing apoptosis or necrosis depending on the ATP levels.

The role of mitochondria-mediated apoptosis and necrosis in the aetiology of many diseases is well established and the increased rate of apoptosis/necrosis typical of pathological stress conditions correlates with the MPT and loss of mitochondrial integrity.

Inhibitors of PTP are thus regarded as potential therapeutic agents, particularly for the prevention and/or treatment of diseases and conditions characterized by ischemia/reperfusion, oxidative or degenerative tissue damage.

In this account we report on the identification and early development of a series of substituted cinnamic anilides that represents a novel class of PTP inhibitors.



This series was identified through a high-throughput mitochondrial swelling assay. Subsequent class expansion led to the establishment of the basic structural requirements for activity and to the identification of derivatives with inhibitory potency higher than that of the standard inhibitor cyclosporine-A (CSA). These compounds can inhibit mPTP opening in response to several stimuli including calcium overload, oxidative stress and cross-linkers. The activity of the cinnamic anilides PTP inhibitors turned out to be additive with that of CSA, suggesting for these inhibitors a molecular target different from cyclophilin-D.

POSTER COMMUNICATIONS – PC.355

STRUCTURAL AND ENZYMATIC STUDY OF TRYPTOPHAN 2,3- DIOXYGENASE (TDO), A POTENTIAL TARGET IN ANTICANCER IMMUNOTHERAPY

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Immunotherapy is a promising novel strategy for cancer therapy. It consists of the therapeutic vaccination of cancer patients to stimulate their (natural) immune system against cancer cells. This approach, however, showed limited efficacy in vivo. Cancer cells are actually able to develop enzymatic mechanisms allow-

ing tumours to resist or escape the immune rejection. Among the enzymes involved, the indoleamine 2,3-dioxygenases IDO and TDO represents potential actors. [1-4] These enzymes catalyse the rapid degradation of tryptophan (Trp) through the kynurenine (KYN) pathway to form quinolinic acid (QA). This results in a local Trp depletion that severely affects the proliferation of T lymphocytes and is thereby profoundly immunosuppressive.



Figure: Metabolism of L-tryptophan to N-formyl-L-kynurenine catalyzed by IDO or TDO.

This project was funded in part by Télémie (FNRS grant 7.4.543.07) and led in collaboration with the team of Pr Benoit Van Den Eynde (LICR, UCL) and Pr Moreno Galeni (CIP, ULG) aims at developing novel TDO inhibitors using a rational approach. These inhibitors will allow a better understanding of the role of TDO in the phenomenon of immunosuppression, especially in cancerous tumors. (see poster Dolušić E. *et al.*)

In this poster, the structural and enzymatic study of TDO from *Ralstonia metallidurans* will be presented.

REFERENCES

1. Uyttenhove, C., and *al.*, (2003), **Nat Med**, 9, 1269-1274
2. Van den Eynde, B., and *al.*, (2010), WO2010008427.
3. Van den Eynde, B., and *al.*, (2009), U.S. application S.N.:61/247,372.
4. Batabyal D. and *al.*, (2007), **JACS**, 129, 15690-15701

POSTER COMMUNICATIONS – PC.356

TARGETING HIF-1 α WITH SMALL-MOLECULE INHIBITORS

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Hypoxia-inducible factor 1 (HIF-1) activates the transcription of genes that are involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion. Intratumoral hypoxia and genetic alterations can lead to HIF-1 α overexpression, which has been associated with increased patient mortality in several cancer types. Over the past decades, many efforts have been given in the identification of novel strategies aimed to inhibit HIF-1 in human cancer. In this exciting and promising avenue for cancer therapy we engaged in development of HIF-1 inhibitors from natural products and

chemical library. Based on our finding that moracin O (IC₅₀ = 0.14 nM) and P (IC₅₀ = 0.65 nM) exhibited excellent inhibitory activity against hypoxia inducible factor (HIF-1), we first accomplish total synthesis moracin O and P. During screening the HIF-1 inhibitors from synthetic library, we found a hit and carried out SAR studies using a cell-based assay. Many agents have been shown to inhibit HIF-1 α protein expression and HIF-1 targeted genes. Ongoing efforts should lead to the identification of more selective HIF-1 inhibitors in the near future.

REFERENCES

1. Semenza GL. *Nat. Rev. Cancer*, **2003**, 3, 721–32.
2. Giaccia A, Siim BG, Johnson RS. *Nat. Rev. Drug Discov.*, **2003**, 2, 803–11.
3. Navneet Kaur, Yan Xia, Yinglan Jin, Nguyen Tien Dat, Kondaji Gajulapati, Yongseok Choi, Young-Soo Hong, Jung Joon Lee and Kyeong Lee, *Chem. Commun.*, **2009**, (14), 1879 – 1881.
4. Kyeong Lee, Jeong Hyung Lee, Shanthaveerappa K. Boovanahalli, Yinglan Jin, Mijeoung Lee, Xuejun Jin, Jin Hwan Kim, Young-Soo Hong, and Jung Joon Lee, *J. Med. Chem.*, **2007**, 50 (7), 1675–1684.

POSTER COMMUNICATIONS – PC.357

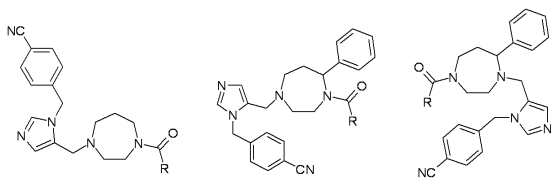
DESIGN, SYNTHESIS OF NOVEL FARNESYLTRANSFERASE INHIBITORS AS NOVEL MICROTUBULE-DESTABILIZING AGENTS IN PC3 CELLS

*N. Włodarczyk*¹, *D. Ryckewaert*¹, *P. Gilleron*¹, *A. Lemoine*¹, *A. Farce*², *C. Furman*¹, *J. Dubois*³, *P. Chavatte*², *J.-P. Henichart*¹, *R. Millet*¹

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The maturation of many proteins requires one or more post-translational modification(s). Around 60 oncoproteins (Ras, Rheb, RhoB, CENP-E, -F...) were activated by a series of post-translational modifications including prenylation, proteolysis and carboxymethylation. The prenylation is catalyzed by zinc metalloenzymes, such as farnesyltransferase (C15) (FTase) or geranylgeranyltransferase (C20) (GGTase-I). The isoprenoid chain is added to the cysteine residue located at the C-terminal tetrapeptidic sequence called the "CA₁A₂X box" (A₁A₂: aliphatic amino acids; X: methionine or serine for FTase, leucine or isoleucine for GGTase-I). The prenylation gives the sufficient hydrophobicity to translocate the protein to the adequate cellular localisation. Then, intense studies have been focused to design farnesyltransferase inhibitors (FTis). However, the exact biological mechanism of FTis remains still unknown. Yet, the excellent efficacy and low toxicity in preclinical animal models of FTis present these inhibitors as promising agents in cancer therapy.

One of the strategies to inhibit this enzyme consists in the design of peptidomimetics of the “CA₂A₂X box”. The acknowledgment of the FTase allowed the evolution of FTis from thiol-containing peptidomimetics to non-thiol, non-peptidic molecules, which may or may not interact with the zinc atom. On the basis of this model and molecular modelling studies, we have synthesised three series of original compounds, in which the 1-(4-cyanobenzyl)-5-methylimidazole zinc chelator is constant where the spacer is the 1,4-diazepane substituted or not by a phenyl and in which hydrophobic moiety is the modulated component.



Enzymatic and cellular (DU145 and PC3) assays of the synthesized compounds allowed us to establish structure-activity relationships, and thus to design new appropriate structures. CENP-E and CENP-F, centromere-associated proteins, are pertinent targets of FTis since their functional association with mitotic spindle requires farnesylation. We would so like to evaluate any selected FTis for their effects on the CENP.

REFERENCES

- Houssin, R.; Pommery, J.; Salaün, M.-C.; Deweer, S.; Goossens, J.-F.; Chavatte, P.; Hénichart, J.-P. *J. Med. Chem.* 45:533–536; 2002
- Millet, R.; Domarkas, J.; Houssin, R.; Gilleron, P.; Goossens, J.-F.; Chavatte, P.; Logé, C.; Pommery, J.; Pommery, J.; Hénichart, J.-P., *J. Med. Chem.* 47:6812–6820, 2004.
- Włodarczyk N., Gilleron P., Millet R., Houssin R., Hénichart J.-P. *Tetrahedron Lett.*, 2007, 48, 2583–2586

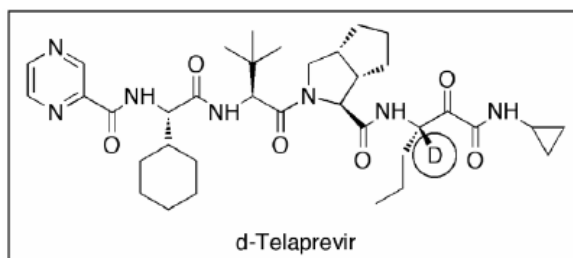
POSTER COMMUNICATIONS – PC.358

IN VITRO AND IN VIVO ISOTOPE EFFECTS WITH HEPATITIS-C PROTEASE INHIBITORS: ENHANCED PLASMA EXPOSURE OF DEUTERATED TELAPREVIR VS. TELAPREVIR IN RATS

François Maltais, Young Chun Jung, Minzhang Chen, Jerry Tanoury, Robert B. Perni¹, Nagraj Mani, Leena Laitinen, Hui Huang, Shengkai Liao, Hongying Gao², Hong Tsao, Eric Block, Chien Ma, Rebecca S. Shawgo, Christopher Town, Christopher L. Brummel, David Howe, S. Pazhanisamy, Scott Raybuck, Mark Namchuk, and Youssef L. Bennani.

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Telaprevir (VX-950), an inhibitor of the hepatitis C virus (HCV) NS3-4A protease, is in Phase 3 clinical trials. One of the major metabolites of telaprevir is its P1-(R)-diastereoisomer, VRT394, containing an inversion at the chiral center next to the α -ketamide on exchange of a proton with solvent. VRT394 is significantly less active against HCV protease. In an attempt to suppress the epimerization of telaprevir without losing activity against the HCV protease, the proton at that chiral site is replaced with deuterium (d). The d-telaprevir is as active as telaprevir in *in vitro* inhibition of protease activity and viral replication (replicon assay). The kinetics of *in vitro* stability of d-telaprevir and telaprevir in buffered solutions and plasma samples, including human plasma, suggest that d-telaprevir is significantly more stable. Oral administration (10 mg/kg) in rats resulted in a 13% increase of AUC for d-telaprevir.



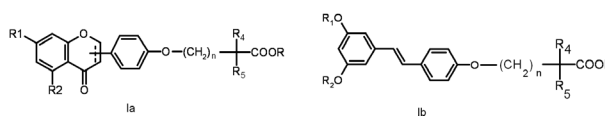
POSTER COMMUNICATIONS – PC.359

NEW ANTIDYSLIPIDEMIC DRUGS CONSTRUCTED BY INCORPORATING THE PHARMACOPHORE OF FIBRATES INTO NATURE MOLECULES WITH CARDIOVASCULAR ACTIVITY

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Fibrates are known to exhibit their beneficial effects by activating peroxysomal proliferator-activated receptor- α (PPAR α) and used in the treatment of dyslipidemia and atherosclerosis and for the prevention of heart failure. Flavonoids and resveratrol are natural antioxidants with cardioprotection activity. By incorporating the pharmacophore of fibrates into flavonoids or resveratrol, two series of α -alkyl-substituted aryloxyalkanoic acids (a and b) were prepared as agonists of peroxisome proliferator-activated receptors.



PPAR agonist activity assay revealed that compounds from b series exhibited full agonist activity for all subtype PPARs, while

fenofibric acid only show activity for PPAR α . Compounds from a series exhibited weaker activity for PPARs. Compounds with higher agonist activity were further evaluated for in vivo efficacy. Compounds from Ib₁₄ and Ib₁₅ showed excellent oral hypolipidemic activity in three hamster models and anti-hyperglycemic efficacy in KK mouse model. Structure–activity relationship studies indicated that the substituent at the distal benzene ring play key roles in determining the potency of PPAR subtype trans-activation and hypolipidemic efficacy.

POSTER COMMUNICATIONS – PC.360

3-TRIFLUOROMETHYL-2-CARBONYLQUINOXALINE DI-N-OXIDE DERIVATIVES AS POTENT ANTI-*T. CRUZI* AGENTS

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Chagas disease, caused by the protozoan *T. cruzi*, is the principal parasitic of the American continent. It affects approximately 8 million persons, from south of the United States to Chile^{1,2}. In spite of the efforts made by public health programs to eliminate the vector in some of the Latin American countries, this disease continues to be endemic in many areas. Each year, 12.500 persons die because of this parasitosis, with approximately 50.000 new cases arising. Only two drugs are used for treating this disease, nifurtimox (Nfx) and benznidazole³, both of which have important toxic effects and relative clinical efficacy. Therefore, it is essential to develop new drugs that are safer and more efficient.

Based on the demonstrated anti-infective capability of the 1,4-quinoxaline di-N-oxide (QDO) system against a large number of microorganisms, our research team has been working in the area of neglected diseases for many years, more specifically, with Chagas disease^{4,5}. We have found QDO to have good selectivity against *T. cruzi* and we determined the structural requirements in order to optimize the activity. A new series of 3-trifluoromethyl-2-carbonylquinoxaline di-N-oxide derivatives is presented; this series showed excellent results for activity against different strains of *T. cruzi*, proving itself to be even better than Nfx (Fig. 1). In addition, many of these derivatives have shown activity against different strains of *Leishmania*. Studies carried out on the mechanism of action shown that the active QDO affect the mitochondrial dehydrogenases.

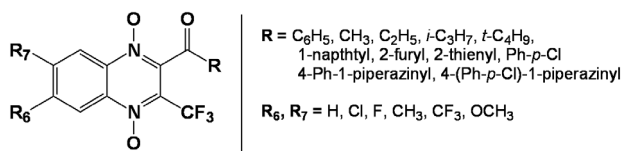


Figure 1: General structure of 3-trifluoromethyl-2-carbonylquinoxaline di-N-oxide derivatives

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REFERENCES

1. A. Rassi Jr., A. Rasi and J.A. Marin Nieto. *Lancet* 2010, 375, 1388.
2. H Cerecetto, M González. *Mini-Rev Med Chem* 2008, 8, 1355.
3. LRS Dias, MB Santos, S de Albuquerque, HC Castro, AMT de Souza, ACC Freitas, MAV DiVaio, LM Cabral, CR Rodrigues. *Bioorg Med Chem* 2007, 15, 211.
4. G Aguirre, H Cerecetto, R Di Maio, M González, ME Montoya, A Jaso, B Zarranz, M Ortega, I Aldana, A Monge. *Bioorg Med Chem Lett* 2004, 14, 3835.
5. S Ancizu, E Moreno, E Torres, A Burguete, S Pérez-Silanes, D Benítez, R Villar, B Solano, A Marín, I Aldana, H Cerecetto, M González, A Monge. *Molecules* 2009, 14, 2256.

POSTER COMMUNICATIONS – PC.361

ALLOSTERIC MODULATION OF GPCRS. SODIUM IONS ACT AS FINGERTIP TOGGLING THE ROTAMER SWITCH

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G-protein coupled receptors (GPCRs), the largest family of proteins in the human genome, are involved in many signaling pathways regulated by orthosteric ligand binding and allosteric modulation. The sodium-sensitive dopaminergic D₂ receptors which belong to the class A family of GPCRs take part in the control of various neurological processes and are therefore important drug targets in the treatment of central nervous system disorders (e.g. schizophrenia, Parkinson disease). Recent studies indicate that sodium ions allosterically modulate the dopaminergic D₂ receptors affecting mainly agonist binding at the orthosteric binding site (1,2). In an effort to understand the mechanisms of allosteric modulation of sodium ions for the dopaminergic D₂ receptor, we performed microsecond scale all-atom molecular dynamics simulations to characterize sodium

binding and receptor modulation. We found that sodium ions enter the receptor from the extracellular side and bind at the residue Asp2.50, an allosteric site deep inside the receptor. Most importantly, the presence of a sodium ion at this allosteric site induces a conformational change of the rotamer switch Trp6.48 which locks in a state identical to the partially inactive state of the crystallized human β_2 adrenergic receptor (3). The observed conformational change in which sodium ions act like a fingertip toggling the rotamer switch constitutes a completely novel aspect of sodium-induced effect for GPCRs. Moreover, this finding provides an original viewpoint for drug discovery of allosteric modulators targeting the dopaminergic D_2 receptor in particular and GPCRs in general.

REFERENCES

1. Ericksen, S.S.; Cummings D.F.; Weinstein H.; Schetz J.A. *J. Pharmacol. Exp. Ther.* **2009**, 328, 40-54.
2. Schetz J.A. *Mini. Rev. Med. Chem.* **2005**, 5, 555-61.
3. Selent J., Sanz F.; Pastor M.; De Fabritiis G. **2010** (submitted).

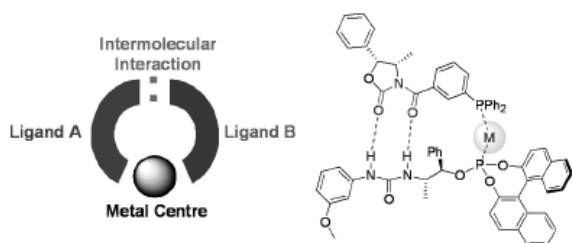
POSTER COMMUNICATIONS – PC.362

AUTOMATED SYNTHESIS & SCREENING OF SUPRAMOLECULAR CATALYSTS

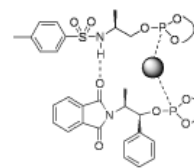
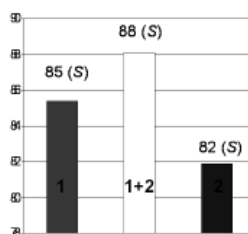
Bert Sandee¹, Lidy van der Burg¹, Jurjen Meeuwissen¹, Frederic Patureau¹, Nina Dovalil¹, Joost Reek¹; Peter Berben², Annie Schnyder³

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To circumvent the often tedious modification of novel bi- or polydentate ligands, a modular and supramolecular approach might be a potential alternative: Two monodentate ligands interact with each other in a well-defined manner, e.g. one being a hydrogen bond acceptor (A-Type), the other a hydrogen bond donor (D-Type), resulting in a quasi bidentate ligand. In this manner, a large number of different ligands are accessible by simply combining different monodentate ligands with each other, as exemplified below.



Some 500 catalytic asymmetric hydrogenation reactions were performed to screen the different ligand-ligand combinations. With the right combination of donor-acceptor ligand units, an amplification of enantioselectivity of such hetero-bidentate ligands relative to the homo-bidentate ligands was observed.



Use of Chemspeed's CATSCREEN 96 allows for the parallel, automated synthesis of such ligand building blocks and for the parallel, automated screening of the resulting catalyst library.

POSTER COMMUNICATIONS – PC.363

PROTEIN-PROTEIN INTERACTIONS BETWEEN A KINASE AND ITS SUBSTRATE

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The role of protein kinases in signal transduction pathways has been extensively studied over the past years, revealing their ability to switch various cellular processes on or off. As a consequence of abnormal kinase-mediated phosphorylation, many diseases arise such as cancer, diabetes, rheumatoid arthritis and hypertension,¹ making kinases targets of choice for medicinal chemistry programs. Although most of the efforts in the development of inhibitors concern ATP mimetic compounds,² many disadvantages have been identified such as kinome selectivity, high concentration of ATP in cells and an increasingly congested IP situation. As a consequence, alternative approaches for kinase inhibition have been envisaged such as the elaboration of potent and selective non-ATP site binders.³

Disrupting the interactions between the kinase and its peptidic substrate represents another alternative to modulate kinase function and several substrate site ligands have been reported over the last years.⁴ One general strategy for the identification of such substrate site binders consists in the elaboration of peptidic substrates of high affinity which are subsequently turned into non-peptidic inhibitors at a later stage, a methodology successfully applied by Ellman et al. in the phosphatase field.⁵

As a prologue to the development of a general combinatorial strategy for the generation of substrate-site inhibitors, we set out to map the substrate binding site of two highly homologous model kinases. We used size- and alanine-scans of known peptidic substrates to determine the key residues and minimal size required for recognition and efficient phosphorylation. An enzymatic assay was developed and used to rank substrates according to their Michaelis-Menten constants (K_M , V_{max}). Correlations

between initial velocities at fixed substrate concentrations and substrate efficiency enabled the rapid screening of substrate libraries. Notably, we were able to identify efficient semi-peptidic substrates consisting of less than five amino acids. Initial chemical strategies for the generation of inhibitors based on these optimized substrates will be presented.

REFERENCES

1. Cohen, P. *Nat. Rev. Drug. Disc.* **2002**, *1*, 309.
2. For recent examples, see: (a) Davies, S.P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95. (b) Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. *Biochem. J.* **2003**, *371*, 199.
3. For a review on allosteric inhibitors see: Eglen, R. M.; Reisine, T. *Expert Opin. Drug Discov.* **2010**, *5*, 277
4. For a review on the development of substrate binding site inhibitors on Src tyrosine kinase, see: Ye, G.; Tiwari, R.; Parang, K. *Curr. Opin. Investig. Drugs* **2008**, *9*, 605.
5. Soellner, M. B.; Rawls, K. A.; Grundner, C.; Alber, T.; Ellman, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 9613.

POSTER COMMUNICATIONS – PC.364

A NOVEL TUBULYSIN-DENDRIMER ANALOG CONJUGATE IN CHEMOTHERAPEUTIC EVALUATION USING C26 TUMOR BEARING MICE

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Current methods of cancer chemotherapy suffer from several limitations. The drugs used typically have poor water solubility, which limits their administration to the patient. Once the drug is injected into the body, a large portion is filtered from the bloodstream by the kidneys, and so multiple doses are required to maintain an effective drug concentration. From the circulatory system, the drug can travel anywhere within the body, leading to side effects like fatigue, nausea, and hair loss. One possible way to address these limitations is to attach a drug to a suitable macromolecular carrier, which would add water solubility, reduce the toxicity of the drug towards healthy tissue, cause the drug to accumulate at higher levels in the area surrounding the tumor, and release the drug in its native form at the tumor site.

Currently dendritic polymers as vehicles for anticancer drug delivery are under investigation. It is known that in general large molecules (> 40 kDa) will accumulate selectively in tumor tissue due to the Enhanced Permeation and Retention (EPR) effect. Dendrimers, however, can carry multiple copies of a drug and thus increase the effective dose per carrier and can selectively release the drug under acidic conditions.

Among the tubulysins, tubulysin D shows the highest anti-cancer activity. Thus suitably substituted tubulysin derivative has been synthesized and attached to a dendritic carrier with an acid labile acyl hydrazone linkage. The drug-dendrimer conjugate is under biological evaluation for further chemotherapeutic studies.

POSTER COMMUNICATIONS – PC.365

THE DISCOVERY AND ANTI-TUMOUR ACTIVITY CHR-3996 – A NOVEL, ORALLY AVAILABLE INHIBITOR OF CLASS 1 HISTONE DEACETYLASES

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The hyperacetylation of histones occurs in many cancers and consequently the inhibition of histone deacetylases (HDACs) has emerged as a powerful new treatment modality in a number of types of cancer, exemplified by the approval of Istodax (Celgene) and Zolinza (Merck) for the treatment of cutaneous T-cell lymphoma.¹ *In vitro* inhibition of HDACs has been shown to lead to cell cycle arrest, reactivation of tumour suppressor genes, and induction of apoptosis in a broad variety of cancer cell lines. Herein we describe the discovery and SAR of CHR-3996 a novel, orally available Class I selective HDAC inhibitor with superior pharmaceutical properties. Modulation of the Polar Surface Area (PSA) proved to be important for obtaining good cellular potency and was incorporated into the design process.

CHR-3996 shows excellent anti-proliferative activity against a broad range of human cancer cell lines *in vitro*, with good anti-tumour activity in subcutaneous tumour models. Potent synergy between CHR3996 and other anti-cancer agents, including the novel aminopeptidase inhibitor tosedostat² has also been demonstrated in a number of *in vitro* and *in vivo* models of cancer. CHR-3996 has entered clinical trials and is currently under investigation in a Phase 1 study, and this poster represents the first disclosure of its structure.

REFERENCES

1. Paris et. al., "Histone Deacetylase Inhibitors: From Bench to Clinic" *J. Med. Chem.*, **2008**, 51(6), pp. 1505-1529
2. Smith et. al. "Inhibition of HDACs and aminopeptidases is highly synergistic in myeloma cells resulting in cell death via the upregulation of BIRC3, a key mediator of NF-kappa B signalling" *Blood (ASH Annual Meeting Abstracts)*, **2009**, 114, Abstract 607

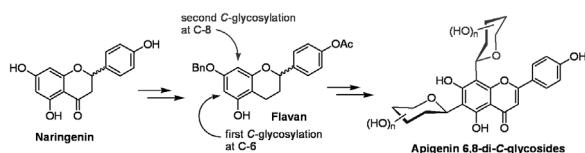
POSTER COMMUNICATIONS – PC.366

BIOACTIVE SMALL MOLECULES AND POLYSACCHARIDES IN MEDICINAL PLANT *DENDROBIUM HUOSHANENSE*

Jim-Min Fang, Jiun-Jie Shie, Yves S.-Y. Hsieh, Chih-Chien Lin, Chih-An Chen, Angela Fay Ku, Cheng Chien, Chia-Chuan Chang, Ting-Jen Rachel Cheng, Wen-Bin Yang, and Chi-Huey Wong

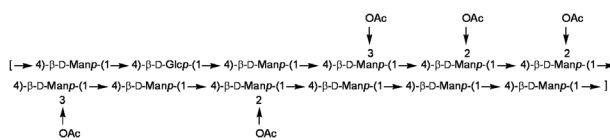
The Genomics Research Center, Academia Sinica, Taipei, 115, Taiwan, and Department of Chemistry, National Taiwan University, Taipei, 106, Taiwan.

Dendrobium huoshanense C.Z. Tang et S.J. Cheng (Orchidaceae) is a valuable herbal plant used in traditional Chinese medicine. We recently isolated four 6,8-di-C-glycosylflavones with a core structure of apigenin bearing pentoside (arabinoside or xyloside) and rhamnosyl-hexoside (glucoside or galactoside) substituents. Three methods were applied to the synthesis of di-C-glycosylflavones. The prepared 6,8-di-C-glycosylflavones were found to exhibit anti-inflammation activities by inhibiting TNF- α expression and NO production. Introduction of glucosyl moieties improved the anti-inflammation activities compared with the parent compound of apigenin. Our study also indicates that acetylation may help the availability of the compounds to suppress TNF- α expression.



In another study, we also showed that the mucilage polysaccharide exhibited specific functions in murine splenocytes. The mucilage induced several cytokines, including IFN- γ , IL-10, IL-6, and IL-1 α , and hematopoietic growth factors GM-CSF and G-CSF. However, the deacetylated mucilage obtained from an alkaline treatment failed to induce cytokine production. The extract of mucilage was further fractionated by chromatography on anion-exchange DEAE-cellulose and Sephacryl size-exclusion columns. The bioactive polysaccharide fraction was determined to have an average molecular weight of ~ 10 KDa, and its composition and structure were rigorously determined by a combination of chemical, enzymatic and spectroscopic methods.

This is the first study that provides clear evidence for the structure and activity relationship of the polysaccharide in *D. huoshanense*.



REFERENCES

1. Hsieh, Y. S.-Y.; Chien, C.; Liao, S. K.-S.; Liao, S.-F.; Hung, W.-T.; Yang, W.-B.; Lin, C.-C.; Cheng, T.-J. R.; Chang, C.-C.; Fang, J.-M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, 16, 6054–6068.
2. Chang, C.-C.; Ku, A. F.; Tseng, Y.-Y.; Yang, W.-B.; Fang, J.-M.; Wong, C.-H. *J. Nat. Prod.* **2010**, 73, 229–232.

POSTER COMMUNICATIONS – PC.367

DISCOVERY FROM A CELL PHENOTYPE SCREEN OF A SPECIFIC INHIBITOR OF THE MITOTIC DRUG TARGET SERINE-PROTEIN PHOSPHATASE I (PPI)

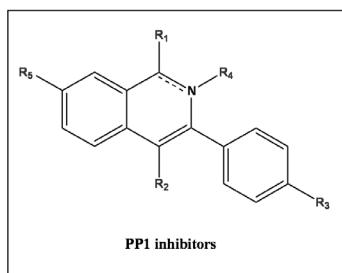
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Although there are approximately 25 serine/threonine protein phosphatases in an average mammalian genome, protein phosphatase 1 and 2A (PP1 and PP2A) together account for the majority of serine-threonine phosphatase activity within cells. PP1 consists of a catalytic subunit and regulatory polypeptides. The PP1 catalytic subunits are part of the most conserved eukaryotic proteins (PP1 beta isoform shows 99% amino acid identity between *Homo sapiens* and *Xenopus laevis*). The PP1 regulatory subunits are numerous (over 50) and can act as targeting proteins or substrates. All three PP1 isoforms localize to mitotic spindle where they are found on the centrosome, kinetochores, microtubules and chromosomes. Importantly, during cytokinesis, PP1-alpha and gamma are found on the cleavage furrow, suggesting a role in cytokinesis.

Numerous inhibitors of both protein phosphatase 1 (PP1) and 2A (PP2A) are known and are extensively used for research purposes. Most of these molecules are naturally occurring toxins, most are not selective or inhibit preferentially PP2A. Finding a selective PP1 inhibitor should be very useful for evaluation of this protein as an anti-mitotic drug target.

We isolated the initial hit compound D5 in the course of a phenotypical drug screen using live cells expressing YFP-tubulin. As a source of small molecules in the screening assays we used the highly diverse proprietary Institut- Curie/CNRS library. At the screening dose (25 µM) the compound was not toxic to cells in interphase, but induced MT depolymerization and arrested cells in mitosis. We verify that the hit did not interfere with MTs assembly *in vitro*. This meant that it induced MT depolymerization in cells by modifying the activity of another(s) than tubulin protein(s). During cell division, the Hit induced ectopic furrows and blocked cells in mitosis. In interphase HeLa cells, apart of microtubule depolymerization, it induced extensive cell membrane "blebbing", a phenomenon described in cells treated with phosphatase inhibitors. The spindle and cleavage furrow localization of PP1 have been described which could be related to the drastic effects of D5 on spindle formation and cytokinesis failure. Taken altogether, the above evidence and other phenomena lead us to propose that PP1 could be the target of D5. The hit has been tested in a range of concentrations in an *in vitro* inhibition assay with PP1 and PP2A. In these experiments, while a known non-selective PP1/PP2A inhibitor, okadaic acid, inhibited both enzymes, only PP1 was efficiently and dose-dependently inhibited with an IC₅₀ of 1 µM. The screen, the structure-activity relationships and synthetic access to this new PP1 inhibitor will be discussed.



POSTER COMMUNICATIONS – **PC.368**

ONE-POT SYNTHESIS OF UNNATURAL AMINO ACIDS FROM SERINE DERIVATIVES AND THEIR APPLICATION TO THE PREPARATION OF BIOACTIVE PRODUCTS

Juan Antonio Gallardo and Alicia Boto

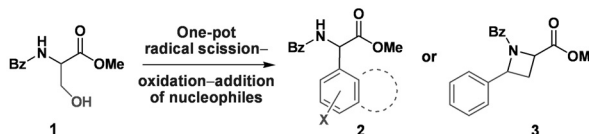
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Non-proteinogenic amino acids are important building blocks in the synthesis of alkaloids, peptides, and other biologically active compounds.¹ Thus, they are components of glycopeptide and β-lactam antibiotics, glutamate antagonists, and other drugs. These amino acids have been incorporated into peptides and proteins to modulate their biological properties or to improve their hydrolytic stability or bioavailability.^{1,2}

In previous reports from our group, the one-pot conversion of serine derivatives **1** into aryl glycines **2** was described.³ The

sequential process was initiated by a domino radical scission-oxidation process, which was coupled to the arylation step. The procedure took place under mild conditions and in good to excellent yields.³ Some of these compounds showed interesting cytotoxic properties.^{3b}

In this communication we report the conversion of serine derivatives into 2-azetidine carboxylic acids (such as compound **3**), and their utility in the preparation of drugs or biologically active compounds.



ACKNOWLEDGMENTS

This work was supported by the Investigation Programmes CTQ2006-1426/PPQ and CTQ2009-07109 (Plan Nacional I+D, Spanish Ministry of Science) and FEDER funds. J.A.G. thanks CSIC for a JAE-DOC postdoctoral contract.

REFERENCES

- (a) Pace, J. L.; Yang, G. *Biochem. Pharmacol.* **2006**, *71*, 968–980. (b) Bridges, R. J.; Esselinger, C. S. *Pharmacol. Therap.* **2005**, *107*, 271–285. (b) Van Bambeke, F. *Curr. Opin. Pharmacol.* **2004**, *4*, 471–478.
- Chen, P.R.; Groff, D.; Guo, J.; Ou, W.; Cellitti, S.; Geierstanger, B.H.; Schultz, P.G. *Angew. Chem.Int.* **2009**, *48*, 4052–4055.
- (a) Boto, A.; Hernández, R.; Montoya, A.; Suárez, E. *Tetrahedron Lett.* **2004**, *45*, 1559–1563. (b) Boto, A.; Gallardo, J. A.; Hernández, R.; Ledo, F.; Muñoz, A.; Murguía, J. R.; Menacho-Márquez M.; Orjales, A.; Saavedra, C. J. *Biorg. Med. Chem. Lett.* **2006**, *16*, 3073–3077. (c) Boto, A.; Gallardo, J. A.; Hernández, D.; Hernández, R. J. *Org. Chem.* **2007**, *72*, 7260–7269.

POSTER COMMUNICATIONS – **PC.369**

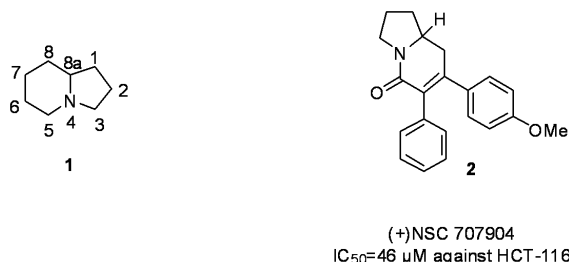
SYNTHESIS OF SECOPHENANTROINDOLIZIDINE ALKALOIDS FROM 4-HYDROXYPROLINE DERIVATIVES USING A ONE-POT DECARBOXYLATION-ALKYLATION PROCESS

Javier Miguélez Ramos and Alicia Boto.

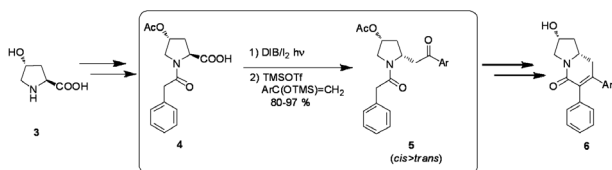
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Among the alkaloids with an indolizidine core (bicycle **1**), many phenantro- and secophenantro- indolizidine ones and their synthetic analogues (such as compound **2**) have displayed potent cytotoxic activities against various cancer lines.¹ The stereochemistry of these products is related to their biological activity,

and hence the development of stereoselective syntheses has elicited the interest of several groups.



In this poster we report a one-pot decarboxylation-alkylation process² which allows the transformation of readily available hydroxyproline derivatives **4** into 2-substituted pyrrolidines **5** which are converted in few steps into the secophenanthroline analogues **6**.



ACKNOWLEDGMENT

This work is funded by the research project CTQ2006-1426/PPQ and CTQ2009-07109 (Plan Nacional I+D, Spanish Ministry of Science) and FEDER funds. In addition, J.M.R. thanks CSIC (Spanish Research Council) for his JAE Predoc Fellowship.

REFERENCES

1. a) Michael, J. P. *Nat. Prod. Rep.* **2008**, 25, 139-165. b) Kimball, F. S.; Tunoori, A. R.; Victory, S. F.; Dutta, D.; White, J. M.; Himes, R. H.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **2007**, 17, 4703-4707.
2. a) For preliminary results, see: Boto, A.; Hernández, D.; Hernández, R. *Tetrahedron Lett.* **2009**, 50, 3974-3977. b) Boto, A.; de León, Y.; Gallardo, J. A.; Hernández, R. *Eur. J. Org. Chem.* **2005**, 3461-3468.

POSTER COMMUNICATIONS – PC.370

DEVELOPING NEW ANTIBIOTICS BASED ON THE IRON UPTAKE PATHWAY

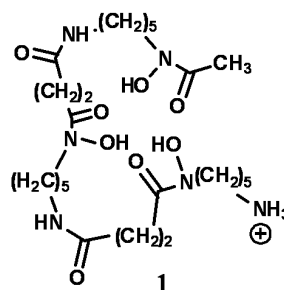
Daniel Obando, Lauren J. Brites, Chengjie Shi, Rachel Codd

School of Medical Science (Pharmacology) and Bosch Institute, The University of Sydney, NSW 2006, Australia

There is a rapid increase in the development of antibiotic resistant microbes.¹ Therefore, it is widely recognised that new anti-bacterial drugs with a novel mode of action are required. Siderophores are low molecular weight compounds produced by non-pathogenic and pathogenic bacteria in response to iron deprivation, which have been recognized as novel virulence fac-

tors. If an iron uptake inhibitory agent could be designed, then the iron uptake pathway in the bacteria could be antagonized, leading to a narrow spectrum antibiotic. Our model is based on the siderophore desferrioxamine B (DFOB) (**1**) produced by non-pathogenic gram positive bacterium *Streptomyces pilosus*.

A suite of seven DFOB-based homodimers have been synthesized with the flanking DFOB motifs separated by linker units that vary in length and rigidity. The compounds are described here in addition to study their antagonist effect against the iron pathway of *S. pilosus* and their potential as effective antibiotics.



REFERENCES

1. Maki, G.; Safdar, N.; Ebert Steven, C 2007; *Pharmacotherapy*. **27**: 121S-125S.
2. Miller, MJ; Zhu, H; Xu, Y; Wu, C; Walz, AJ; Vergne, A; Roosenberg, JM; Moraski, G; Minnick, AA; McKee-Dolence, J; Hu, J; Fennell, K; Kurt Dolence, E; Dong, L; Franzblau, S; Malouin, F; Mollmann, U 2009; *BioMetals*. **22**: 61-75.

POSTER COMMUNICATIONS – PC.371

MOLECULAR MODELING OF INTERACTIONS BETWEEN FENOTEROL STEREOISOMERS AND DERIVATIVES AND β_2 -ADRENERGIC RECEPTOR

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The β_2 adrenergic receptor (β_2 -AR) has become a model system for studying the ligand recognition process and mechanism of GPCR activation. In the present study we use stereoisomers of fenoterol and some of its derivatives as a molecular probe to identify differences in stereo-recognition of structurally similar agonists. The influence of stereochemistry of fenoterol and fenoterol derivatives on their binding to β_2 -AR is studied. Fenoterol is a selective full β_2 -AR agonist existing as four stereoisomers which significantly differ in their β_2 -AR binding affinities and selectivities. During the previous study four

stereoisomers of fenoterol and its 18 derivatives were synthesized. The submicromolar binding affinities to the β_2 -AR and the subtype selectivity to the β_1 -AR (K_{β_1} -AR/ K_{β_2} -AR) were examined. The K_d (dissociation constant) values were determined by using two types of radioligand 1) β_2 -AR antagonist, [^{3} H]CGP-12177, and 2) β_2 -AR agonist, [^{3} H] methoxy-fenoterol. The activity (EC_{50}) of the selected isomers in the stimulation of cAMP accumulation in HEK cells containing human β_2 adrenergic receptors (EC_{50cAMP}) and in a cardiomyocyte contractility model system ($EC_{50cardio}$) were examined. The experimentally calculated data were used to build the CoMFA models [1,2]. One of these models was used to design the most potent compound, i.e. (R,R)-4-methoxy-1-naphthylfenoterol. This derivative appeared to have very low K_i value (0.28 μ M), and exceptionally high subtype selectivity (the K_{β_1}/K_{β_2} ratio equal to 578) [2]. The thermodynamics of the binding of fenoterol derivatives to β_2 -AR were determined. The results indicate that the binding of (S,S)- and (S,R)-fenoterol were predominately enthalpy-driven processes while the binding of (R,R)- and (R,S)-fenoterol were entropy-driven [3]. Moreover, the coupling of β_2 -AR to the Gs and Gi proteins depends on the stereochemistry of the molecule. (R,R)-fenoterol selectively activates only the Gs protein, while (S,R)-fenoterol activates both the Gi and Gs proteins [4]. Stereoselectivity of binding to β_2 -AR were demonstrated in several docking procedures (Molegro Virtual Docker 6.03) and molecular dynamics simulations (GROMACS 3.3 package, GROMOS96 force field). Fenoterol stereoisomers and derivatives ($n = 32$), in case of which the enantioselectivity plays a crucial role, were docked to three molecular models of β_2 -AR. Agonist molecules occupy the same binding region, located between TM3, TM4, TM5, TM 6 and TM7. The following residues identified by us during docking procedure were experimentally indicated in functional and biophysical studies as being very important for formation of the hydrogen bonds: Ser204, Ser207, Asp113, Asn293, Tyr308. Correlations between the scoring function values and the compound binding affinities (the latter expressed as K_i values) were examined. According to our study, docking in Molegro Virtual Docker offers a good prediction of the binding energies (expressed as the scoring function values). The correlation between the MolDock Score and the experimentally determined pK_i is depicted by the determination coefficient $R^2 = 0.427$ for the crystal model of β_2 -AR (PDB 2RH1). The results of both the experimental and theoretical studies confirm that the (R,R)- configuration of fenoterol derivatives is the most effective one [1,2].

REFERENCES

1. Jozwiak K, Khalid C, et al., J Med Chem. 2007;50(12):2903-15.
2. Jozwiak K, Woo YH, et al., Bioorg Med Chem. 2010;18(2):728-736.
3. Jozwiak K, Toll L, et al., Biochem. Pharmacol. 2010; 79: 1610-1615.
4. Woo YH, Wang TB, et al., Mol Pharmacol. 2009; 75: 158-165.

POSTER COMMUNICATIONS – PC.372

ANTI-KINETOPLASTID BENZENESULFONAMIDES

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Leishmaniasis and Chagas disease, caused by the kinetoplastid parasites *Leishmania spp.* and *Trypanosoma cruzi*, respectively, are among the most important parasitic diseases, affecting millions of people and considered to be within the most relevant group of neglected tropical diseases. Currently chemotherapeutic treatments are far from being satisfactory. Toxic side effects, route of administration, long-term treatments and the generation of resistance mechanisms, highlight the urgent need to develop new antikinetoplastid drug candidates. Recently we demonstrated that 4-nitro-*N*-pyrimidin-2-ylbenzenesulfonamide **1** and 4-nitro-*N*-pyrazin-2-ylbenzenesulfonamide **2** showed excellent *in vivo* efficacy against *L. infantum*¹ (Fig 1).

In continuation of our interest on sulfonamides with antiprotozoal activities, a new series of benzenesulfonamides has been synthesized and assayed against *T. cruzi* and *Leishmania spp.* In this communication, we will present the discovery of some benzenesulfonamides as potent anti-kinetoplastid compounds. The SAR of these sulfonamides will be discussed.

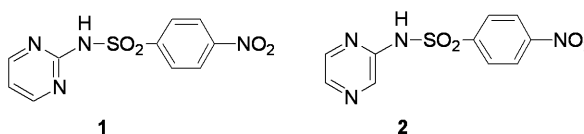


Figure 1

REFERENCES

1. Dea-Ayuela, M.A.; Castillo, E.; Gonzalez-Alvarez, M. Vega, C.; Rolón, M.; Bolás-Fernández, F.; Borrás, J.; González-Rosende, M. E. *Bioorg. Med. Chem.* **2009**, 17, 7449.

POSTER COMMUNICATIONS – PC.373

DYNAMICAL MODEL OF THE *E. COLI* MurD ENZYME AND *IN SILICO* DESIGN OF NOVEL INHIBITORS

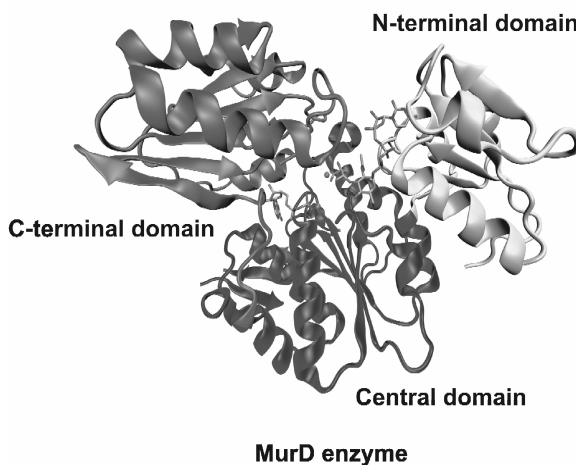
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The increasing incidence of bacterial resistance to the available antibiotics renders the discovery of novel antibacterials urgent. An essential bacterial component, peptidoglycan, is traditionally an optimal target with respect to selective toxicity. In the peptidoglycan biosynthetic pathway Mur ligases catalyze the intracellular construction of its peptide moiety. MurD enzyme in particular catalyses an incorporation of the D-Glu into the UMA precursor coupled with the concurrent ATP hydrolysis.

A complex dynamical model of the *E. coli* MurD enzyme was derived along with *in silico* structure-based discovery of novel Mur inhibitors. Targeted molecular dynamics (TMD) simulations were performed to examine the substrates binding process and gain insight into the closure of the C-terminal domain.¹ Off-path simulation (OPS) technique was initiated for the energy evaluation of the TMD-generated pathways. QM/MM molecular modeling approach was utilized, to evaluate reaction pathways leading to the tetrahedral intermediate formation – a frequent drug design starting point.² Calculated models confirmed the expected reaction order; first taking place between ATP and UMA resulting in the acyl-phosphate intermediate following by the addition of the D-Glu which most likely enters the enzyme reaction in its deprotonated form. Binding free energies calculated for a series of MurD *N*-sulphonyl-glutamic acid inhibitors revealed non-polar van der Waals interactions as the main driving force for the binding of these inhibitors.³ Based on the available structural data for the MurD and MurE enzymes a virtual screening campaign was performed, resulting in the identification of a novel class of glutamic acid surrogates – benzene 1,3-dicarboxylic acid derivatives possessing dual MurD and MurE inhibitory activity.⁴



REFERENCES

1. Perdih, A.; Kotnik, M.; Hodoscek, M.; Solmajer, T. *Proteins: Structure, Funct. Bioinf.*, **2007**, 68, 243-254.
2. Perdih, A.; Hodoscek, M.; Solmajer, T. *Proteins: Structure, Funct. Bioinf.*, **2009**, 74, 744-759.
3. Perdih, A.; Bren, U.; Solmajer, T. *J. Mol. Model.*, **2009**, 15, 983-996.
4. Perdih, A.; Kovač, A.; Wolber, G.; Blanot, D.; Gobec, S.; Solmajer, T. *Bioorg. Med. Chem. Lett.*, **2009**, 19, 2668-2673.

POSTER COMMUNICATIONS – PC.374

FUNCTIONALIZED 3-AMINOMETHYLINDOLES AS POTENT INHIBITORS OF ISOPRENYLCYSTEINE CARBOXY METHYLTRANSFERASE

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The enzyme isoprenylcysteine carboxyl methyltransferase (Icmt) plays an important role in the post-translational modification of proteins involved in the regulation of cell growth and oncogenesis. Evidence from the biological consequences of Icmt inhibition strongly support the potential of the enzyme as a novel therapeutic target for oncogenesis. Hence, there is a compelling rationale for developing specific Icmt inhibitors as pharmacological tools to investigate the functional effects of inhibition, and more importantly, as potential therapeutic agents for cancer. The indole acetamide cysmethynil is by far the most potent and widely investigated Icmt inhibitor. While it is the most promising inhibitor reported to date, we have noted its poor water solubility, strong binding to plasma proteins and less than optimal drug-like properties. Less lipophilic analogs will have a better solubility-lipophilicity balance which may translate to an improved pharmacokinetic profile and bioavailability. On the other hand, excess-

sive reduction in lipophilicity may result in compounds that are not able to gain access to the membrane bound Icmt and thus fail to bring about adequate inhibition. In an effort to reconcile these conflicting requirements, we have synthesized cysmethynil analogs with a 10^4 fold variation in lipophilicities and evaluated them for Icmt inhibitory activities as well as antiproliferative activity on breast cancer MDA-MB-231 cells. Key modifications were the replacement of the acetamide side chain by tertiary amino groups, the n-octyl side chain by isoprenyl and the 5-m-tolyl ring by fluorine. In this way, we have succeeded in identifying several compounds that are at least equivalent to cysmethynil in terms of Icmt inhibition but have significantly greater antiproliferative activity on MDA-MB-231 cells. The gains in potency were achieved in many instances without an increase in lipophilicity and this may translate to an improved solubility-lipophilicity balance leading to a more desirable pharmacokinetic profile. Hence, the findings of the present investigation may be usefully employed to guide future synthetic efforts aimed at deriving clinically useful potent inhibitors of Icmt.

REFERENCES

1. Winter-Vann, A.M.; Casey, P.J. Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat Rev Cancer* **2005**, 5, 405-12.
2. Winter-Vann, A. M.; Baron, R. A.; Wong, W.; dela Cruz, J.; York, J. D.; Gooden, D. M.; Bergo, M. O.; Young, S. G.; Toone, E. J.; Casey, P. J. A small-molecule inhibitor of isoprenylcysteine carboxyl methyltransferase with antitumor activity in cancer cells. *Proc Natl Acad Sci U S A* **2005**, 102, 4336-41.

POSTER COMMUNICATIONS – PC.375

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW CHOLINE KINASE (ChoK) INHIBITORS

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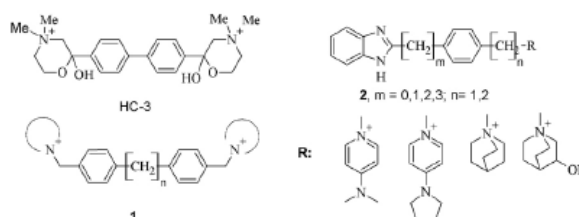
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The design and synthesis of analogues of the hemicholinium-3 (HC-3) have been the starting point in the development of ChoK inhibitors with activity antitumor.¹ Our research group has developed different bis-quaternary compounds represented by general formula **1**, which show good both ChoK inhibitory activity and antiproliferative activity.² Some of these compounds have also demonstrated to be less toxic than HC-3.

Docking studies performed on the crystalline structure of human ChoK indicate that these molecules can bind simultaneously in both the coline and ATP binding sites. In these studies, it was found that one of the cationic head of these inhibitors mimic the

choline quaternary N atom while the other is situated in the adenine binding site, which are not appropriate to stabilize the second cationic head.³

For these reasons, new non-symmetric inhibitors have been designed, and in this communications we report the synthesis and biological evaluation of new molecules of general formula **2**, bearing one cationic head and a benzimidazole moiety that could mimic the ATP adenine fragment.



REFERENCES

1. Cannon, J. G.; Lee, T. M. L.; Nyalda, A. M.; Bhattacharyya, B.; Long, J. P. *Drug Design and Delivery*, **1997**, 1, 209.
2. Conejo-García, A.; Báñez-Coronel, M.; Sánchez-Martín, R.M.; Rodríguez-González, A.; Ramos, A.; Ramírez de Molina, A.; Espinosa, A.; Gallo, M.Á.; Campos, J.M.; Lacal, J.C. *J. Med. Chem.*, **2004**, 47, 5433.
3. Malito, E.; Sekulic, S.; Too, W. C. S.; Konrad, M.; Lavie, A. *J. Mol. Biol.*, **2006**, 364, 136.

POSTER COMMUNICATIONS – PC.376

NEW INHIBITORS OF CHOLINE KINASE AS ANTIPROLIFERATIVE AGENTS

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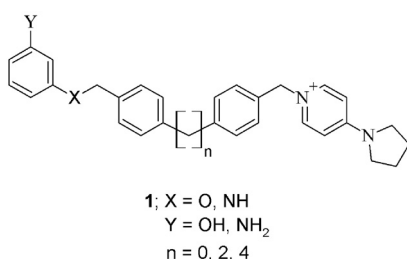
It has been described that phosphocholine (PCho) level are increased in Ras-transformed cells as a consequence of an activation of Choline Kinase (ChoK). Inhibition of ChoK by hemicholinium-3 (HC-3), a known inhibitor of this enzyme, prevents the entry in S phase of those cells that have been previously stimulated growth factors.

Our research group has reported more than 250 symmetrical bis-cationic compounds, based on structural modifications of hemicholinium-3 (HC-3), as choline kinase inhibitors showing good antiproliferative activity.

The reported crystal structures of both *Caenorhabditis elegans* and human ChoK, the last one complexed with ADP or PCho, provide new opportunities to rationalize the knowledge regarding the inhibition of this enzyme.

Docking studies performed on a homology model of human Chok, based on the X-ray crystallographic structure of *C.elegans*, indicate that inhibitors can simultaneously bind the binding sites of both substrates (ATP and Choline). The nature of both (Choline and ATP) binding sites are really different, and hence new non symmetrical inhibitors bearing a positively charged group that could be stabilized into the Choline binding sites, and a non charged aromatic moiety that mimics the ATP adenine moiety, connected by an appropriated spacer, can be designed.

In this communication the design, synthesis and biological evaluation of a series of **1-[4-(aminophenoxy)methylbenzyl]-4-(1-pyrrolidinyl)pyridinium** bromides represented by the general formula **1**, are described.



REFERENCES

1. J. M. Campos, R. M. Sánchez-Martín, A. Conejo-García, A. Entrena, M. A. Gallo, A. Espinosa, *Curr. Med. Chem.* **2006**, *13*, 1231-1238
2. D. Peisach, P. Gee, C. Kent, Z. Xu, *Structure*, **2003**, *11*, 703-713.
3. Malito, N. Sekulic, W. C. S. Too, et al. *JMB*, **2006**, *364*, 136-151.
4. L. Milanese, A. Espinosa, J. Campos, et al. *ChemMedChem*, **2006**, *1*, 1216-1228.

POSTER COMMUNICATIONS – PC.377

SYNTHESIS OF NEW 2,3-DIHYDRO-1H-QUINAZOLIN-4-ONE DERIVATIVES AS NITRIC OXIDE SYNTHASE INHIBITORS

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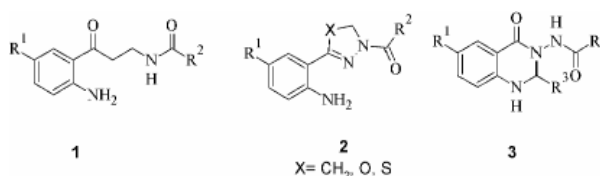
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Nitric oxide (NO), which is produced from *L*-arginine by the nitric oxide synthase (NOS) family of enzymes,¹ is an important second messenger molecule involved in a wide range of physiological functions as well as pathophysiological states. NO overproduction by nNOS has been associated with neurodegenerative pathologies such as Parkinson's, Alzheimer's and Huntington's diseases.² In addition, enhanced formation of NO following the induction of iNOS is implicated in chronic inflammatory diseases such as arthritis,³ and septic shock.⁴ Therefore, nNOS and iNOS are interesting targets in the developing of new compounds with

potential therapeutic interest. Consequently, selective inhibition of these enzymes by means of synthetic derivatives has become an interesting objective in the potential treating of these diseases.

Looking for new nNOS and iNOS inhibitors with structural relation with the main brain metabolite of melatonin, the N-acetyl-5-methoxykinurenamine **1** (R¹ = OCH₃, R² = CH₃)⁵ we have synthesized and evaluated several families of rigid analogue compounds **2**, that include 4,5-dihydro-1H-pyrazoles (X = CH₂)⁶, 2,3-dihydro-1,3,4-thiadiazoles (X = S) and 2,3-dihydro-1,3,4-oxadiazoles (X = O).

Herein we report the synthesis of a new family of 2,3-dihydroquinazolin-4(1H)-one derivatives represented by the general formula **3**. In these molecules, the benzene 2-NH₂ group present in other inhibitors has been included in a six-membered ring in order to restrict the conformational freedom, with the object of investigating the influence of this cyclization on the NOS inhibitory activity.



REFERENCES

1. Stuehr, D.J.; Griffith, O.W. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1992**, *65*, 287.
2. Schulz, J. B.; Matthews, R. T.; Klockgether, T.; Dichgans, J.; Flint, B. *M. Mol. Cell. Biochem.*, **1997**, *174*, 193.
3. Bingham, C. O. III. *J. Rheumatol. Suppl.*, **2002**, *65*, 3.
4. Petros, A.; Bennett, D.; Vallance, P. *Lancet*. **1991**, *338*, 1157.
5. Entrena, A.; Camacho, M. E.; Carrión, M. D.; López-Cara, L. C.; Velasco, G.; León, J.; Escames, G.; Acuña-Castroviejo, D.; Tapias, V.; Gallo, M. A.; Vivó, A.; Espinosa, A. *J. Med. Chem.* **2005**, *48*, 8174.
6. Camacho, M. E.; León, J.; Entrena, A.; Velasco, G.; Carrión, M. D.; Escames, G.; Vivó, A.; Acuña, D.; Gallo, M. A.; Espinosa, A. *J. Med. Chem.* **2004**, *47*, 5641.

POSTER COMMUNICATIONS – PC.378

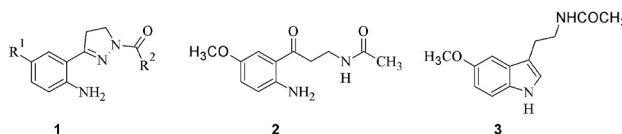
SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 4,5-DIHYDRO-1H-PYRAZOLE DERIVATIVES AS SELECTIVE INHIBITORS OF NEURONAL NITRIC OXIDE SYNTHASE

M. D. Carrión; M. E. Camacho; L. C. López-Cara;
A. Entrena; M. J. Pineda de las Infantas;
M. E. García-García; M. Chayah; M. A. Gallo; A. Espinosa

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Nitric oxide (NO) is a well-known biologically active compound that acts as a cell messenger with important regulatory functions in the nervous, immune and cardiovascular systems.¹ In mammals, NO is synthesized from *L*-arginine by a family of nitric oxide synthase (NOS) isoenzymes.² The constitutive endothelial (eNOS) and neuronal (nNOS) isoforms are calcium/calmodulin dependent and the inducible iNOS isoform is activated by basal intracellular calcium concentrations.³ NO overproduction by nNOS produces neurotoxicity, and this fact has been associated with several neurological disorders.⁴

Previously, we have described the synthesis of a series of 4,5-dihydro-1H-pyrazole derivatives **1**,⁵ as a new type of nNOS inhibitors. These compounds behave like a rigid analogue of the main brain metabolite **2** of the hormone melatonin **3**.



In order to find new compounds with neuroprotective activity and nNOS/iNOS selectivity, here we present the synthesis and biological evaluation of a new series of 4,5-dihydro-1H-pyrazole derivatives **1**. In these new molecules the benzene ring is substituted with one or several electron-withdrawing (5-Cl, 4,5-Cl₂) or electron-donating [5-OMe, 4,5-(OMe)₂ and 4,5,6-(OMe)₃] substituents with the purpose of analysing the influence of the aromatic ring charge distribution in the inhibition activity. On the other hand, the size of R² has also been increased (-CH₂Ph, -CH₂-CH₂Ph) in order to test the importance of the steric effects in the NOS selectivity.

REFERENCES

1. Moncada, S.; Palmer, R.M.J.; Higgs, E. *Pharmacol. Rev.*, **1991**, 43, 109-142.
2. Nathan, C.; Xie, Q.W. *Cell*, **1994**, 78, 915-918.
3. Dawson, T.M.; Snyder, S.H. *J. Neurosci.*, **1994**, 14, 5147-5159.
4. Vallance, P.; Leiper, J. *Nature Reviews Drug Discovery*, **2002**, 1, 939-950.
5. Camacho, M.E.; León, J.; Entrena, A.; Velasco, G.; Carrión M.D.; Escames, G.; Vivó, A.; Acuña-Castroviejo, D.; Gallo, M.A.; Espinosa, A. *J. Med. Chem.*, **2004**, 47, 5641-5650.

POSTER COMMUNICATIONS – PC.379

CAPTURE COMPOUND MASS SPECTROMETRY (CCMS) - A UNIQUE TECHNOLOGY ADDRESSING TARGET IDENTIFICATION, LEAD OPTIMIZATION AND DRUG SAFETY EVALUATION

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For drug development, medicinal chemists are in great need to specify the protein target of drug candidates and to predict toxic side effects. As a unique cost and time saving technology, CCMS addresses these challenges by the swift and unambiguous identification of drug-protein interactions in any biological sample (cell lines and tissues).

Applying CCMS, we were able to robustly isolate and identify the target proteins from (human) cell lysates for a variety of drug molecules targeting different types of proteins (e. g. Entacapone/Tolcapone, Sertindole, Dabigatran/Melagatran).

In a CCMS case study, we revealed the molecular basis of hepatotoxicity of the anti-Parkinson catechol-O-methyltransferase inhibitor Tolcapone for the first time by specifying all its relevant (on- and off-target) proteins.

In current ImproMed™ studies, we improve withdrawn drug candidates in two easy steps. First, we generate a set of equally active structural analogues of the drug with the assistance of computational drug design. Second, CCMS is directly used in profiling selected candidates by means of activity and selectivity.

The Capture Compound Mass Spectrometry (CCMS) approach allows the isolation and identification of proteins based on their specific interaction with drug candidates or other small molecules as one structural feature of the Capture Compound™. Capture Compounds are tri-functional molecules that consist of i) the drug candidate as selectivity group, ii) a photo-reactive functionality for covalent bond forming reaction to proteins and iii) a sorting function which allows to sort out captured proteins from complex protein mixtures. Offering an exceptional level of detection sensitivity, the CCMS process is highly reproducible and results can be obtained within 24 hours.

CCMS offers outstanding advantages over well established drug R&D techniques (e.g. affinity chromatography, pull-down experiments, in-vitro screens) as CCMS technology analyzes complex protein mixtures by probing the functional protein surface in almost native environment of the proteins and in presence of all toxicologically relevant proteins. In summary, CCMS has the potential to fill a technological gap in drug target identification,

drug safety assessment and helps to reengineer or to reject drugs at an early pre-clinical stage.

POSTER COMMUNICATIONS – PC.380

PYRIDO[2,3-*d*]PYRIMIDINE-4(1*H*)-ONES: A NOVEL CLASS OF GABA-A ALFA5 RECEPTOR INVERSE AGONISTS

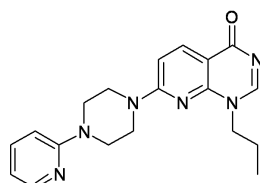
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The $\alpha 5$ subunit-containing GABAA ($\alpha 5$ GABAA) receptors are highly expressed in the hippocampus. Thus, it is suggested that $\alpha 5$ GABAA receptors play an important role in hippocampal functions such as learning and memory.

In our laboratories, pyrido[2,3-*d*]pyrimidine-4(1*H*)-one derivatives were identified as $\alpha 5$ GABAA ligand through HTS. As a result of modification of 1- and 7-position of pyrido[2,3-*d*]pyrimidine-4(1*H*)-one nucleus, compound A was found as a lead compound having $\alpha 5$ GABAA inverse agonistic activity. Compound A (1 mg/kg, p.o.) significantly improved scopolamine-induced cognitive impairment. In addition, Compound A didn't show the anxiogenic and convulsant effects associated with non-selective inverse agonistic activity. These results suggested that the selective $\alpha 5$ GABAA receptor inverse agonists such as Compound A have strong therapeutic potential for cognitive impairment and may provide a novel treatment for Alzheimer's disease.

In our presentation, SAR and pharmacological activity will be disclosed in detail.



Compound A

$\alpha 5$ Ki = 37 nmol/L

$\alpha 5$ efficacy (%) = -75 / -11
(at 10^{-5} / 10^{-6} mol/L)

Y-maze MED = 1 mg/kg, p.o.

REFERENCES

1. S. Uchida et al, Effects of $\alpha 5$ GABAA receptor inverse agonists in several animal models of cognitive function, 9th international conference AD/PD. **2009**

POSTER COMMUNICATIONS – PC.382

TOWARDS GREEN BIOCIDES: NEW BORON SPECIES AND EVALUATION OF THEIR BIOLOGICAL PROPERTIES

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The current environmental concern encourages us to find new biocides, intrinsically toxic, that would be more ecofriendly. The insecticide properties of boric acid have been known for a long time¹ and many boron species have been pointed out for their antibacterial,² antifungal,³ antiviral⁴ and antineoplastic⁵ activities (Bortezomib, Velcade®). Some of them are also described as protease⁶ and quorum-sensing inhibitors⁷.

Very few systematic biological studies of organoboron compounds have been carried out so far but none concerning the heterocyclic series. The latest have been the subject of the syntheses performed in our lab⁸⁻¹² (sylvain.rault@unicaen.fr) whose results led us to the creation of a library of boronic acids and esters liable to interest screening labs. Consequently, our research program does not only focus on the investigation of the biocides properties of these new boron species but also on their environmental acceptability. The biological evaluation is conducted in partnership with the "Ecole Nationale Supérieure de Chimie de Paris" (Paris - France), the "Institut de Chimie des Substances Naturelles" (Gif-sur-Yvette - France), the "Institut Pasteur" (Paris - France), the "Equipe de Recherche et d'Etudes en Mycologie" (Caen - France), and the "Institut National de l'Environnement Industriel et des Risques" (Paris - France).

The first screening results on bacterial activity allowed us to identify several hits. On the basis of these results, we undertook a hit-to-lead chemistry program for these particular compounds. We managed to set up structure-activity relationships in order to bring out the requested chemical structures for biological activity.

REFERENCES

1. Settepani J.A. et al. *J.Med.Chem.* **1970**, 13, 128.
2. Grassberger M.A. et al. *J.Med.Chem.* **1984**, 27, 947.
3. Hui X. et al. *J.Pharm.Sci.* **2007**, 96, 2622.
4. Kohno J. et al. *Biosci.Biotech.Biochem.* **1996**, 60, 1036.
5. Teicher B.A. et al. *Clin. Cancer Res.* **1999**, 5, 2638.
6. Morandi F. et al. *JACS*, **2003**, 125, 685.
7. Ni N. et al. *Chem.Biol.Drug.Des.* **2009**, 74, 51.

8. Bouillon A. *et al. Tetrahedron*, **2002**, 58, 2885. *Idem, ibidem*, **2002**, 58, 3323. **2002**, 58, 4369. **2003**, 59, 10043.
9. Bouillon A. *et al. J.Org.Chem.* **2003**, 68, 10178.
10. Voisin A.S. *et al. Tetrahedron*, **2006**, 62, 11734.
11. Cailly T. *et al. Tetrahedron Lett.* **2005**, 46, 135. *Idem, Synlett* **2006**, 53. *Idem, Tetrahedron* **2006**, 62, 5862.
12. Primas N. *et al. Tetrahedron* **2008**, 64, 4596. *Idem, ibidem*, **2009**, 65, 5739. **2009**, 65, 6348.

POSTER COMMUNICATIONS – PC.383

NAPHTHYRIDINE N-OXIDES AS POTENT, ORALLY BIOAVAILABLE INHIBITORS OF p38 α MAP KINASE: DESIGN, SYNTHESIS, SAR AND IN VIVO ANTI-INFLAMMATORY ACTIVITY

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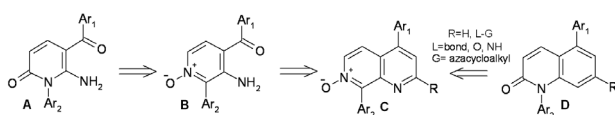
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The p38 α mitogen-activated protein (MAP) kinase is an intracellular serine/threonine (Ser/Thr) kinase that is activated by a range of environmental stimuli such as TNF α , IL-1 β and stress.¹ The proven ability of p38 α MAP kinase to efficiently regulate both the release and the activity of those pro-inflammatory cytokines has attracted numerous pharmaceutical companies and independent researchers into pursuing p38 α inhibitors primarily as novel anti-inflammatory drugs.²

In this poster, the design, synthesis and ability to inhibit p38 α MAP kinase of a novel series of naphthyridine *N*-oxides will be described. Some of these compounds showed a significant reduction in the LPS-induced TNF α production in human whole blood. Structure-activity relationship studies revealed that *N*-oxide oxygen was essential for activity and was probably a determinant factor for its marked selectivity against other related kinases.

Starting from known p38 α inhibitor pyridinones **A**, an isosteric change was carried out leading to aminopyridine *N*-oxides **B**.³ Based on the X-ray studies of these structures in the catalytic site of the enzyme, a bicyclic core emerged as a logical step forward in the design of conformationally more rigid p38 α inhibitors, keeping the unique binding mode through the *N*-oxide oxygen atom. Additionally, Merck's quinolinones **D** confirmed that a bicyclic core could provide access to good p38 α inhibitors.⁴

Naphthyridine *N*-oxides **C** proved to be significantly more potent p38 α inhibitors in the enzymatic and cellular assays than their corresponding aminopyridine *N*-oxide precursors **B**.



After an extensive SAR exercise, several compounds from the naphthyridine *N*-oxide series were identified as very potent p38 α inhibitors. *In vivo* efficacy of some derivatives was demonstrated in reducing TNF α levels in an acute murine model of inflammation (ED₅₀ = 0.5 mg/kg in LPS-induced TNF α production when dosed orally 1.5 h prior to LPS administration). The oral efficacy was further demonstrated in a chronic model of adjuvant arthritis in rats with established disease when administered orally (ED₅₀ <1 mg/kg).

REFERENCES

1. Raingeaud, J.; Gupta, S.; Rogers, J. S.; Dickens, M.; Han, J.; Ulevitch, R. J.; Davis, R. J. *J. Biol. Chem.* **1996**, 271, 2886-2891.
2. Kumar, S.; Boehm, J.; Lee, J. C. *Nature Rev. Drug Disc.* **2003**, 2, 717-726.
3. Lumeras, W.; Caturla, F.; Vidal, L.; Esteve, C.; Balagué, C. *et al. J. Med. Chem.* **2009**, 52, 5531-5545.
4. Fitzgerald, C. E.; Patel, S. B.; Becker, J. W.; Cameron, P. M. *et al. Nat. Struct. Biol.* **2003**, 10, 764-769.

POSTER COMMUNICATIONS – PC.384

A STRUCTURE-BASED DRUG DESIGN STRATEGY TO TARGET THE COLLAGEN – VON WILLEBRAND FACTOR - GPIIb/IIIa BINDING AXIS

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The Von Willebrand Factor (VWF) is an important mediator in the blood coagulation process. Upon internal vascular wall damage at places of high shear stress such as arteries and subsequent exposure of the subendothelial matrix, the VWF-A3 domain binds to collagen. This enables the multimeric VWF to unfold and bind, through its A1 domain, to the GPIIb/IIIa platelet receptor on the surface of circulating platelets. As a consequence, the platelets are tethered and a pathological thrombus is formed. The antithrombotic monoclonal antibodies 82DA3 [1] and 6B4 [2] interfere with the collagen – VWF - GPIIb/IIIa complex. 82D6A3 disrupts the VWF-A3 – collagen interaction by adhesion to the collagen binding site on VWF-A3, and 6B4 inhibits binding of VWF-A1 to GPIIb/IIIa by blocking the A1 interaction site on GPIIb/IIIa. We propose a strategy for structure-based drug design based on the crystal structures of the antibodies and their target proteins. Structural information, derived from the epitopes and paratopes, was converted into pharmacophore queries, which were used in screenings of commercial available small molecule databases. The design of the pharmacophore queries and the screenings were carried out with the Molecular Operating Environment software. The obtained hits were docked into their putative binding sites with Gold. Docking results were filtered based on different scoring functions and visu-

al inspection, and the remaining selection was ordered for in vitro testing. We were able to identify a small molecule that most likely binds to the VWF-A1 - Gplb α complex. This compound needs further optimization to increase its activity and specificity.

REFERENCES

1. Staelens S., Hadders M.A., Vauterin S., Platteau C., De Maeyer M., Vanhoorelbeke K., Huizinga E.G., Deckmyn H. (2006) Paratope determination of the antithrombotic antibody 82D6A3 based on the crystal structure of its complex with the von Willebrand factor A3-domain. *J. Biol. Chem.* 281:2225-2231.
2. Fontayne A., De Maeyer B., De Maeyer M., Yamashita M., Matsushita T., Deckmyn H. (2007) Paratope and epitope mapping of the antithrombotic antibody 6B4 in complex with platelet glycoprotein Ibalph α . *J. Biol. Chem.* 2007 282:23517-23524.

POSTER COMMUNICATIONS – PC.385

HMG-CoA REDUCTASE INHIBITORS FROM SECONDARY METABOLITES OF MALAYSIAN RAINFOREST PLANTS

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In the biosynthesis route of cholesterol through mevalonate pathways, utilizes the enzyme HMG-CoA reductase as the rate limiting enzyme in the conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mavalonic acid. The commercially available blockbuster drugs known as statins work by inhibiting HMG-CoA reductase to lower serum cholesterol. With the wide and expending use of statins, adverse side effects have become common. The needs for a lower risk statins have lead us to consider ethnopharmacological plants as sources of HMG-CoA reductase inhibitors. The plant *Pithecellobium ellipticum* (UNMC35), *Gonocaryum gracile* (UNMC43), *Mallotus kingii* (UNMC44) and *Garcinia urophylla* (UNMC45) were collected from Sungai Congkak Forest Reserve, Hulu Langat, Malaysia. Extraction of crude were done on the leaves and bark by soaking in solvents hexane, ethyl acetate, ethanol and water in order of increasing polarity. The crude extracts were then subjected to specific inhibitory assay of HMG-CoA reductase by measuring the NADPH uptake at 340 nm using a microplate reader. It was found that the leaves extract of UNMC35, leaves and bark extract of UNMC43 and UNMC45 gives specific activity that ranges from 31.25 μ g/ml to 500 μ g/ml. The crude extract in ethyl acetate from leaves of UNMC43 gives inhibition nearest to the standard used, *Pravastatin*, at 70% followed by ethanol extract in leaves of UNMC35 at 60% and hexane extract in leaves of UNMC45 at 48% inhibition. A preliminary phytochemical screening of the three crude extract indicate the presence of secondary metabolites saponin, flavonoid, terpenoid, steroid and phenolic constituents. Interestingly the presence of

tannins was not detected in all the three crude extract. Based on the specific activity of the three crude extract, further fractionation and isolation of specific secondary metabolites were conducted and we will report the specific activity together with quantitative inhibitory results followed by structural elucidation to postulate the pharmacophores guided by inhibition activity.

KEYWORDS

HMG-CoA reductase inhibitors, statins, plant extract, specific activity, ethnopharmacology

REFERENCES

1. Christophe Wiart, *Medicinal Plants From the East*, Nottingham University Press (2009).
2. Volpe, R., Gavita, R., Grassi, M. and Rossetti, A., Two better than one: Plant sterols plus red yeast rice to achieve superior efficacy in patients with moderate hypercholesterolemia, *Nutrition, Metabolism and Cardiovascular Diseases*, S35-S65 (2008).
3. Wang, G., Tang, W. and Bidigare, R., Terpenoids as therapeutic drugs and pharmaceutical agents. IN ZHANG, L. & DEMAINE, A., *Natural products: Drug discovery and therapeutics medicine*. Humana Press Inc., p. 371(2005).
4. Sung J, S-J Lee, K Park and T Moon, Isoflavones inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase *in vitro*, *Bio.sci. Biotechnol. Biochem.* 68 (2) p. 428-432 (2004).
5. Weidner C, M Krempf, J-M Bard, M Cazaubiel and D Bell, Cholesterol lowering effects of soy drink enriched with plant sterol in a French population with moderate hypercholesterolemia, *Lipids in Health and Disease* 7 p. 35

POSTER COMMUNICATIONS – PC.386

HYDROSOLUBLE CANNABINOID LIGANDS: SYNTHESIS, BIOLOGICAL EVALUATION AND METABOLISM STUDIES

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Metabolic disfunctions such as type 2 diabetes, cardiovascular diseases and cancer associated with obesity are now becoming a massive clinical and public health challenge. The therapies included sibutramine, a noradrenaline and serotonin reuptake inhibitor drug and rimonabant, a cannabinoid receptor antagonist. Both were removed from the market due to adverse effects. Orlistat, a gastrointestinal lipase inhibitor, is the only currently

marketed anti-obesity drug with limited prescription due to undesirable side effects.

Cannabinoid CB1 receptor antagonists have proved to be efficient for treating obesity and associated metabolic disorders. However rimonabant, first in this class therapy, has shown to induce depressed mood disorders. Since cannabinoid CB1 receptors are highly abundant in the brain, they mediate these adverse effects limiting the therapeutic value of such drug. CB1 receptors are also present in peripheral tissues involved in metabolic control including endocrine pancreas, skeletal muscle and liver. Thus, peripheral CB1 receptors represent an important target by which CB1 antagonists regulate energy balance. Therefore, there is a real need for developing a peripherally restricted CB1 receptors antagonist.¹

Our approach to the design of novel CB1 antagonists focused on conjugating a poly(ethylene glycol) (PEG) polymer to the pyrazole scaffold of rimonabant. This polymer is nontoxic, soluble in water and FDA approved.² Based on this strategy, a series of PEGylated pyrazole derivatives was synthesized. These new compounds exhibited very promising pharmacological profile. Moreover, unlike most of the cannabinoid ligands they are water-soluble.

We report the preparation and the biological and metabolism data of these new cannabinoids.

REFERENCES

1. D. R. Janero, A. Makriyannis. Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opin. Emerg. Drugs*, 2009; 14: 43-65.
2. F. M. Veronese, G. pasut. PEGylation, successful approach to drug delivery. *DDT*, 2005; 10: 1451-1458.

POSTER COMMUNICATIONS – PC.387

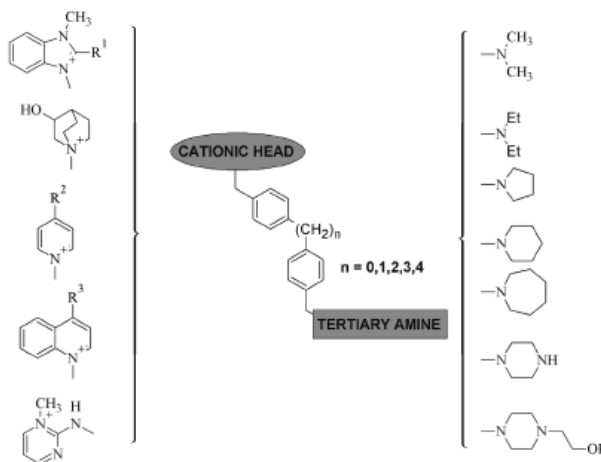
DESIGN AND SYNTHESIS OF NEW MONOQUATERNIZED HETEROCYCLIC DERIVATIVES AS CHOK INHIBITORS WITH ANTIPROLIFERATIVE ACTIVITY

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Theoretical docking studies of the ChoK-HC-3 complex have been necessary to design five new series of potential selective ChoK inhibitors.¹ Following our line of work we have designed a new series of monocationic compounds, with a cationic head in one of their termini and a tertiary amine at the other extreme. The cationic head allows the binding of the compounds to the active site of the enzyme, while the tertiary amine is directed towards the outer part of the complex. This last moiety of the compounds

can be solvated, generating a stable ligand-enzyme complex. In between them the two termini there is a biphenyl-type spacer which increases the affinity for the enzyme by setting π - π bonding interactions with aromatic residues at the active site of ChoK. These new compounds are being tested in the HepG2 cell line and their biological activity is being theoretically explained by using several tools such as Docking, Molecular Dynamics² and free energy estimations. These programs will help to give clues to the possible improvements of the ChoK inhibition.



REFERENCES

1. Conejo García, A. Doctoral Thesis. University of Granada. **2002**.
2. Sybyl 7.0 is available from Tripos Associates, Inc. St. Louis, MO. <http://www.tripos.com>.

POSTER COMMUNICATIONS – PC.388

ADAMANTYLALKYL PHENYL ETHERS AS LONG ACTING β_2 ADRENERGIC AGONISTS

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The search for new ultra long acting β_2 -adrenoreceptor agonists (LABA) has become a very active area in the last years and some compounds are currently under registration or in advanced development stage for a once daily dosing^{1,2}.

The design, synthesis, and in vitro / in vivo SAR of an adamantylalkyl phenyl ether series of aryl ethanolamine β_2 adrenergic agents (Fig. 1) will be presented. All compounds are highly β_2/β_1 selective (assessed as the ratio between IC_{50} for relaxation of Guinea-Pig tracheal rings and rat left atria).

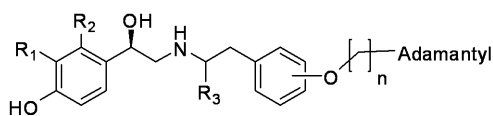


Fig. 1

Some compounds exhibit good potencies and duration of action in the reversion of acetylcholine-induced bronchoconstriction model in Guinea-Pig.

The effect of the adrenergic head, α -amino substitution and position of attachment of the adamantyl group on activity/duration will be discussed.

REFERENCES

1. J. Med. Chem. XXXX, XXX, 000-000. DOI: 10.1021/jm100068m
2. J. Med. Chem. XXXX, XXX, 000-000. DOI: 10.1021/jm100326d

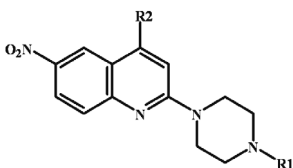
POSTER COMMUNICATIONS – PC.389

WHY N-4'-METHYLNITROQUIPAZINES POSSESS MUCH LOWER AFFINITY THAN NONMETHYLATED NITROQUIPAZINES

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Nitroquipazine (**1a**, R1 = R2 = H) possess much higher affinity to serotonin transporter (SERT) than quipazine ($K_i = 0.17$ nM and 30 nM, respectively [1]). That also concerns other derivatives of nitroquipazine bearing substituents at the aromatic rings and possessing nanomolar affinity to SERT [1,2] (for instance **1b**, R1 = H, R2 = Cl, $K_i = 0.03$ nM [2]). However, when methyl or ethyl substituent is introduced at N-4' nitrogen the affinity drops dramatically (**1c**, R1 = CH₃, R2 = Cl, $K_i = 17$ nM [2]; **1d**, R1 = CH₃, R2 = H, $K_i = 1300$ nM [3]; **1e**, R1 = C₂H₅, R2 = H, $K_i = 86$ nM [3]; **1f**, R1 = n-C₃H₇, R2 = H, $K_i = 495$ nM [3]).



1a-1f

To explain the impact of an alkylation on the SERT affinity we performed *ab initio* (Hartree-Fock 6-31** calculations on N-4'-

protonated 6-nitroquipazine and N-4'-methyl-6-nitroquipazine. Two low energy conformations have been identified for each compound: with piperazine ring in a chair conformation and with piperazine ring in a boat conformation. In the latter conformation N-4'-hydrogen atom formed intramolecular hydrogen bonding with quinoline nitrogen atom. Calculations of molecular electrostatic potential for all conformations revealed substantial differences in charge distribution between methylated and nonmethylated compounds explaining different accessibility N-4'-hydrogen atom for binding with Asp98 residue in SERT. The results were confirmed by docking of the compounds to a SERT model.

This study was supported by a grant PNRF-103-AI-1/07 through the Norwegian Financial Mechanism.

REFERENCES

1. Lee B.S., Chu S., Lee B.C., Chi D.Y., Choe Y.S., Jeong K.J., Jin C. Synthesis and binding affinities of 6-nitroquipazine analogues for serotonin transporter. Part I. *Bioorg. Med. Chem. Lett.* **10**, 1559 (2000).
2. Moon B. S., Lee B. S., Chi D. Y. Synthesis and binding affinities of 6-nitroquipazine analogues for serotonin transporter. Part 4: 3-Alkyl-4-halo-6-nitroquipazines. *Bioorg. Med. Chem.* **13**, 4952-4959 (2005).
3. Chilmonczyk Z., *et al.* Unpublished results.

POSTER COMMUNICATIONS – PC.390

COMPUTER-AIDED DRUG DESIGN OF COMPETITIVE INHIBITORS OF L-DOPA-DECARBOXYLASE, A TARGET FOR THE TREATMENT OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative condition involving the loss of dopaminergic neurons of the *substantia nigra*; its main symptoms are muscle rigidity, bradykinesia and akinesia (1).

Modern treatment of PD is based on the administration of L-DOPA, which is transformed by the pyridoxal-5'-phosphate (PLP)-dependent enzyme L-DOPA decarboxylase (DDC) into dopamine in dopaminergic neurons (1). However, only 1-5 % of L-DOPA reaches the brain, while the rest is metabolized by the peripheral DDC, causing various side effects. In order to increase central concentration of L-DOPA and prevent its side effects, a DDC-inhibitor (e.g. Carbidopa) that does not pass the blood-brain barrier, is co-administered (1). Unfortunately, Carbidopa is not selective for DDC, but reacts irreversibly with both free PLP-cofactor and other PLP-dependent enzymes, causing diverse side effects (2,3).

The aim of this work is to develop new, more selective and competitive inhibitors of DDC by means of computer-assisted techniques. Pharmacophore modeling and molecular docking were used to filter a public database of eight million compounds (4) in order to retrieve DDC inhibitors.

A structure and ligand based approach were integrated to predict the essential chemical features of a competitive inhibitor and to create a 3D pharmacophoric hypothesis (PH). The latter was then used to filter the database in order to select compounds sharing those features. About half a million compounds were processed through rigid docking into the active site of DDC; 19 compounds displaying the best docking score, while fulfilling at the same time the spatial restraints imposed by the PH, were purchased and tested *in vitro*.

Three of the five compounds acting as competitive inhibitors of DDC and showing a K_i in the lower micromolar range will be the subjects of further investigation.

REFERENCES

1. Hauser RA. Eur Neurol 2009; 62: 1-8
2. Bender DA *et al.* Biochem Pharmacol 1980; 29: 707-12
3. Bender DA. Br J Nutr 1999; 81: 7-20
4. Irwin JJ *et al.* J. Chem. Inf. Model 2005; 45: 177-182

POSTER COMMUNICATIONS – PC.391

PROPOSAL OF NOVEL NAD⁺-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE INHIBITORS BASED ON A MOLECULAR MODELING STUDY

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Inhibitors of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase could be beneficial in conditions where elevated concentration of the PGE₂ prostaglandin is desired. It has been postulated that such inhibitors could be effective as specific anticancer agents [1]. Recently, thiazolidinedione derivatives have been reported as inhibitors of this enzyme [2]. These compounds were designed based on ciglitazone, and reported as being not competitive for NAD⁺ or the substrate. Based on these data, the site finder module of MOE 2009.10 [3] was used in order to assess the possible binding site of the ligands. 14 sites were found, from which the first one was the cofactor binding site. All sites were tested with the docking program Autodock Vina [4], by using the best ligand. Based on docking scores and visualization of ligand's interactions, sites #5 and #7 were chosen for further studies, with the use of six of the best and worse ligands. Compounds devoid of thiazolidine-

dione structure (e.g chalcone¹) were also checked. The best docking poses indicated the possibility of adding interactions with the receptor in case of placing substitutions on the thiazolidinedione-based ligands from the thiazolidinedione end. A fragment search was then performed for the receptor, and suitable fragments, including acetaldehyde added to the best thiazolidinedione ligand. Novel compounds generated with this method were then docked into the receptor and the best ones retained.

REFERENCES

1. H.Cho, H.-H.Tai. Prostaglandins, Leukotrienes and Essential FattyAcids 67 (2002) 461-465
2. Y. Wu, H-H. Tai, H. Cho. Bioorganic & Medicinal Chemistry 18 (2010) 1428-1433
3. Chemical Computing Group Inc., Montreal, Canada
4. O. Trott, Aj Olson. Journal of Computational Chemistry 31:455-461

POSTER COMMUNICATIONS – PC.392

PROBING THE ORTHOSTERIC GABA_A RECEPTOR BINDING SITE BY 1-HYDROXYPYRAZOLE ANALOGUES OF THE PARTIAL GABA_A AGONIST 4-PIOL

Bente Frølund, Henriette A. Møller, Tommy Sander, Anders A. Jensen, and Thomas Balle.

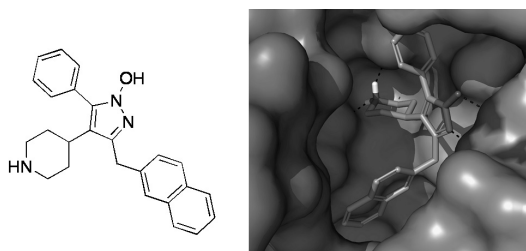
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Synaptic inhibition in the brain is largely a result of 4-aminobutyric acid (GABA) signaling, where the fast inhibitory actions of GABA are mediated by activation of GABA_A receptors. GABA_A receptors are clinically relevant drug targets for anti-convulsant, anxiolytic and sedative-hypnotic agents. Moreover, deficits in the functional expression of GABA_A receptors are critical in epilepsy, anxiety disorders, cognitive deficits, schizophrenia, depression and substance abuse.

The orthosteric binding site has been intensively investigated using a range of techniques, which have implicated numerous amino acids that may be important in the binding site. Based on these studies and the structural knowledge recently obtained from the several high-resolution X-ray structures of ACh-binding proteins from various snails, hypotheses on the ligand binding mode, mainly focusing on GABA, have been proposed. However, since GABA is a small and flexible molecule and the binding site is still very spacious and vague defined, these hypotheses are still very ambiguous.

To enhance our knowledge of the architecture of the orthosteric binding site of the GABA_A receptor and the binding mode of the ligands, we have identified the 1-hydroxypyrazole ring as bioisostere for the 3-hydroxyisoxazole ring in the partial GABA_A agonist 4-PIOL. A series of 3- and 5- and 3,5-substituted 1-

hydroxypyrazol analogs were synthesized and pharmacological characterized in [^3H]muscimol displacement at native GABA_A receptors and in the FLIPR® Membrane Potential Blue (FMP) assay at the $\alpha_1\beta_2\gamma_2$ GABA_A receptor subtype. All of the 3- and 5-substituted 1-hydroxypyrazole analogs were shown to be moderate to highly potent antagonists (K_i 5 μM – 3 nM), which indicate space above and below the orthosteric binding site able to accommodate large hydrophobic substituents.



The present and previously structure-activity studies were rationalized on the basis of preliminary homology models of the ligand binding domain of the GABA_A $\alpha_1\beta_2$ dimer. A probable binding mode of the new compounds was proposed and hydrophobic cavities associated to the binding site were identified able to account for the pharmacological data.

These results offer new knowledge to the architecture of the binding site and pharmacological tools for focused studies regarding sequence alignment and receptor flexibility.

POSTER COMMUNICATIONS – PC.393

SYNTHESIS, ACTIVITY TESTING AND Mo(VI) COMPLEXATION OF A SERIES OF SCHIFF BASES INVESTIGATED AS NOVEL XANTHINE OXIDASE INHIBITORS

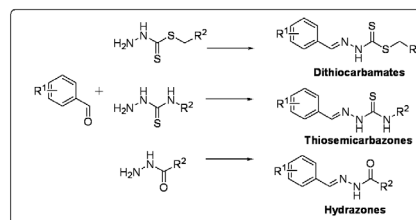
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Xanthine oxidase catalyses the formation of uric acid from dietary and endogenous purines. High serum levels of uric acid can lead to hyperuricemia and gout. It has also been shown to contribute to postischemic tissue damage as it gives rise to superoxide formation.¹ Toxicity issues in renal patients related to the use of the current benchmark for chronic gout treatment, allopurinol, as well as its time-dependent inhibition, have driven the search for novel drugs with improved safety and efficacy profiles.²

Our aim is the synthesis of a library of Schiff bases as potential xanthine oxidase inhibitors. The synthetic approach involves the condensation of a range of substituted benzaldehydes with thiosemicarbazides, dithiocarbazates and aryl hydrazides (Scheme 1). The two sites of diversity on the aldehyde and amine moieties allow the design of a structurally diverse library. The synthesis, inhibitory activity and SAR study will be presented. In

addition, the extraction and purification of xanthine oxidase for co-crystallisation trials with promising inhibitors will be reported. Mo(VI) complexation of the reported Schiff bases (Figure 1) will also reveal the potential of direct binding to the molybdenum(VI)-pterin active site of the enzyme. Several of the Schiff base ligands synthesised have shown promising inhibitory activity surpassing the benchmark, allopurinol.



Scheme 1. Synthetic pathway for inhibitor synthesis

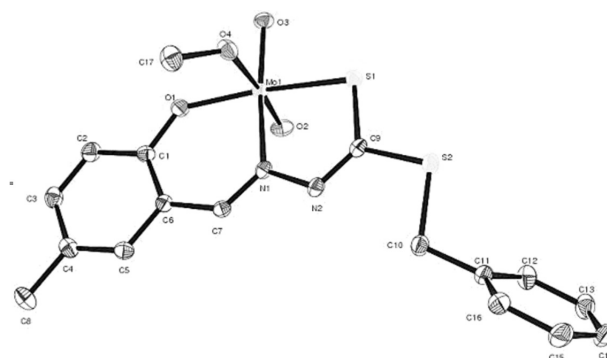


Figure 1. Mo(VI) -Schiff base complex

ACKNOWLEDGMENTS

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REFERENCES

- (a) P. Pacher, A. Nivorozhkin and C. Szabó, *Pharmacol. Rev.*, 2006, **58**, 87-114 (b) M.B. Grisham, L.A. Hernandez and D.N. Granger, *Am. J. Physiol.*, 1986, **251**, G567-G574.
- (a) J.Z. Singer and S.L. Wallace, *Arthritis Rheum.*, 1986, **29**, 82-87 (b) K.R. Hande, R.M. Noone and W.J. Stone, *Am. J. Med.*, 1984, **76**, 47-56.

POSTER COMMUNICATIONS – PC.394

USING PROXIMITY LIGATION ASSAYS TO EXPLORE PROTEIN-INTERACTIONS AND PTMS AS DRUG TARGETS

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Measurements in single cells are required to identify cell-to-cell variation in a heterogeneous cell population such as a tissue section. The most commonly used method determines expression levels of one or a few protein (i.e. immunohistochemistry). However, to obtain a more accurate view of the activity status of the cell simultaneous measurements of multiple parameters such as protein levels, protein activity and mRNA expression is required. The activation status of proteins is in most cases regulated by post-translational modifications (PTMs) such as phosphorylation that cause structural changes in the proteins and thereby expose catalytic sites or promote interactions with other proteins. By binding partner proteins, i.e. forming protein complexes, they will gain additional function such as the ability to target substrate molecules. The activity status of a protein or signaling pathway can be visualized with *in situ* Proximity Ligation Assays (*in situ* PLA) using a pair of antibodies targeting the interacting proteins or PTM, using an attached DNA molecule to the antibodies to template the creation of a circular DNA molecule that is a surrogate marker for the interaction. It can then be amplified by rolling circle amplification (RCA) and be detected, with a single-molecule resolution, in fixed cells or tissues (Söderberg et al., Nature Methods, 2006, 3(12): 995). Padlock probes also utilize RCA for generating signals in situ for detection of DNA and mRNA with a single-nucleotide resolution (Larsson et al., Nature Methods, 2010, 7(5): 395). Combining *in situ* PLA with padlock probes now gives us the tool to simultaneously detect different types of biomolecules and to monitor cellular activity status (i.e. protein levels, protein interactions, PTMs and mRNA expression) in clinical material at a single-cell level. The advantage with these methods is that the same method can be used for high-content analyses and to monitor drug efficacy in tissue sections from animal models or patients (Leuchowius et al., Mol Cell Proteomics, 2010, 9(1): 178).

POSTER COMMUNICATIONS – PC.395

BISQUATERNARY NAPHTHALIMIDES – NOVEL ACTIVE COMPOUNDS AGAINST PLASMODIA, TRYPANOSOMA AND STAPHYLOCCOCI

Tischer, M.¹, Menzel, T.², Sologub, L.², Pradel, G.², Ohlsen, K.², Holzgrabe, U.¹

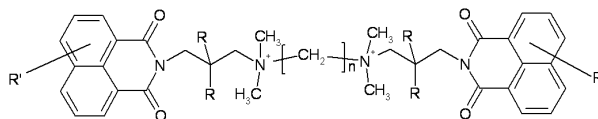
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Malaria, an infectious disease caused by *Plasmodia* spp., is the world's most prevalent tropical disease. It causes about a half billion new infections and up to three million casualties each year. The majority of those are young children of Sub-Saharan Africa. The currently available drugs are showing increasing resistance and they are partially too expensive for the "poor market" of Africa. Hence new lead structures and easily accessible compounds are urgently needed. The situation of *Trypanosoma* spp. is comparable.

The bisquaternary bisnaphthalimides are a versatile class of compounds being active against *Plasmodium falciparum*¹, *Trypanosoma brucei brucei*² and *Staphylococcus* strains³ in the low micromolar and nanomolar range of concentration. The qualitative analysis of the structure-activity relationships revealed the importance of a long methylene middle chain of at least 8 methylene groups between the two bisquaternary naphthalimides or a monoquaternary naphthalimide consisting of a long alkyl chain attached to the positively charged nitrogen atom (for *Plasmodia* and *Trypanosoma*).

As a singularity one bisnaphthalimide exhibits a high activity against several strains of *Staphylococcus*, especially multi-resistant strains. Radioactive labeling, surface plasmon resonance and gel retardation experiments revealed a direct and unspecific binding to the DNA of *Staphylococci*. The cytotoxicity of these compounds studied here was evaluated and found to be very low. The structure activity relationships of cytotoxicity on the one hand and activity against *Plasmodia*, *Trypanosoma* and *Staphylococci* on the other hand are different. The mode of action in *Plasmodia* and *Trypanosoma* remains still to be elucidated.

General structure of the bisnaphthalimides



REFERENCES

- Holzgrabe, U.; Antitrypanosomal activity of quaternary naphthalimide derivatives; *Bioorg. Med. Chem. Lett.* 2007, 17, 1590-1593
- Tischer, M.; The Bisnaphthalimides as New Active Lead Compounds against *Plasmodium falciparum*; *Bioorg. Med Chem* 2010, 18, 2987-3352
- Menzel, T. M.; The novel bisquaternary bisnaphthalimide MT02 and its antibacterial mode of action against *Staphylococcus aureus*; *Antimicrob. Agents Chemother.* 2010, [submitted]

POSTER COMMUNICATIONS – PC.396

BIOINFORMATICS ANALYSIS OF CELL-FREE PROTEIN PRODUCTION FOR FUNCTIONAL AND STRUCTURAL PROTEOMICS

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Cell-free protein synthesis is being used increasingly in structural and functional proteomics. Cell-free systems of protein synthesis offer numerous advantages over cell-based expression. The cell-free format allows direct access to the protein synthetic reaction and controlling reaction conditions without concerns about cell

growth and viability. The productivity of the bacterial cell-free synthesis reaches several milligrams of protein per milliliter of reaction mixture [1] and allows protein production on a scale sufficient for functional and structural studies. However, the factors determining expression success are poorly understood. Recently, computational approaches to predict protein propensity for expression and solubility in an *E. coli* host have been reported [2,3]. However, so far, no methods to predict the success of protein expression in a cell-free system have been developed, in part because of the small number of proteins expressed in the datasets.

In the present study, we evaluated the expression of 3,066 human proteins and their domains in a bacterial cell-free system. Screening for expressed recombinant proteins was performed in a 96-well format to allow simultaneous processing of multiple samples. The main steps of the protein synthesis according to the developed protocol included linear template generation by the two-step PCR from the source human cDNA clones, batch-mode coupled transcription/translation protein synthesis in the cell-free extract of *E. coli*, separation of soluble and insoluble reaction products by centrifugation, and estimation of protein yields and solubility by SDS PAGE and protein staining. The expression scores were assigned to all experimentally expressed proteins. Based on this information, we analyzed the correlation of cell-free protein expression with multiple physicochemical and structural properties of proteins. As a result of bioinformatics analysis performed, the most influential features that affect protein amenability to cell-free expression were determined. They include protein length, hydrophobicity, pI, content of charged, non-polar and aromatic residues, cysteine content, solvent accessibility, presence of coiled coil, content of intrinsically disordered and structured (alpha-helix and beta-sheet) sequence, number of disulfide bonds and functional domains, presence of transmembrane regions, PEST motifs and signaling sequences. This study represents the first comprehensive bioinformatics analysis of heterologous protein synthesis in a cell-free system. It provides important insights into rationalization of cell-free protein production and can be of practical use for protein engineering with the aim of increasing expression success.

REFERENCES

1. Kigawa, T., Endo, M., Ito, Y., Shirouzu, M., Kikuchi, A., and Yokoyama, S. (1999) *FEBS Letters* **442**, 15-19.
2. Bertone, P., Kluger, Y., Lan, N., Zheng, D., Christendat, D., Yee, A., Edwards, A. M., Arrowsmith, C. H., Montelione, G. T., and Gerstein, M. (2001) *Nucleic Acids Res.* **29**, 2884-2898.
3. Idicula-Thomas, S., Kulkarni, A. J., Kulkarni, B. D., Jayaraman, V. K., and Balaji, P. V. (2006) *Bioinformatics* **22**, 278-284.

POSTER COMMUNICATIONS – PC.397

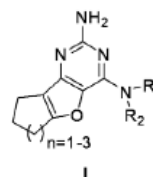
DISCOVERY AND SAR OF TRICYCLIC PYRIMIDINE DERIVATIVES AS HISTAMINE H4 RECEPTOR ANTAGONISTS

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Histamine H4 receptor is a seven-transmembrane G-protein coupled receptor, highly expressed in cells of the haematopoietic lineage, in particular eosinophils, mast cells, basophils, dendritic cells and T-cells. Its distribution and data from several pre-clinical *in vitro* and *in vivo* proof-of-principle studies, suggest that this novel target plays a key role in the immuno-inflammatory response. It is therefore expected that H4 receptor antagonists will be useful for the treatment or prevention of several allergic, immunological and inflammatory diseases such as allergic rhinitis, asthma, pruritus and atopic dermatitis.

In the context of our H4 receptor antagonist program, tricyclic aminofurypyrимidine derivatives **1** have been synthesized and identified as potent histamine H4 receptor antagonists, following an initial benzofurypyrимidine hit identified by us and others. The initial benzofurypyrимidine series, although highly potent, suffered from strong affinity for the hERG channel. Optimization of this series led to the discovery of this new structural class of highly potent and selective H4 antagonists which demonstrated reduced affinity for the hERG channel. The impact of the saturated ring size and the nature of the amine on the hERG channel affinity will be discussed. The synthesis and SAR (including the binding affinity and the functional activity) of this new structural class will be presented as well.



POSTER COMMUNICATIONS – PC.398

SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL TLR7 AGONIST WITH AN ANTIDRUG STRATEGY

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Toll-like receptor 7 (TLR7) recognizes single-stranded RNA and we have reported 8-oxoadenine compounds as TLR7 agonists¹ which have been considered to be effective for the treatment of allergic diseases by modulating Th1/Th2 immune responses. However, systemic administration of the currently available TLR7 agonists is associated with flu-like symptoms caused by induction of cytokines such as IL-6, IL-12 and type I IFN.

In order to overcome this undesired activity, we hypothesised that introduction of an “antedrug” into the TLR7 agonist would reduce the systemic side effects. An “antedrug” is defined as a biologically active form which is quickly metabolized to an inactive form upon entry into the circulation. In this case, we planned to introduce a labile ester as an “antedrug” functionality into 8-oxoadenine compounds. Our aim was to identify the ester compound which had potent TLR7 agonistic activity and was quickly metabolized to the corresponding carboxylate with significantly less activity.

A series of 8-oxoadenines containing various types of ester were synthesised and their TLR7 agonistic activities and plasma stabilities evaluated. As a result, we found that **SM-324405** exhibited good *in vitro* activity (human TLR7 EC₅₀ 50 nM) and lability (human plasma T_{1/2} 2.6 min). *In vivo* efficacy was evaluated by a Brown Norway rat model. The number of eosinophils in the bronchoalveolar lavage fluid was significantly reduced by intratracheal administration at 0.1 mg/kg, and as we expected, no systemic IFN was induced even at 10 mg/kg². R848, the referenced “non-ante” compound showed efficacy and systemic cytokine induction at the same dose. This means **SM-324405**, the TLR7 agonist with antedrug characteristics, satisfies the criteria we set and can be considered to be an excellent lead compound for the immunotherapy of allergic diseases without cytokine derived systemic side effects.

In this poster, we describe in detail the successful design of a novel TLR7 agonist containing an “antedrug” strategy.

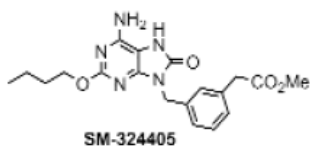


Figure 1

REFERENCES

1. Y. Isobe, A. Kurimoto, M. Tobe, K. Hashimoto, T. Nakamura, K. Norimura, H. Ogita, and H. Takaku *J. Med. Chem.* **2006**, 49, 2088-2095.
2. A. Kurimoto, K. Hashimoto, T. Nakamura, K. Norimura, H. Ogita, H. Takaku, R. Bonnert, T. McNally, H. Wada, and Y. Isobe *J. Med. Chem.* **2010**, 53, 2964-2972.

POSTER COMMUNICATIONS – PC.399

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF NOVEL CYTOTOXIC CARBAMATE AND N-ACYLHETEROCYCLIC BEARING DERIVATES OF BETULIN AND BETULINIC ACID

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Natural products have been used to treat human disease for thousands of years and play an increasingly important role in drug discovery and development. In fact, the majority of anti-cancer and anti-infectious agents are of natural origin.^{1,2}

Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and structure-activity relationship (SAR) analyses.³ To date, several reports have been published demonstrating that either simple or advanced modifications may be performed without loss of the desired biological properties.⁴ The natural occurring lupane-type triterpenoids betulin and betulinic acid have been thoroughly investigated during the past years for their promising medical properties,⁵⁻⁸ and particularly their chemopreventive and antitumor activities.^{9,10}

In this communication,¹¹ we report the synthesis of novel 2'-methylimidazole and triazole carbamates and N-acylheterocyclic bearing derivatives of betulin and betulinic acid, and their *in vitro* cytotoxic activity against the human cancer cell lines HepG2, Jurkat, HeLa, HT-29, PC-3 as well as on fibroblasts. The compounds were also screened for their ability to inhibit topoisomerase I.

REFERENCES

1. Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug. Discov.* **2005**, 4, 206.
2. Li, J. W. H.; Vederas, J. C. *Science* **2009**, 325, 161.
3. Petronelli, A.; Pannitteri, G.; Testa, U. *Anti-Cancer Drug.* **2009**, 20, 880.; Salvador J. A. R., Ed., *Pentacyclic Triterpenes as Promising Agents in Cancer*, Nova Science Publishers, USA, New York, 2010, ISBN: 978-1-60876-973-5.
4. Tolstikova, T. G.; Sorokina, I.V.; Tolstikova, G.A.; Tolstikov A.G.; Flekhter, O. B. *Russ. J. Bioorg. Chem.* **2006**, 32, 37.
5. Cichewicz, R. H.; Kouzi, S. A. *Med. Res. Rev.* **2004**, 24, 90.
6. Patocka, J.; Stirobova, M. *Chem. Listy* **2004**, 98, 185.
7. Dzubak, P.; Hajdich, M.; Vydra, D.; Hustova, A.; Kvaniscka, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J. *Nat. Prod. Rep.* **2006**, 23, 294.
8. Sami, A.; Taru, M.; Salme, K.; Jari, Y. K. *Eur. J. Pharm. Sci.* **2006**, 29, 1..

9. Eiznhamer, D. A.; Xu, Z.-Q. *IDrugs* **2004**, 7, 359.
10. Mukherjee, R.; Kumar, V.; Srivastava, S.K.; Agarwal, S. K.; Burman, A. C. *Anticancer Agents Med. Chem.* **2006**, 6, 271.
11. Santos, C. S.; Salvador, J. A. R.; Marin, S.; Cascante, M.; Moreira, J. N.; Dinis, T. C. P. *Bioorg. Med. Chem.* **2010**, in press. doi:10.1016/j.bmc.2010.04.085

POSTER COMMUNICATIONS – PC.400

SYNTHESIS AND CYTOTOXIC EVALUATION OF TRIHYDROXYSTERIODS

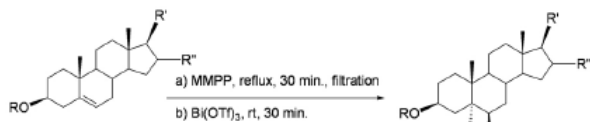
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Evidence of the anticancer potential of polyhydroxysteroids, either endogenous (oxysterols) or isolated from diverse natural sources has been provided by different lines of research. The 3 β ,5 α ,6 β -trihydroxy pattern is present in several steroids with cytotoxic activity, specifically the well known oxysterol cholestane-3 β ,5 α ,6 β -triol and diverse steroids from marine origin.

Within our framework on the synthesis and biological evaluation of polyhydroxysteroids [1,2,3], we explore herein the influence of structural variations at C-17 on the cytotoxic outcome by synthesizing several 3 β ,5 α ,6 β -trihydroxysteroids with different side chains.

A convenient, fast and high-yielding process to synthesize 5 α ,6 β -dihydroxysteroids directly from the corresponding Δ^5 -steroids was developed [3]. The reaction protocol consists in the conjugation of a readily available and stable oxidant, magnesium bis(monoperoxyphthalate) hexahydrate with the non-toxic bismuth(III) triflate in acetone to afford the *trans*-diaxial hydroxylation product in a stepwise fashion and in excellent yields.



A library of 3 β ,5 α ,6 β -trihydroxysteroids was obtained and screened against HT-29 cells. Cholestane-3 β ,5 α ,6 β -triol, its 3 β -acetyl and 3 β -hemisuccinyl derivatives were cytotoxic at the low micromolar range. The presence of oxygenated substituents at C-17, C-20 or C-16 did not afford cytotoxicity. The C-24 ramification of the cholesterol side chain, a structural characteristic of phytosterols, as well as its C-22 unsaturation led to a significant decrease in activity. Therefore, we conclude that the saturated side chain of cholesterol, which has been reported as essential for cellular proliferation [4], is also required for cytotoxicity.

ACKNOWLEDGMENTS

Thanks are due to Fundação para a Ciência e Tecnologia (FCT), Portugal, through POCl. JFS Carvalho also thanks FCT for a PhD grant (SFRH/BD/18263/2004).

REFERENCES

1. JFS Carvalho, MMC Silva, JN Moreira, S Simões, ML Sá e Melo, *J. Med. Chem.*, 2009, 52, 4007-4019.
2. JFS Carvalho, MMC Silva, ML Sá e Melo, *Tetrahedron*, 2009, 65, 2773-2781.
3. JFS Carvalho, MMC Silva, ML Sá e Melo, *Tetrahedron*, 2010, 66, 2455-2462.
4. F Xu, SD Rychnovsky, JD Belani, HH Hobbs, JC Cohen, RB Rawson, *Proc. Natl. Acad. Sci. USA* 2005, 102, 14551-14556.

POSTER COMMUNICATIONS – PC.401

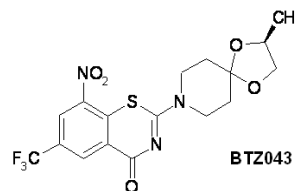
BENZOTHAZINONE DERIVATIVES. A NEW CLASS SYNTHETIC ANTITUBERCULOSIS COMPOUNDS

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Loss of human lives to tuberculosis (TB) continues essentially unabated as a result of poverty, devastating synergy with the HIV/AIDS pandemic and the emergence of multidrug- and extensively-drug resistant strains of *Mycobacterium tuberculosis*. Unlike most other areas of anti-infective agent discovery, some activity has been maintained in the TB field and, for the first time in over 40 years, there is a semblance of a drug development pipeline. Better drugs are required to counter the tuberculosis (TB) pandemic.

Here we report the synthesis and characterization of 1,3-benzothiazin-4-ones (BTZ), a new potent class of antimycobacterial agents, a new high potent class of antimycobacterial agents, that rapidly kill actively multiplying *Mycobacterium tuberculosis* *in vitro*, *ex vivo*, in murine and guinea pigs models of TB. One compound **BTZ043** (2-[2S-methyl-1,4-dioxo-8-azaspiro[4.5]dec-8-yl]-8-nitro-6-(trifluoromethyl)-4H-1,3-benzothiazin-4-one) is under active development now. Several original methods of the synthesis of target compound were discovered and will be presented.



Its activity was detail studied on wild clinical isolated strains and resistant mutants were not found. Using genetics and biochemistry, the enzyme decaprenylphosphoryl- β -D-ribose 2'-

epimerase was identified as a major target of the most advanced compound, BTZ043. Inhibition of this enzymatic activity abolishes formation of decaprenylphosphoryl-arabinose, a key precursor required for the synthesis of the cell-wall arabinans of many actinobacteria, thus provoking cell lysis and death.

Structure-activity relationship as well as metabolic transformation most active agents will be discussed during presentation.

This work was done as part of RFBR grant 09-04-12108 and EU project NM4TB LSHP-CT-2005-018923.

REFERENCES

1. V. Makarov, G. Manina, K. Mikusova, et al. Benzothiazinones Kill *Mycobacterium tuberculosis* by Blocking Arabinan Synthesis, *Science*, 2009, **324**, 801.

POSTER COMMUNICATIONS – PC.402

DESIGN OF ALLOSTERIC/ORTHOSTERIC HYBRID COMPOUNDS AS ANTAGONISTS FOR MUSCARINIC RECEPTORS

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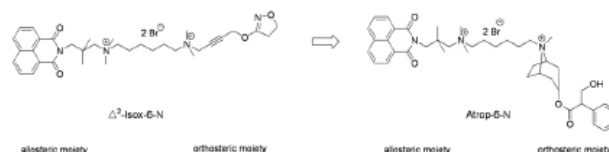
Muscarinic receptors, belonging to the superfamily of G-protein-coupled receptors, are classified in 5 subtypes (M_1 - M_5) which differ in tissue distribution and G-protein-coupling specificity. Among the five subtypes the amino acid sequence of the orthosteric acetylcholine-binding site is highly conserved so that drugs that target the orthosteric site generally lack therapeutically relevant subtype selectivity.^[1] However, there is a second (allosteric) binding site that is less conserved among the different subtypes. Allosteric modulators that bind to this region have the ability to affect equilibrium binding of orthosteric agonists or antagonists.

Fusion of appropriate building blocks was shown to yield a new class of highly selective and active muscarinic receptor agonists that combines allosteric subtype selectivity with orthosteric receptor activation.^[2] In order to translate this concept directly to muscarinic receptor antagonists, the orthosteric agonistic building block, i.e. iperoxo, was replaced by the orthosteric antagonists atropine and scopolamine, respectively. By conversion of phthalic and naphthalic acid anhydride derivatives with a corresponding propane-1,3-diamine derivative and alkylation of the obtained amine with dibromoalkane the allosteric moieties were built. The following coupling with the antagonist moieties via alkylation of the basic tropine nitrogen resulted in the allosteric/orthosteric hybrid antagonists.

Using [³H]N-methylscopolamine ([³H]NMS) as an orthosteric probe the binding affinities of the different compounds were determined at human receptors or mutant receptors, respective-

ly, both being expressed in CHO cells. As the allosteric building blocks have highest affinity for the M_2 receptor subtype and lowest for M_5 , binding affinity to these two subtypes was measured. The antagonist hybrid Atrop-6-N displayed a relatively small but still significant M_2/M_5 subtype selectivity of 0.7 log units of affinity, whereas the antagonist atropine itself shows no appreciable selectivity. Additionally there is evidence that the appropriately designed antagonists also bind – in analogy to agonists – in a dualsteric mode at muscarinic receptors.

The proof-of-principle is provided by this study, the M_2/M_5 selectivity has to be optimized in the future. The synthesis of corresponding derivatives is in progress.



ACKNOWLEDGMENTS

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REFERENCES

1. De Amici et al. *Med Res Rev.* 2010, 30(3) 463-549
2. Antony et al. *FASEB J* 2009, 23(2) 442-450

POSTER COMMUNICATIONS – PC.403

SYNTHESIS OF NEW GABA UPTAKE INHIBITORS

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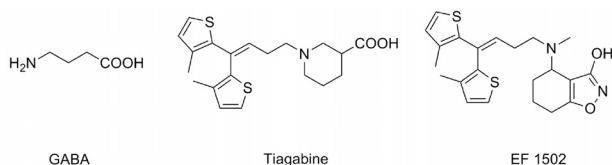
GABA (gamma-aminobutyric acid) is the most important inhibitory neurotransmitter in the central nervous system (CNS). Upon release into the synapse GABA activates $GABA_A$ and $GABA_B$ receptors. The signal transmission is terminated by re-uptake of GABA into neurons and glia cells by GABA transporters (GATs). Generally, increased GABA transmission is known to depress neuronal activity. Accordingly, drugs that increase inhibitory activity are used clinically as anxiolytics, hypnotics, anaesthetics, anti-epileptics, and muscle relaxants.

Four cloned GABA transporter subtypes (GAT1-GAT4) exist belonging to the 12 transmembrane family of Na^+/Cl^- dependent transporters (termed the SLC family), which also includes transporters for other neurotransmitters including dopamine, serotonin, norepinephrine and glycine. However, little is known about the physiological function of the transporter subtypes GAT2-GAT4. This is mainly due to the lack of specific and potent

inhibitors for these subtypes. The major goal of the project is to develop such novel GAT2, GAT3, and GAT4 selective inhibitors.

Recently the compound EF 1502 has been developed that selectively targets both GAT1 and GAT2 (1). Extensive animal studies with this compound have revealed that this combination could be important and give rise to new therapeutic possibilities, especially in combination with Tiagabine, which is a GAT1 selective inhibitor that is applied clinically as an anti-convulsive (2). These results emphasize the importance of clarifying the roles of other transporter subtypes than GAT1.

Comparison of the chemical structures of EF 1502 and Tiagabine shows that they share a lipophilic aromatic sidechain. While limiting the variation in the lipophilic sidechain extensive variation will be introduced in the amino acid moiety of the molecule by conformational restriction of the amino acid in combination with bioisosteric substitution. This strategy is opposed to the huge variation that has been introduced in the lipophilic aromatic part of this class of molecules and usually based on a few amino acids especially nipecotic acid and guvacine (3). It is therefore the primary object of the current project to generate new GABA and beta-alanine analogues that can be selective GABA transport inhibitors and expanding the pharmacological exploration by the addition of lipophilic aromatic sidechains to the amino group.



Four objects are pursued by the addition of a lipophilic sidechain: increased potency, blood-brain barrier penetration properties, induce inhibitory properties, and altered pharmacological profile.

REFERENCES

1. Clausen, R. P. et. al. (2005). *Bioorg. Med. Chem.* **13**, 895-908.
2. White, H. S. et. al. (2005). *J. Pharmacol. Exp. Ther.* **312**, 866-874.
3. Borden, L. A. (1996). *Neurochem. Int.*, **29**, 4, 335-356.

POSTER COMMUNICATIONS – PC.404

STRUCTURE-BASED DRUG DESIGN OF DIKETO ACID DERIVATIVES INHIBITING INFLUENZA VIRAL METALLOENZYME RNA POLYMERASE

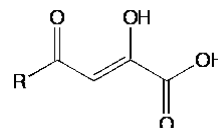
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Epidemics of influenza A virus is a global threat every year. Whereas neuraminidase inhibitors *Tamiflu* and *Relenza*, and M2

protein blocker *Symmetrel* are effective and clinically used, the viral drug resistance has been emerged. Thus, the development of effective anti-influenza agents having a different mechanism of action is needed.

Tomassini et al. reported anti-influenza virus activities of a series of diketo acid derivatives.^{1,2)} Because the action is due to the inhibition of endonuclease activity of influenza viral RNA polymerase, the enzyme is currently regarded as a promising target for anti-influenza virus agents.³⁾



Influenza viral RNA polymerase consists of three subunits, PA, PB1 and PB2. Recently, the crystallography of the PA subunits made it clear that PA_N, the N-terminal domain of PA, contains a Mg ion or two Mn ions in its endonuclease active site.^{4,5)} The endonuclease activity of PA_N is inhibited by a diketo acid derivative.⁵⁾

Our objective of this research is to rationalize the inhibitory mechanism of the diketo acid derivatives to the metalloenzyme RNA polymerase, PA_N, and to design new diketo acid derivatives showing more potent inhibitory activity. To do so, we carried out molecular docking simulation to predict the binding modes of the diketo acid anti-influenza compounds to the PA_N subunit.

The structural modeling of the diketo acid derivatives was done using AMPAC8. The structural optimization and electrostatic potential calculation were performed using Gaussian03. The RESP charge fitting of the electrostatic potentials of the optimized structures were done with the *antechamber* module in AMBER9. The crystal structure of the PA_N with two Mn ions (ID:2w69) was obtained from PDB. All water molecules were eliminated from the coordinates, and then hydrogen atoms were added through WHAT IF web server. Molecular docking was performed using AUTODOCK4.

We carried out molecular docking simulation between the PA_N subunit having two Mn(II) ions and diketo acid derivatives with their various inhibition activities. The binding modes and activity-docking energy relationship of the diketo acids, and the structure-based drug design of new diketo acid derivatives will be presented. The molecular docking analysis and structure-based drug design certainly help the discovery of new anti-influenza drugs.

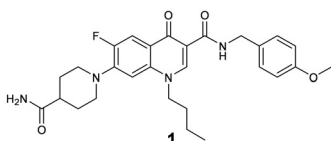
REFERENCES

1. Tomassini, J. et al., *Antimicrob. Agents Chemother.* **38**, 2827 (1994).
2. Hastings, J. C. et al., *Antimicrob. Agents Chemother.* **40**, 1304 (1996).
3. Nakazawa, M. et al., *Antiviral Res.* **78**, 194 (2008).
4. Yuan, P. et al., *Nature*, **458**, 914 (2009).
5. Dias, A. et al., *Nature*, **458**, 909 (2009).

POSTER COMMUNICATIONS – PC.405

NEW QUINOLONE-TYPE MOLECULES AGAINST *TRYPANOSOMA BRUCEI**Hiltensperger, G.¹, Niedermeier, S.¹, Stich, A.², Holzgrabe, U.¹*¹Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, 97074 Wuerzburg, Germany; ²Medical Mission Institute, Salvatorstraße 7, 97067 Wuerzburg, Germany

Human african trypanosomiasis (HAT) is caused by infection with one of two parasites, which differ in their geographical range and etiopathology. While *T.b. gambiense* HAT is primarily a human chronic disease and occurs in Western and Central Africa, *T.b. rhodesiense* HAT is primarily zoonotic with a huge animal reservoir and causes the acute form of sleeping sickness in Eastern and Southern Africa. Current estimations show that in the prevalence sub-Saharan area about 60 million people are at risk and 50000 – 70000 cases occur annually¹. Both forms of HAT show two clinical stages, whereas the first corresponds to the multiplication of the parasite in the blood and lymphatic system. After crossing the blood-brain barrier the trypanosoma attack the central nervous system and neurological symptoms appear. Without medical treatment, coma and finally death results. Due to a low number of available drugs which suffer from severe side effects, new and easily accessible active compounds are urgently needed. Therefore we synthesized a quinolone-type library and tested them against *Trypanosoma brucei*. The 4-oxo-quinolone skeletons are build up using the Gould-Jacobs procedure with subsequent installation of the piperidine residue at C7 by nucleophilic aromatic substitution. The final step includes the amidation at C3 with different benzyl- and phenethylamines. The biological evaluation shows that the 4-oxo-quinolone-3-carboxylates are inactive. The essential increase in activity is obtained by amidation at C3. Moreover the piperidine residue at C7 and longer alkyl chains at N1 lead to further enhancement. Compound **1**, which exhibits an IC₅₀ value of 0.78 µM, represents a promising lead structure for further structural modification.



REFERENCES

1. E. M. Fèvre, B. v. Wissmann, S. C. Welburn, P. Lutumba, The Burden of Human African Trypanosomiasis, *PLoS Negl. Trop. Dis.*, 2008, 2 (12).

POSTER COMMUNICATIONS – PC.406

HETEROARYL SUBSTITUTED PYRIDO[2,3-b]PYRAZINES AS NOVEL SELECTIVE ERK INHIBITORS - A STRUCTURE-GUIDED OPTIMIZATION APPROACH

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The MAPK (Ras/Raf/Mek/ERK) pathway controls a number of fundamental cellular process including cell survival, proliferation, motility and differentiation. Deregulation of this pathway by mutations or amplifications is frequently observed in several tumor types including lung, colon, pancreas, kidney, and ovary.

A number of companies are actively developing MAPK pathway inhibitors. Sorafenib, an inhibitor of Raf kinase was approved in 2005 for the treatment of renal cell carcinoma. Two highly selective non-ATP-competitive Mek inhibitors, PD-0325901 and AZD6244 are currently undergoing clinical development.

ERK is pivotal in the pathway downstream of Ras, Raf and Mek, acting as central point where multiple signaling pathways coalesce to drive transcription. ERK1 and ERK2 share 88% sequence identity, and the residues in the ATP site are conserved.

Our goal was to design novel and selective ATP-competitive inhibitors of ERK that would enable control over the penultimate step in this key signal transduction pathway.

Here we present the structure class of heteroaryl substituted pyrido[2,3-b]pyrazines with high selectivity for ERK2.

This poster describes our efforts in combining X-ray crystallography, molecular modeling and medicinal chemistry towards the discovery and optimization of heteroaryl substituted pyrido[2,3b]pyrazines as novel selective ERK inhibitors. So far, we have identified compounds with ERK inhibitory activity in the low nanomolar range. Presented herein are the synthesis, structure-activity relationships, physicochemical data and ADME parameter of this structural class.

POSTER COMMUNICATIONS – PC.407

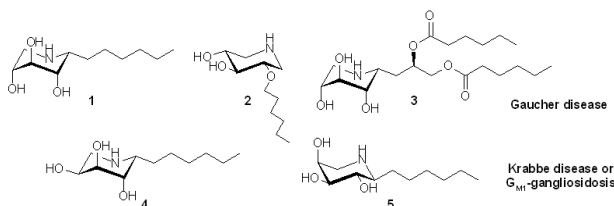
IMINOGLYCOLIPIDS AS PHARMACOLOGICAL CHAPERONES FOR LYSOSOMAL STORAGE DISORDERS

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Lysosomal Storage Disorders (LSD) are rare inherited diseases caused by a mutation in one of the glycosidases involved in the catabolism of glycosphingolipids (GSL). The resulting dysfunction leads to the accumulation of partially degraded GSL in cells and organs, which induces serious, often fatal symptoms. An innovative and very promising approach to the treatment of LSD is the use of small molecules acting as pharmacological chaperones:¹ such compounds, which are usually potent inhibitors of the involved glycosidases, help stabilize the mutant protein and save it from degradation; this results in an increased enzymatic activity in the lysosome and a concomitant decrease of the symptoms. Our previous investigations have shown that α -1-C-alkyl derivatives of iminoxylitols such as **1** are potent inhibitors of β -glucocerebrosidase (GCase) and act as effective pharmacological chaperones of the N370S mutant form of GCase from Gaucher disease patients.²

We have further explored the field of "iminoglycolipids" (iminosugars carrying a lipophilic substituent) with the goal of finding new derivatives with improved biological activity towards various lysosomal glycosidases. We have prepared O-alkyl iminoxylitol derivatives such as **2** and glucosylceramide mimics such as **3**, which are potent GCase inhibitors (nM range) with good chaperone effects. These results pave the way to new therapeutic agents for Gaucher disease. Similar synthetic methodologies have also been applied in *galacto*-like series to yield compounds such as **4** and **5** as potential inhibitors of β -galactocerebrosidase involved in Krabbe disease, or lysosomal β -galactosidase involved in G_{M1} -gangliosidosis.



The synthesis of these compounds and the results of preliminary biological investigations, performed in the laboratories of Dr. Asano (Hokuriku University, Kanazawa, Japan) and of Prof. Orlicchio (University of Perugia, Italy) will be described in the presentation.

REFERENCES

1. Fan, J.-Q. *Biol. Chem.* **2008**, 389, 1-11.
2. Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006**, 7, 1356-1359.

POSTER COMMUNICATIONS – PC.408

SYNTHESIS OF ESTRADIOL RING-D DERIVATIVES WITH POTENTIAL NEUROPROTECTIVE ACTIVITY

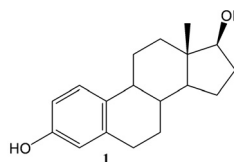
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A growing body of evidence has accumulated supporting the effects of estradiol, **1**, as neuroprotector, acting through either genomic or non-genomic mechanisms, and the clinical value of estradiol in ischemia and neurodegeneration has been much discussed.

The synthesis of estradiol derivatives arises as an interesting strategy to gain insights into the mechanisms of neuroprotection and to disclose new neuroprotective compounds.

Within our framework of new reactions and processes towards bioactive steroids [1,2,3] we have been exploring the selective modification of key positions of the estradiol scaffold to prepare new derivatives.



In this communication we report the synthetic strategies followed to access vicinal diols at the estradiol ring-D. Selective ring opening of epoxides has been explored to obtain beta-hydroxy ether derivatives. Finally, our results on the combined use of lipases in organic media to prepare the corresponding monoacylated derivatives starting from either *cis*- or *trans*-diols are discussed.

ACKNOWLEDGMENTS

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REFERENCES

1. Leitão AJL, Salvador JAR, Pinto RMA, Sá e Melo ML *Tetrahedron Lett.* **2008**, 49, 1694-1697
2. Carvalho, JFS, Silva MMC, Melo ML *Tetrahedron* **2009**, 65, 14, 2773-2781
3. Carvalho, JFS, Silva MMC, Melo ML *Tetrahedron* **2010**, 66, 13, 2455-2462

POSTER COMMUNICATIONS – PC.409

NOVEL SEMISYNTHETIC DERIVATES OF BETULIN AND BETULINIC ACID WITH CYTOTOXIC ACTIVITY

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Cancer is the second most important disease leading to death in both the developing and developed countries nowadays. Numerous experimental and epidemiological studies have shown that several plant derived natural products may serve as effective anticancer drugs, among which are plant triterpenes, for example betulin and betulinic acid.^{1,2}

Previous reports revealed that betulinic acid is a melanoma-specific cytotoxic agent,³ however recent evidence has indicated that betulinic acid possesses a broader spectrum of cytotoxic activity against other cancer types.⁴⁻⁷ This lupane triterpene is currently undergoing clinical trials at the National Cancer Institute (NCI).⁸ Nevertheless, the poor aqueous solubility of lupane triterpenes such as betulinic acid, which compromises blood serum solubilization, has limited the exploitation of their potential in both the medical and pharmaceutical areas.⁹ For this reason a huge amount of effort has been directed towards performing modifications of betulinic acid in order to increase and broaden its range of biological activities. In contrast to betulinic acid, betulin has no significant cytotoxic activity.^{10,11} However, some derivatives were found to be active.¹¹

In this communication,¹² we describe the synthesis of novel lupane imidazole carbamates and *N*-acylimidazole derivatives of betulin and betulinic acid, and their screening for *in vitro* cytotoxicity activity against the human cancer cell lines HepG2, Jurkat and HeLa.

REFERENCES

1. Salvador J. A. R., Ed., *Pentacyclic Triterpenes as Promising Agents in Cancer*, Nova Science Publishers, USA, New York, 2010, ISBN: 978-1-60876-973-5.
2. Kinghorn, A. D.; Su, B. N.; Jang, D. S.; Chang, L. C.; Lee, D.; Gu, J. Q.; Carcache-Blanco, E.J.; Powlus, A. D.; Lee, S. K.; Park, E. J.; Cuendet, M.; Gills, J. J.; Bhat, K.; Park, H.S.; Mata-Greenwood, E.; Song, L. L.; Jong, M. H.; Pezzuto, J. M. *Planta Med.* **2004**, *70*, 691.
3. Pisha, E.; Chai, H.; Lee, I. S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Dasgupta, T. K.; Pezzuto, J. M. *Nat. Med.* **1995**, *1*, 1046.
4. Schmidt, M. L.; Kuzmanoff, K. L.; Indeck, L. L.; Pezzuto, J. M. *Eur. J. Cancer* **1997**, *33*, 2007.
5. Fulda, S.; Jeremias, I.; Steiner, H. H.; Pietsch, T.; Debatin, K.-M. *Int. J. Cancer* **1999**, *82*, 435.
6. Thurnher, D.; Turhani, D.; Pelzmann, M.; Wannemacher, B.; Knerer, B.; Formanek, M.; Wacheck, V.; Selzer, E. *Head Neck* **2003**, *25*, 732.
7. Kessler, J. H.; Mullauer, F. B.; de Roo, G. M.; Medema, J. P. *Cancer Lett.* **2007**, *251*, 132.
8. Development therapeutics program national cancer institute/national institutes of health, B., MD, USA.
<http://dtp.nci.nih.gov/dtpstandards/cancerscreeningdata/index.jsp>.
9. Cichewicz, R. H.; Kouzi, S. A. *Med. Res. Rev.* **2004**, *24*, 90.
10. Kvasnica, M.; Sarek, J.; Klinotova, E.; Dzubak, P.; Hajdych, M. *Bioorg. Med. Chem.* **2005**, *13*, 3447.
11. Sarek, J.; Kvasnica, M.; Urban, M.; Klinot, J.; Hajdych, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4196.
12. Santos, C. S.; Salvador, J. A. R.; Marín, S.; Cascante, M. *Bioorg. Med. Chem.*, *17*, 6241.

POSTER COMMUNICATIONS – PC.411

DEVELOPMENT OF CYANOPYRROLIDINES AS INHIBITORS OF FIBROBLAST ACTIVATION PROTEIN

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Fibroblast activation protein (FAP, also known as seprase) is a serine protease, structurally related to dipeptidyl peptidase IV (DPP IV). It belongs to the DPP IV gene family along with other prolyl peptidases: DPP IV, DPP8 and DPP9. FAP expression has been described to be associated with normal or excessive wound healing, tissue remodeling sites *in vivo* and with malignant tumour growth and chronic inflammation, including human liver cirrhosis. [1] The restricted expression and distribution of the enzyme add to its potential as a target for drug development. To date, this potential has largely been studied in the context of oncology. [2]

Inhibitors with maximal FAP-selectivity with respect to other DPP IV gene family members have not been reported in literature. Nonetheless, such compounds could prove highly useful for further investigations of the involvement of FAP activity in the pathologies mentioned, or even as potential drugs. The most potent inhibitors of FAP described in literature are dipeptide derived diphenyl phosphonates (e.g. Gly-Pro^P(OPh)₂; K_i 59.4 nM and boronic acid inhibitors (e.g. PT100 /Val-boro-Pro; K_i 6.2 nM [1]). However, both are non-selective and with regards to the clinically investigated Val-boro-Pro, safety has been questioned.

Based on our expertise in inhibitor development for related DPP enzymes and previous results, we designed a library of acylated dipeptide based inhibitors with a cyanopyrrolidine P1 fragment.

Most of the tested inhibitors showed good to excellent selectivity for FAP with regards to related DPPs. A promising lead-candidate for further structural optimization of these compounds has been discovered and will be discussed.

REFERENCES

1. B. B. Wolf, C. Quan, Th. Tran, Ch. Wiesmann, D. Sutherland, On the edge of validation – cancer protease fibroblast activation protein. *Mini-Reviews in Medicinal Chemistry*, **2008**, 8, 719-727.
2. K. Narra, S. R. Mullins, H.-O. Lee, B. Strzemkowski-Brun, K. Magalong, V. J. Christiansen, P. A. McKee, B. Egleston, S. J. Cohen, L. M. Weiner, N. J. Meropol, J. D. Cheng, Phase II trial of single agent Val-boroPro (Talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. *Cancer Biology & Therapy*, **2007**, 6(11), 1691-1699.

POSTER COMMUNICATIONS – PC.412

DISCOVERY OF 5- AND 6-SUBSTITUTED QUINOLONES: A NEW CLASS OF METABOTROPIC GLUTAMATE 2 RECEPTOR POSITIVE ALLOSTERIC MODULATORS (mGluR2 PAM)

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mGluR2 is a G-protein coupled receptor (GPCR) expressed on presynaptic nerve terminals where it negatively modulates glutamate and GABA release. Mixed mGluR2/mGluR3 orthosteric agonists such as LY354740 have shown activity in a range of pre-clinical animal models of anxiety and schizophrenia. Early clinical work with LY354740 demonstrated activity in a CO₂ inhalation study suggesting the usefulness for the treatment of anxiety related disorders. Subsequently, a related prodrug LY2140023 demonstrated improvements in positive and negative symptoms in patients suffering from schizophrenia. These molecules exhibit combined mGluR2/mGluR3 activity although there is evi-

dence from knock-out studies that preclinical anti-psychotic effects may be mediated *via* the mGluR2 receptor. An alternative avenue for modulating GPCRs is to act *via* allosteric mechanisms, binding at a different site from the orthosteric agonist. Since the discovery of early mGluR2 positive allosteric modulators such as 2,2,2-TEMPS and BINA multiple families of mGluR2 modulators have been reported and several have entered into clinical development. We report in this communication on a novel series of 5- and 6-substituted quinolones (Figure 1) identified as mGluR2 PAM *via* the proprietary Addex high throughput screening (HTS) platform. The subsequent synthesis and initial SAR exploration will be described.

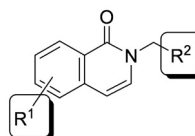


Figure 1

POSTER COMMUNICATIONS – PC.413

DEVELOPMENT OF 3-HYDROXYQUINOLEIN-2(1H)-ONES AS INHIBITORS OF HIV-1 INTEGRASE AND RIBONUCLEASE H FUNCTION OF REVERSE TRANSCRIPTASE

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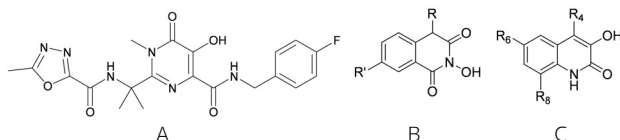
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Since it appeared in the eighties, AIDS has been responsible for the death of more than 25 million people and has become the most challenging pandemic of the 21st century. The current standard of treatment, Highly Active Antiretroviral Treatment (HAART), consists of a cocktail of drugs which includes reverse transcriptase (RT) inhibitors, protease inhibitors (PI), and/or a fusion inhibitor. This regimen can achieve a significant reduction of the viral load in HIV-1 infected patients but is noticeably limited by the emergence of multidrug resistant viral strains.

New strategies aimed at inhibiting virus replication are still necessary. In 2007, the FDA approved raltegravir **A** (IsentressTM, Merck & Co., Inc.), the first drug inhibiting the HIV-1 integrase (HIV-1 IN), led to strongly encouraging clinical results but resistant viral strains have already appeared after a 3 year use.¹ Important therapeutic alternatives may consist of novel HIV-1 IN inhibitors with different profiles from that of raltegravir or of multi-targeted drugs.

In our laboratory, we decided to investigate the last alternative by designing dual inhibitors of HIV-1 integrase and ribonuclease H function of reverse transcriptase.² Integrase, the enzyme responsible for integration, catalyses the insertion of viral DNA within the host cell genome whereas the ribonuclease H function of reverse transcriptase catalyses the hydrolysis of the RNA strand in the RNA/DNA duplex. These two key steps of the viral replication are promoted by structurally-related catalytic cores, which are able to chelate two magnesium cations.

We recently developed a series of 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones **B** able to complex magnesium cations with a 1/1 stoichiometry.³ The majority of the synthesized compounds consisted of average dual inhibitors of RNase H and IN (micromolar IC₅₀ values) with high cellular cytotoxicity. In the same field, we investigated a series of 3-hydroxyquinolin-2(1*H*)-ones **C** with metal complexing properties.⁴



Herein we present the synthesis and biological activities (integrase and RNase H inhibitory activities, viral replication inhibition and cytotoxicity on MT-4 cells) of some candidates of the series of 30 derivatives, together with their magnesium chelating properties.

REFERENCES

1. Ramkumar, K. *et al. Med. Res. Rev.* **2010**, 10.1002/med.20194
2. Andréola, M.L. *Curr Pharm Des.* **2004**, 10, 3713-3723
3. Billamboz, M. *et al. J. Med. Chem.* **2008**, 51, 7717-7730
4. Strashnova, S.B. *et al. Russ. J. Coord. Chem.* **2008**, 34, 775-779.

POSTER COMMUNICATIONS – **PC.414**

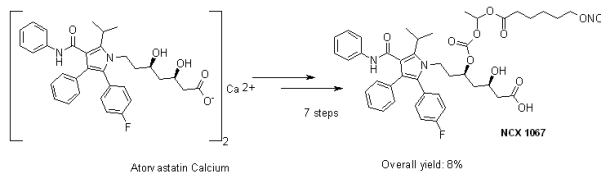
NCX 1067: A NOVEL NITRIC OXIDE (NO)-DONATING ATORVASTATIN ANALOG

Laura Storoni, Annalisa Bonfanti, Raffaella Maucci, Daniela Miglietta, Francesca Oliva

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Inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), commonly referred to statins, are widely used for the treatment of hypercholesterolemia. Evidence suggests that statins have some beneficial effects beyond their cholesterol lowering properties, which are believed to be derived from their propensity to enhance nitric oxide (NO) biosynthesis.¹ In view of this background, new pharmacological agents combining the properties of statins with those of nitric oxide were developed and characterized for their anti-proliferative, anti-inflammatory and anti-thrombotic properties which turned out to be superior to those of the respective parent compound.^{2,3}

NCX 1067 is the prototype of a new class of NO-statin bearing the NO-releasing moiety on 5-hydroxy position of atorvastatin. The compounds in this series possess the same free carboxylic acid moiety as the parent, atorvastatin, and could therefore potentially be formulated as calcium salts using the same formulation approach.



The strategy applied towards the synthesis of NCX 1067 takes advantage of the favourable lactonization process of atorvastatin and is used as the first step in order to differentiate the two secondary alcohols. The synthesis of NCX 1067 then proceeded via a suitable common intermediate, which was also used as the precursor for the synthesis of other analogs in this series.

The interesting *in vitro* profile of NCX 1067 has made this compound a suitable candidate for further investigations.

REFERENCES

1. Takemoto, M., Liao, J.K. "Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors." *Arterioscler.Thromb.Vasc.Biol.* 2001, 21, 1712-1719.
2. Ongini, E., Impagnatiello, F., Bonazzi, A., Guzzetta, M., Govoni, M., Monopoli, A., Del Soldato, P., and Ignarro, L.J. "Nitric oxide (NO)-releasing statin derivatives, a class of drugs showing enhanced anti-proliferative and anti-inflammatory properties ." *PNAS*, 2004, 101, 22, 8497-8502.
3. Momi, S., Impagnatiello, F., Guzzetta, M., Caracchini, R., Guglielmini, G., Olivieri, R., Monopoli, A., Gresele, P. "NCX 6560, a nitric oxide-releasing derivative of atorvastatin, inhibits cholesterol biosynthesis and shows anti-inflammatory and anti-thrombotic properties." *Eur. J. Pharmacol.* 2007, 570,115-124.

POSTER COMMUNICATIONS – **PC.415**

NEW BIFUNCIONAL COMPOUNDS WITH POTENTIAL NEUROPROTECTIVE ROLES, AS ANTIOXIDANTS AND ACETYLCHOLINESTERASE INHIBITORS

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by the progressive and irreversible loss of memory followed by complete dementia. Since the main hall-

mark of the disease is the deposition of extracellular amyloid plaques, the dysfunctional beta-amyloid ($A\beta$) has been the pivotal pharmacological target for AD. However, the only approved therapy is for improving the memory impairment and cognitive deficit by acetylcholinesterase (AChE) inhibitors, but the benefits of its conjugation antioxidants have been also proposed to mitigate the associated neurotoxic oxidative stress.[1]

As part of an ongoing project,[2] we have designed and developed a series of polyfunctional compounds, which have a benzothiazole (BTA) unit as $A\beta$ targeting sensor. That unit was linked to an adequate spacer, and then coupled with a caffeic acid derivative as an antioxidant to scavenge free radicals and their oxidative roles. The BTA-spacer was modeled, by aid of computational tools, in order to optimize the binding interactions with the active site of enzyme, and thus lead to effective AChE inhibition.

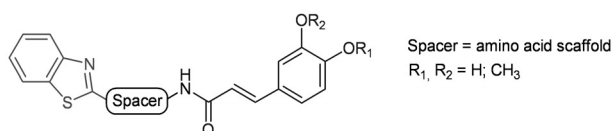


Figure 1

The target compounds (Figure 1) were prepared and characterized by standard techniques. Then they were assessed for their lipo-hydrophilic and antioxidant properties, and also bioassayed to study their inhibitory activity against AChE. The most promising compounds shall further be tested *in vitro* with neuronal cells, in order to evaluate their neuroprotecting/rescuing roles.

REFERENCES

1. V.Tumiatto, M.L. Bolognesi, A. Minarini, M. Rosini, A. Milleli, R. Matera, C. Melchiorre, *Expert Opin. Ther. Patents* **2008**, 18, 387-401.
2. J. Šebestík, S.M. Marques, P.L. Falé, S. Santos, D.M. Arduíno, S.M. Cardoso, C.R. Oliveira, M.L.M. Serralheiro, M.A. Santos, *under publication*.

POSTER COMMUNICATIONS – **PC.417**

THE IONIC LOCK ACTIVATION MECHANISM OF THE HUMAN β_1 -ADRENOCEPTOR

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The human β_1 -adrenoceptor (β_1 -AR) is a seven helices transmembrane (7TM) protein (Figure 1A), member of the large G-protein-coupled receptors (GPCRs) superfamily, which has an essential role in the signal transduction pathway [1,2]. The binding of an agonist (activating molecule) to its extracellular domain leads to a

conformational change in the inner portion of the receptor, triggering the activation of G proteins and raising the intracellular levels of cAMP and Ca^{2+} [1,2]. Despite its importance, the activation mechanism of this receptor still unknown. Many experiments have identified well conserved regions in the GPCRs superfamily and observed that its disruption could lead to a change in the equilibrium between their inactive-active states. The most studied and widely accepted activation mechanism is the disruption of the salt bridge interaction between a TM3 arginine and a TM6 glutamic acid, called ionic lock (Figure 1B) [3]. Therefore, using the state of the art molecular modeling tools, we have provided in this work a functional human β_1 -AR model equilibrated in a membrane environment showing how the ionic lock mechanism works. The results have shown that differently from the antagonist (a molecule that blocks the receptor activation), an agonist disturbs the β_1 -AR causing a rotation of the transmembrane helices. This rotary motion occurs in opposite directions in the inner and outer portion of the β_1 -AR, opening the ionic lock.

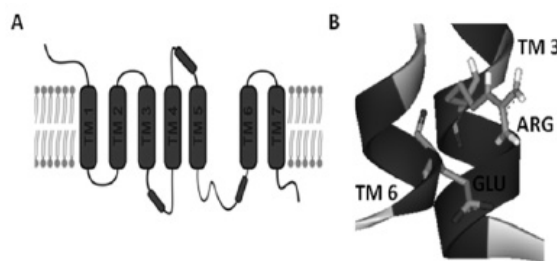


Figure1. A) Schematic representation of the human β_1 -AR showing the 7TM motif. B) Structural representation of the ionic lock.

REFERENCES

1. Baker, J., Proudman, R., Hawley, N., Fischer, P. & Hill, S. (2008). *Molecular Pharmacology*, 1246-1260.
2. Warne, T., Serrano-Vega, M., Baker, J., Moukhametzianov, R., Edwards, P., Henderson, R., Leslie, A., Tate, C. & Schertler, G. (2008). *Nature*, 486-U2.
3. Vanni, S., Neri, M., Tavernelli, I. & Rothlisberger, U. (2009). *Biochemistry*, 4789-4797.

POSTER COMMUNICATIONS – **PC.418**

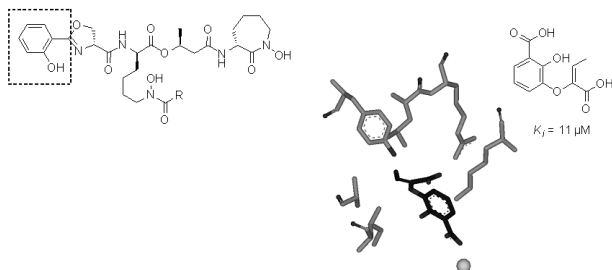
SYNTHESIS AND EVALUATION OF SALICYLATE SYNTHASE INHIBITORS AS TUBERCULOSIS DRUG LEADS

Alexandra Manos-Turvey and Richard J. Payne

School of Chemistry, The University of Sydney, NSW, Australia 2006

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, remains a major cause of morbidity and mortality worldwide with 9.4 million new cases diagnosed and 1.8 million deaths reported in 2008.^[1] The recent elucidation of the *M. tuberculosis* genome has revealed several new TB drug targets.

One promising target is the inhibition of enzymes responsible for the biosynthesis of the iron-chelating siderophore mycobactin T.^[2] Salicylate represents an essential building block of the mycobactin T core and was recently shown to be biosynthesised by a salicylate synthase in *M. tuberculosis*.^[3] Given the essential nature of mycobactin T for the survival of *M. tuberculosis*, salicylate synthase represents an attractive TB drug target.



Mycobactin T (salicylate core in blue)

This research describes the design and synthesis of the first inhibitors of *M. tuberculosis* salicylate synthase.^[4] First generation compounds designed to probe the active site of the enzyme resulted in poor inhibitors. Subsequent *in silico* docking revealed a number of unfavourable interactions in the active site of the enzyme with these compounds. A significant increase in inhibitor potency was achieved in second generation inhibitors based on 3,4- and 2,3-dihydroxybenzoate cores. The 2,3-dihydroxybenzoate compounds, mimics of the enzyme bound intermediate, proved to be the most potent inhibitors of this enzyme, with inhibition constants in the low micromolar range. These compounds are currently being assessed for their ability to inhibit the growth of *M. tuberculosis* *in vitro*.

REFERENCES

1. WHO, WHO report 2009 update, **2009** WHO/HTM/TB/2009.426
2. L. E. N. Quadri, *Infect. Disord.: Drug Targets* **2007**, 7, 230-237.
3. A. J. Harrison et al., *J. Bacteriol.* **2006**, 188, 6081-6091.
4. A. Manos-Turvey, E. M. M. Bulloch, P. J. Rutledge, E. N. Baker, J. S. Lott, R. J. Payne, *ChemMedChem* **2010**, DOI: 10.1002/cmdc.201000137

POSTER COMMUNICATIONS – PC.419

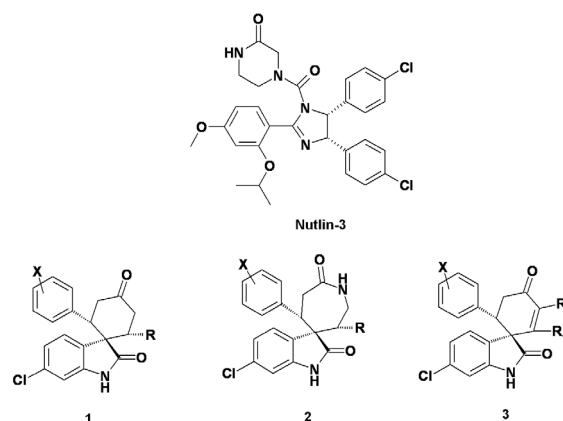
DESIGN, SYNTHESIS AND SAR OF NOVEL SPIROINDOLINONES AS P53/MDM2 INHIBITORS

Jin-Jin Liu, Bradford Graves, Zhuming Zhang, Tina Ross, Jing Zhang, Ted Lambros, Sung-Sau So, Christine Lukacs, Ursula Kammlott, Yang Wen and Lyubomir Vassilev

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Tumor suppressor protein p53 regulates cellular response to stress and plays a central role in protection against the develop-

ment of cancer. It guards cellular integrity and prevents the propagation of permanently damaged cells by the promotion of growth arrest or apoptosis. As a potent cell cycle inhibitor, p53 is tightly regulated at the functional level, through the binding of p53 to MDM2. Inhibition of the p53-MDM2 interaction in tumor cells with wild-type p53 should lead to accumulation of p53, cell cycle arrest and/or apoptosis. MDM2 antagonists, therefore, can offer a novel approach to cancer therapy as single agents or in combination with a broad spectrum of other antitumor therapies. **Nutlin-3**, a representative of an imidazolines series of such antagonists, has been shown to inhibit the MDM2/p53 interactions in the low nanomolar range and shows anti-proliferative activity in cells with functional p53¹. In the search to find non-imidazoline derived MDM2 inhibitors, several novel templates have been identified. This presentation will outline the design, synthesis and SAR of a number of novel spiroindolinones (series 1, 2 and 3) as p53/MDM2 inhibitors.



REFERENCES

1. Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **2004**, 303, 844-848.

POSTER COMMUNICATIONS – PC.420

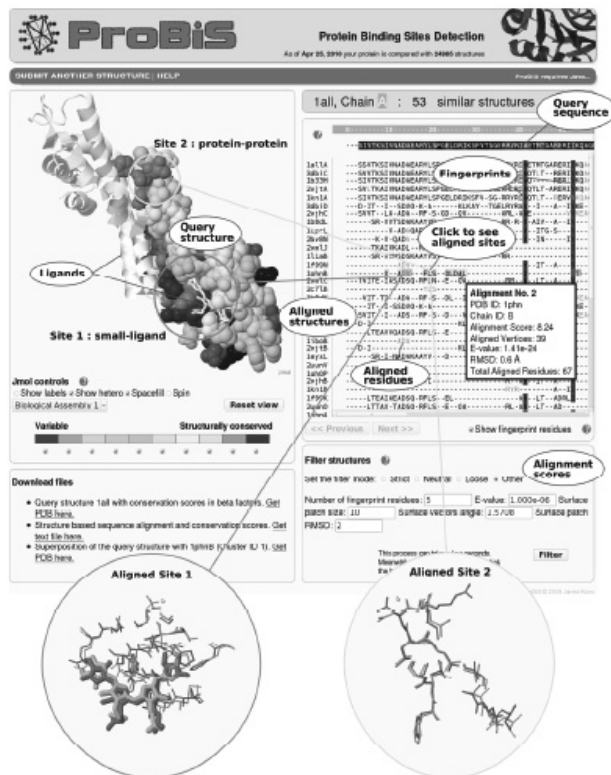
PROBIS: A WEB SERVER FOR DETECTION OF STRUCTURALLY SIMILAR PROTEIN BINDING SITES

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A web server, ProBiS, freely available at <http://probis.cmm.ki.si>, is presented. This provides access to the program ProBiS (Protein Binding Sites), which detects protein binding sites based on local structural alignments. Given a structure of a protein with unknown binding sites, ProBiS suggests the regions on its surface which may be involved in binding with small ligands, proteins, or DNA/RNA. Alternatively, given a protein with an identified binding site, ProBiS finds other proteins with struc-

turally or physicochemically similar binding sites. If used as a pairwise structure alignment program, ProBiS detects and superimposes similar functional sites in a pair of submitted protein structures, even when these do not have similar folds.



REFERENCES

1. Konc, J. and Janežič, D. ProBiS algorithm for detection of structurally similar protein binding sites by local structural alignment. *Bioinformatics* 2010, 26, 1160-1168.
2. Konc, J. and Janežič, D. ProBiS: A web server for detection of structurally similar protein binding sites. *Nucleic Acids Res.* May 25 2010, ASAP.

POSTER COMMUNICATIONS – PC.422

TARGETING AN RNA POLYMERASE-TRANSCRIPTION FACTOR INTERACTION FOR POTENTIAL NOVEL ANTIMICROBIALS

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Transcription is carried out by the highly conserved RNA polymerase (RNAP) and is essential to all forms of life. Bacterial core RNAP consists of 5 subunits, α - β β' ω . The initiation of transcrip-

tion occurs with the binding of a sixth subunit, α , providing the holoenzyme (HE: core RNAP + σ) with the ability to bind to specific promoter regions on DNA. σ -factors are unique to prokaryotes, making their interaction with the core a perfect target for the development of antimicrobial drugs with a novel mechanism of action. A homology model of *Bacillus subtilis* HE was created (based on a *Thermus thermophilus* HE crystal structure [1]) and visualisation of the RNAP- σ^A interface revealed the amino acids responsible for binding. Interestingly, these were predicted to be slightly different to those in *T. thermophilus*, which is important because *B. subtilis* is closely related to pathogens such as *Staphylococcus aureus*. The model aided the design of mutations that may alter the binding capacity of σ^A (an essential 'house-keeping' σ -factor in *B. subtilis*) for RNAP. These mutant σ^A proteins were purified for use in *in vitro* binding studies. Far-Western blots using fragments of β' containing the σ^A binding site, demonstrated the importance of each proposed interacting amino acid. Our results show that E166 is absolutely essential for binding of σ^A to β' , Q165 is very important, and M169 is of some importance. Conversely, mutations of D162 and M172 exhibited no detrimental effects, probably due to their placement towards the ends of the interface. Structure-based pharmacophores were constructed to represent the important binding interactions at the β'/σ interface. *In silico* database searches with these pharmacophores revealed a number of small molecule hits which we propose will be able to inhibit transcription in *B. subtilis*.

REFERENCES

1. Vassylyev et al., (2002) *Nature* **417**: 712-719.

POSTER COMMUNICATIONS – PC.423

LOOKING BEYOND SUBTYPE SELECTIVITY: ALPHA ONE ADRENOCEPTORS AS NEW TARGETS FOR PROSTATE CANCER

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Prostate cancer is one of the most common cancers, accounting for a third of all new cases in males in Australia. Whilst there is a 97% 5-year survival for cancer localised to the prostate, once the tumour is disseminated it is universally lethal, with 5-year survival rates of 16% for distant metastases. Initially, metastatic deposits are usually androgen-dependent, and androgen deprivation therapy will typically halt disease progression for 1.5 to 3 years. However, the tumour inevitably escapes the requirement for male hormone growth factors and progression resumes, leading to death within about 18 months. Unfortunately, standard forms of cytotoxic chemotherapy are ineffective, possibly due to the low proliferative rate of prostate cancer cells (around 2%). Recent studies with the taxane docetaxel, a microtubule poison, indicate that it will prolong life of men with hormone refractory prostate cancer (HRPC) for about 3 months. The side

effects associated with androgen deprivation and taxane treatment can be severe, leading to considerable patient distress. Clearly, on several fronts, there is an urgent need for new approaches to prostate cancer chemotherapy.

Recent research has suggested that noradrenaline and serotonin (5-HT) are growth factors for prostate cancer cell lines, and that blockers of these agents are growth inhibitory in both androgen-sensitive and -insensitive prostate cancer cells. The receptor subtypes involved in stimulating or inhibiting prostate cancer cell proliferation remain to be elucidated. Currently available α_1 adrenergic receptor antagonists are either non-subtype selective, or demonstrate only modest selectivity amongst some of the subtypes and/or display considerable affinity for 5-HT₁ receptors. We are currently finalising the validation of specific α_1 adrenergic and 5-HT₁ receptors as drug targets for prostate cancer therapy. Our second major aim is to design and synthesize novel, specific blockers for these receptors, and we will report on our considerable progress in this area.

This may open the door to a novel non-toxic approach to the treatment of early metastatic prostate cancer.

POSTER COMMUNICATIONS – PC.424

IDENTIFICATION OF NOVEL MACROCYCLES AS POTENT INHIBITORS OF THE HEDGEHOG SIGNALING PATHWAY

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The hedgehog signaling pathway plays a crucial role in embryonic development, cell differentiation, growth, and proliferation. Abnormal activation of the pathway has been implicated in the formation and proliferation of many cancers, therefore inhibition of this pathway could lead to the development of effective cancer therapies. Blocking the interaction between the extracellular protein Sonic hedgehog (Shh) and the transmembrane receptor Patched was targeted by a screening program utilizing small-molecule microarrays of diversity-oriented synthesis libraries. The identification of a macrocycle that binds to Shh and results of medicinal chemistry efforts toward analogs with improved potency and physical properties are presented here.

POSTER COMMUNICATIONS – PC.425

DISCOVERY OF 1,5-DISUBSTITUTED PYRIDONES: A NEW CLASS OF METABOTROPIC GLUTAMATE 2 RECEPTOR POSITIVE ALLOSTERIC MODULATORS (mGluR2 PAM)

Jose María Cid,¹ Guillaume Duvey,² Gregor J. Macdonald,³ Philippe Cluzeau,² Vanthea Nhem,² Karim Macary,² Juan Antonio Vega,¹ Ana Isabel de Lucas,¹ Aránzazu García,¹ Encarnación Matesanz,¹ Hilde Lavreysen,⁴ Alexandre Raux,² Jessica Muller,² Nicolas Poirier,² María Lourdes Linares,¹ Daniel Oehrich,¹ Julen Oyarzábal,⁵ Gary Tresadern,⁵ Andrés A. Trabanco,¹ Robert Lutjens,² Emmanuel Le Poul,² Terry Finn,² Christelle Boléa,² Hassan Imogai,² Jean-Philippe Rocher²

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mGluR2 is a G-protein coupled receptor (GPCR) expressed on presynaptic nerve terminals where it negatively modulates glutamate and GABA release. Mixed mGluR2/mGluR3 orthosteric agonists such as LY354740 have shown activity in a range of pre-clinical animal models of anxiety and schizophrenia. Early clinical work with LY354740 demonstrated activity in a CO₂ inhalation study suggesting the usefulness for the treatment of anxiety related disorders. Subsequently, a related prodrug LY2140023 demonstrated improvements in positive and negative symptoms in patients suffering from schizophrenia. These molecules exhibit combined mGluR2/mGluR3 activity although there is evidence from knock-out studies that preclinical anti-psychotic effects may be mediated via the mGluR2 receptor. An alternative avenue for modulating GPCRs is to act via allosteric mechanisms, whereby small molecules bind selectively but at a different site from the orthosteric agonist. Since the discovery of early mGluR2 positive allosteric modulators (PAM) such as 2,2,2-TEMPs and BINA, multiple families of mGluR2 modulators have been reported and several have entered into clinical development. We report in this communication a novel series of 1,5-disubstituted pyridones (Figure 1) identified as mGluR2 PAM via the proprietary Addex high throughput screening (HTS) platform. The subsequent synthesis and initial SAR exploration will be described.

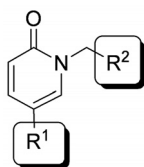


Figure 1

POSTER COMMUNICATIONS – PC.426

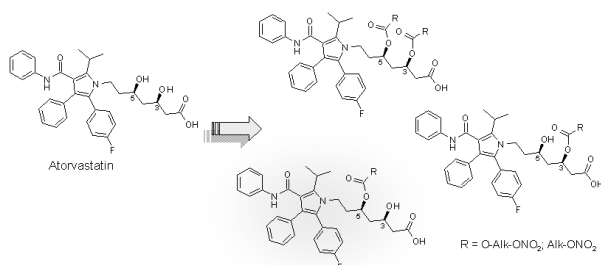
NOVEL NITRIC OXIDE (NO)-DONATING 5-HYDROXY CARBONATE ANALOGS OF ATORVASTATIN

Annalisa Bonfanti, Laura Storoni, Raffaella Maucchi, Daniela Miglietta, Francesca Oliva

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Inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), commonly referred to as statins, are widely used for the treatment of hypercholesterolemia. Evidence suggests that statins have some beneficial effects beyond their cholesterol lowering properties, which are believed to be derived from their propensity to enhance nitric oxide (NO) biosynthesis.¹ In view of this background, new pharmacological agents combining the properties of statins with those of nitric oxide were developed and characterized for their anti-proliferative, anti-inflammatory and anti-thrombotic properties which turned out to be superior to those of the respective parent compound.^{2,3}

Three new classes of NO-donating atorvastatin analogs, where the carboxylic acid moiety of atorvastatin remained free, were synthesized. This free carboxylic moiety was required to formulate these new chemical entities as their calcium salts, as is usual for atorvastatin itself. The 5-hydroxy and 3-hydroxy groups of atorvastatin were functionalized either as esters or carbonates. The mono-functionalized analogs gave better results in pre-clinical studies with respect to the bis-functionalized analogs. On the basis of these results, the 5-hydroxy carbonate series was identified as the best of these new classes, thus meriting further investigation.



Synthetic procedures used for the preparation of these novel NO-donating atorvastatin analogs, as well as preliminary *in vitro* tests will be presented and discussed.

REFERENCES

1. Takemoto, M., Liao, J.K. "Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors." *Arterioscler.Thromb.Vasc.Biol.* 2001, 21, 1712-1719.
2. Ongini, E., Impagnatiello, F., Bonazzi, A., Guzzetta, M., Govoni, M., Monopoli, A., Del Soldato, P., and Ignarro, L.J. "Nitric oxide (NO)-releasing statin derivatives, a class of drugs showing enhanced anti-proliferative and anti-inflammatory properties." *PNAS*, 2004, 101, 22, 8497-8502.
3. Momi, S., Impagnatiello, F., Guzzetta, M., Caracchini, R., Guglielmini, G., Olivieri, R., Monopoli, A., Gresele, P. "NCX 6560, a nitric oxide-releasing derivative of atorvastatin, inhibits cholesterol biosynthesis and shows anti-inflammatory and anti-thrombotic properties." *Eur. J. Pharmacol.* 2007, 570, 115-124.

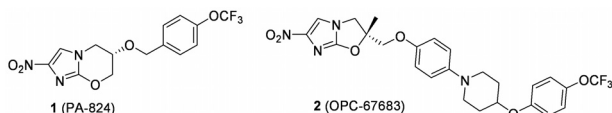
POSTER COMMUNICATIONS – PC.427

HETEROCYCLIC ANALOGUES OF THE NOVEL ANTI-TUBERCULAR DRUG PA-824

Iveta Kmentova,¹ Hamish S. Sutherland,¹ Andrew M. Thompson,¹ Adrian Blaser,¹ Scott. G. Franzblau,² Baojie Wan,² Yuehong Wang,² Zhenkun Ma,³ William A Denny,¹ Brian D. Palmer¹

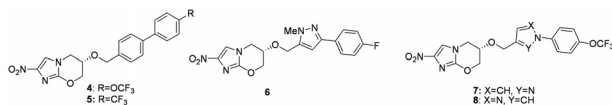
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The (6S)-2-nitroimidazo[2,1-b][1,3]oxazines, exemplified by PA-824 (**1**) and the related 5-nitro-2,3-dihydro[2,1-b]imidazo[4,5-d]oxazoles, exemplified by OPC-67683 (**2**), show potent activity against both replicating and non-replicating cultures of *Mycobacterium tuberculosis* (*M. tb*), the causative agent of tuberculosis. These compounds also show excellent activity against both active and persistent *M. tb* in animal models. Their activity against persistent *M. tb* appears due to an unusual route of reductive metabolism of the nitroimidazole ring, leading to the release of nitric oxide as a toxic and/or signalling species.¹ Both compounds are currently in Phase II clinical trials for the treatment of drug-susceptible and drug-resistant tuberculosis, with the aim of reducing the duration and frequency of drug treatment.



In a program designed to develop back-up clinical candidates for PA-824, we prepared biphenyl analogues of **1**, and showed that within this class there were positive correlations between both overall drug lipophilicity and the electron-withdrawing capability of substituents on the distal ring, with analogues such as **4** and **5** showing superior activity to **1** in both *in vitro* and mouse models.² However, attempts to improve the poor aqueous solu-

bility of the biphenyl series with appended hydrophilic groups led to a loss of potency. In an alternative strategy, we more recently explored the replacement of the proximal phenyl ring in the side chain with a variety of more hydrophilic five-membered ring heterocycles and noted some examples (e.g. **6-8**) which had improved solubility, while retaining *in vitro* and *in vivo* biological activities superior to **4**.³



At this meeting we will present a systematic study of related pyridyl, pyrimidinyl and pyrazinyl nitroimidazooxazines, as more water-soluble derivatives of the biphenyl analogues of **1**. This work has identified several highly *in vivo*-active compounds with excellent PK profiles.

REFERENCES

1. Singh, R.; Manjunatha, U.; Boshoff, H. I. M.; Ha, Y. H.; Niyomrattanakit, P.; Ledwidge, R.; Dowd, C. S.; Lee, I. Y.; Kim, P.; Zhang, L.; Kang, S.; Keller, T. H.; Jiricek, J.; Barry, C. E. PA-824 kills non-replicating *Mycobacterium tuberculosis* by intracellular NO release. *Science* **2008**, 322, 1392-1395.
2. Palmer, B. D.; Thompson, A. M.; Sutherland, H. S.; Blaser, A.; Kmentova, I.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Denny, W. A. Synthesis and structure-activity studies of biphenyl analogues of the nitroimidazooxazine tuberculosis drug PA-824. *J. Med. Chem.*, **2010**, 53, 282-294.
3. Sutherland, H. S.; Blaser, A.; Kmentova, I.; Franzblau, S. G.; Wan, B.; Ma, Z.; Denny, W. A.; Palmer, B. D.; Thompson, A. M. Synthesis and structure-activity relationships of anti-tubercular 2-nitroimidazooxazines bearing heterocyclic side chains. *J. Med. Chem.*, **2010**, 53, 855-866.

POSTER COMMUNICATIONS – PC.428

SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF 2-ANILINONICOTINIC ACID HYDRAZIDES

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of fever, pain and inflammation, particularly arthritis. These compounds suppress biosynthesis of prostaglandins from arachidonic acid (AA) by inhibiting of cyclooxygenase isoforms (COX-1 and COX-2).

Regioisomeric pyridines have been explored previously as replacement of the central core and were found to be useful as an analgesic as well as anti-inflammatory agents and for treating Alzheimer's disease. Synthetic approaches based upon chemical

modification of NSAIDs have been taken with the aim of improving safety profile and in turn therapeutic window of these NSAIDs. Several studies have described the derivatization of carboxylic function with amide or N-acylarylhydrazone having less acidic amide hydrogen as well as the capacity of stabilizing free radicals. Some evidences suggest that the hydrazone moiety possesses a pharmacophoric character for the inhibition of cyclooxygenase.

Therefore, a novel diverse group of N-acylarylhydrazone derivatives of 2-anilinonicotinic acid with different substituents on the terminal phenyl (or heteroaryl) ring were designed and synthesized for their anti-inflammatory activities. They showed moderate to good anti-inflammatory activities in comparison with niflumic acid as the reference drug.

POSTER COMMUNICATIONS – PC.429

IDENTIFICATION OF A NOVEL TLR7 AGONIST ANTEDRUG

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We have previously disclosed a series of 8-oxoadenine compounds containing metabolically labile phenylacetate esters as TLR7 agonists¹. Further exploration of this antedrug series led to the identification of compounds with improved physical chemistry properties whilst maintaining the excellent biological and selectivity profile we sought for potential candidate drugs.

This poster will describe our activities in this field and show the synthesis, in-vitro and in-vivo data for a representative example in the series

REFERENCES

1. A. Kurimoto, K. Hashimoto, T. Nakamura, K. Norimura, H. Ogita, H. Takaku, R. Bonnert, T. McInally, H. Wada, and Y. Isobe *J. Med. Chem.* **2010**, 53, 2964-2972

POSTER COMMUNICATIONS – PC.430

NOVEL ANTITUMOR AMINO-PANCRATISTATIN DERIVATIVES INHIBITORS OF PROTEIN AND DNA SYNTHESIS

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The Amaryllidaceae alkaloids pancratistatin and narciclasine are potent cytotoxic agents but exert marginal antitumor properties *in vivo*, associated with unmanageable toxicities. Despite extensive investigations, research on pancratistatin or analogues did not succeed to provide sufficient therapeutic benefit [1]. A pro-drug strategy applied recently to narciclasine has been reported, with only a limited success [2]. Our long-term interest in anti-cancer plant natural products prompted us to devise an alternative chemistry program aimed at improving the therapeutic activity of this interesting pharmacophore. A complete literature survey suggests that any structural modification of this highly oxygenated isocarbostryl pattern leads to a loss of activity except on position 1 which may be of potential interest according to published structure-activity relationships [3]. We therefore initiated a drug design program based on these assumption.

We report here the synthesis of a series of novel 1-aminopancratistatin derivatives by chemical modification of the naturally available narciclasine extracted from *Narcissus* bulbs. These new compounds exhibit a good pharmaceutical profile facing standard solubility, plasmatic and microsomal stability assays. A sub-set of derivatives demonstrated a drastic enhancement of cytotoxicity as judged from a nM-range potency for molecules tested against a panel of 8 different cancer cell lines. The higher antiproliferative activity translates into the induction of stronger pro-apoptotic signals.

Besides the previously described inhibition of protein synthesis [1], our studies originally demonstrated that part of the mechanism of action involves inhibition of DNA synthesis as well. The contribution of the two pharmacological properties elaborates a structure-activity relationship and segregates our new pancratistatin derivatives into different categories depending on their ability to inhibit protein synthesis, DNA synthesis or both. Based on their differential *in vitro* activity profile, the compounds were selected for evaluation of their *in vivo* antitumor properties on murine and xenografted tumor models. As a major result compound F98604, a dimethylaminomethyl benzamide derivative of pancratistatin, exhibited significant activity on the murine B16 and human A375 melanoma models in terms of tumor growth inhibition as well as increased therapeutic index. The present

medicinal chemistry study suggests that modification of the pancratistatin or narciclasine pharmacophore opens new perspectives for the discovery of new anticancer agents endowed with original pharmacological profiles.

REFERENCES

1. A. Kornienko, A. Evidente *Chem. Rev.* **2008**, 108, 1982
2. L. Ingrassia *et al. J. Med. Chem.* **2009**, 52, 1100
3. G. R. Pettit *et al. J. Org. Chem.* **2001**, 66, 2583

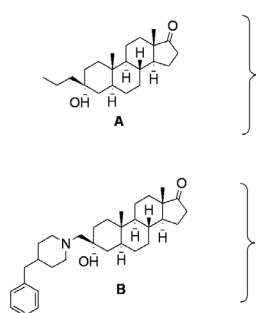
POSTER COMMUNICATIONS – PC.431

NON STEROIDAL MIMICS OF 3 β -SUBSTITUTED-ANDROSTERONE INHIBITORS OF 17 β -HSD TYPE 3

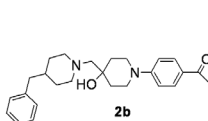
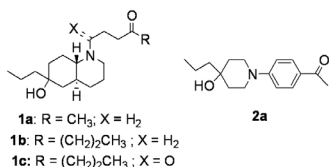
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17 β -Hydroxysteroid dehydrogenase type 3 (17 β -HSD-3) is a steroidogenic enzyme that mainly catalyzes the reduction of 4-androstene-3,17-dione (4-dione) to testosterone (T) in testis. The inhibition of 17 β -HSD-3 is thus a promising approach to block the testicular source of active androgens stimulating the proliferation of prostate cancer cells (endocrine mode). Since 17 β -HSD-3 has also been reported to be expressed in prostate cancer cells,¹ the inhibition of 17 β -HSD-3 could be used to reduce the production of T directly synthesized in the prostate (intracrine mode) from inactive adrenal precursors (4-dione and DHEA).² Our group has developed steroidal inhibitors of 17 β -HSD-3 (see **A**³ and **B**⁴) and we are now interested in developing non steroidal mimics of these inhibitors (see **1** and **2**) to potentially increase their *in vivo* bioavailability as well as to counter their undesirable androgenic effect. Two different mimic scaffolds were designed. The non steroidal compounds **1a-c** were synthesized in 7 to 8 steps from 7-methoxy-1,2,3,4-tetrahydroisoquinoline whereas **2a** and **2b** were synthesized in 4 steps from 4-piperidone. The compounds were evaluated as inhibitors of the transformation of 4-dione to T catalyzed by 17 β -HSD-3. Very weak inhibitions (<10% at 10 μ M) were obtained for compounds **1a**, **1b**, **1c** and **2a**, but compound **2b** shows a significant inhibition of 40% at 10 μ M. The later represents a promising template for future optimization.

Steroid inhibitors of 17 β -HSD-3

Non steroidal mimics



REFERENCES

1. Koh, E.; Noda, T.; Kanya, J.; Namiki, M. *The Prostate*, 2002, 53, 154-159.
2. Luu-The, V.; Zhang, Y.; Poirier, D.; Labrie, F. *J. Steroid Biochem. Molec. Biol.*, 1995, 55, 582-587.
3. Tchédam Ngatcha, B.; Luu-The, V.; Labrie, F.; Poirier, D. *J. Med. Chem.*, 2005, 48, 5257-5268.
4. (a) Maltais, R.; Luu-The, V.; Poirier, D. *J. Med. Chem.*, 2002, 45, 640-653. (b) Maltais, R.; Luu-The, V.; Poirier, D. *Bioorg. Med. Chem.*, 2001, 9, 3101-3111.

POSTER COMMUNICATIONS – PC.432

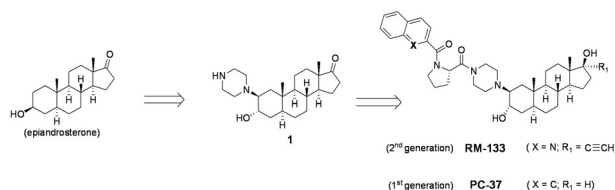
A NEW AMINOSTEROID (RM-133) AS SELECTIVE ANTI-CANCER AGENT: CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITIES

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RM-133 is a second generation of N-derivatives of 2 β -piperazino-5 α -androstane-3 α ,17 β -diol that emerged from a previous structure-activity relationship study performed by our group.¹ The compound was efficiently synthesized in 6 steps from commercially available epiandrosterone. We first dehydrated the epiandrosterone to an alkene with (diethylamino)sulfur trifluoride (DAST), a side reaction for this known fluorinating reagent.² The relatively low yield obtained for this reaction (~30%) was compensated by the high ratio of 2,3- vs 3,4-alkenes (>92% of 2,3-alkene). This later was stereoselectively converted to 2,3 α -epoxide in good yield by using mCPBA in dichloromethane and subsequently opened using an excess of piperazine in water at a high temperature³ to give only the 2 β -piperazino-androsterone (**1**). The NH of piperazine moiety was acylated with the HCTU activated ester of 1-(quinolin-2-ylcarbonyl)-L-proline to give the amide intermediate. The 17 α -ethinyl chain was then introduced using lithium trimethylsilyl ethynyl followed by hydrolysis with potassium carbonate in methanol to give, after purification by chromatography, the RM-133 in high purity (95%) and accept-

able overall yield (12% for 6 steps). RM-133 was evaluated on different cancer cell lines and demonstrated high cytotoxic activities (IC₅₀) notably on HL-60 (0.1-2.0 μ M), MCF-7 (0.1 μ M), T-47D (0.1 μ M) and LNCaP (3.0 μ M). More importantly, when we tested its cytotoxic activity on normal lymphocyte cells and HL-60 cancer cells, we found an interesting selectivity factor > 50. In this particular test, Doxorubicin used as reference showed no selectivity (IC₅₀ = 0.1 μ M both on HL-60 cells and normal lymphocytes). Furthermore, the addition of an ethinyl group and a quinoline group was beneficial for the antiproliferative activity (2 times) and for the rat bioavailability (s.c. injection) (4 times) when compared to the first generation parent compound PC-37 (without these 2 modifications).



Scheme 1. Synthetic route towards RM-133.

REFERENCES

1. Roy, J.; Maltais, R.; Jegham, H.; Poirier, D. *Molecular Diversity*, 2010 (in press)
2. Hudlicky, M. *Organic Reactions*, 1988, 35, 513-641.
3. Roy, J.; DeRoy, P.; Poirier, D. *J. Comb. Chem.*, 2007, 9, 347-358.

POSTER COMMUNICATIONS – PC.433

DISCOVERY OF 4-AMINOPYRAZOLOPYRIDINES AS POTENT, SELECTIVE INHIBITORS OF THE KINASE CMET

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A series of potent, selective inhibitors of the kinase cMet has been discovered and characterized *in vitro* and *in vivo*. Inhibitors in this series showed potent inhibition of cMet mediated phosphorylation of a peptide substrate (PGT) in an ELISA enzyme assay and cMet autophosphorylation in an MKN45 cell assay. A subset of inhibitors showed a decrease in autophosphorylation of cMet *in vivo* in a murine EBC-1 xenograft model. After obtaining crystal structures of our inhibitors bound to the cMet protein, it was observed that in the 4-aminopyrazolopyridine series, the pyrazolopyridine binds to the hinge in a flipped orientation com-

pared to the 4-oxypyrazolopyridines. The series of 4-aminopyrazolopyridine cMet inhibitors showed an excellent selectivity profile against a panel of kinases when compared to a similar series of 4-oxypyrazolopyridines. Groups attached to the amine of the 4-aminopyrazolopyridines modulated both potency and ADME properties. Attachment of the groups to the amine, rather than the pyrazolopyridine, allowed the groups to be positioned closer to the activation loop of the kinase, which is known to have some residues that differ between cMet and KDR. hERG binding was a significant obstacle for this series of potent cMet inhibitors, and efforts were made to mitigate this potential risk.

POSTER COMMUNICATIONS – PC.434

DESIGN AND SYNTHESIS OF CINNAMIC ACID AMIDES AS CALPAIN INHIBITORS

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The involvement of μ -calpain in neurological disorders, such as stroke [1] and Alzheimer's disease [2] has attracted considerable interest in the use of calpain inhibitors as therapeutic agents. In the present study, a series of cinnamoyl acid amides were designed and synthesized as acyclic analogs of calpain inhibitory chromone carboxamides [3]. Cinnamoyl acid amides possessing an alkyl chain at the α -position showed approximately 10-fold more potent μ -calpain inhibitory activities than an unsubstituted derivative ($IC_{50} = 6.07 \mu M$). Among synthesized, the *n*-butyl-substituted cinnamoyl acid amide showed the most potent μ -calpain inhibitory activity ($IC_{50} = 0.13 \mu M$) comparable to those of the chromone carboxamides, indicating that the cinnamoyl skeleton can be regarded as an acyclic variant of the chromone ring. This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by MEST (2010-0016080).

REFERENCES

1. Markgraf, C. G.; Velayo, N. L.; Johnson, M. P.; McCarty, D. R.; Medhi, S.; Koehl, J. R.; Chmielewski, P. A.; Linnik, M. D. *Stroke* 1998, 29, 152-158.
2. Boland, B.; Campbell, V. *Neurobiol. Aging* 2003, 24, 179-186.
3. Lee, K. S.; Seo, S. H.; Lee, Y. H.; Kim, H. D.; Son, M. H.; Chung, B. Y.; Lee, J. Y.; Jin, C. B.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* 2005, 15, 2857-2860.

POSTER COMMUNICATIONS – PC.435

GLYCOSIDASE AND NEURAMINIDASE INHIBITORY OF PHENOLIC COMPOUNDS FROM YOUNG BARLEY LEAVES

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Young barley leaf extract is a good natural source of vitamins and minerals. It is made from barley leaves harvested 2 weeks after seeding. The barley leaves were freeze-dried for 3 d and then ground. The ground leaves contain fat 4.97%, polysaccharide 52.6%, protein 34.1%, and a variety of vitamins, minerals and polyphenolic compounds. In this context, a methanol extract of young barley leaf having antidiabetic activity was examined as a possible α -glucosidase inhibitor, which is a key enzyme involved in diabetic and diabetic complications. Five phenolic compounds, ferulic acid, isovitexin, luteolin, saponarin, and luteonarin were isolated from this methanolic extract, and determined those structures by 1D and 2D nuclear magnetic resonance (NMR), including COSY and HMBC experiments. Among them, isovitexin and luteolin were found to inhibit α -glucosidase with IC_{50} values of 94.4 and 32.1 μM , respectively. Whereas ferulic acid, saponarin, and luteonarin did not inhibit α -glucosidase up to 100 μM . The influenza neuraminidase (E.C. 3.2.1.18) is an antiviral target of high pharmaceutical interest because of its essential role in cleaving sialic acid residues from surface glycoproteins and facilitating release of virions from infected cell. In this context, polyphenolic compounds including ferulic acid, isovitexin, luteolin, saponarin, and luteonarin from young barley leaves were evaluated for their inhibitory effect on recombinant virus H1N1 neuraminidase. Among the test compounds, isovitexin, luteolin, saponarin, and luteonarin inhibited the rvH1N1 neuraminidase with an IC_{50} of 35.6, 8.4, 33.2 and 15.6 μM , respectively. Whereas ferulic acid did not inhibit rvH1N1 neuraminidase up to 100 μM . The inhibition kinetics analyzed by Dixon plots indicates that luteolin and saponarin are noncompetitive inhibitor.

POSTER COMMUNICATIONS – PC.436

SUBSTRATE BASED N^ε-MODIFIED LYSINE CONTAINING INHIBITORS FOR SIRT1 AND SIRT2

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Sirtuins (SIRT1-SIRT7) catalyze the NAD⁺ dependent deacetylation of N^ε-acetyl lysine residues in multiple protein substrates to nicotinamide, O⁶-acetyl-ADP-ribose and N^ε-deacetylated lysine. Therapeutic prospects for sirtuins have been implicated in medically important age-associated diseases, such as cancer and metabolic, cardiovascular and neurodegenerative diseases. Currently, there is intensive research to discover potent activators and inhibitors of SIRT1 and SIRT2 as pharmacological tools in biological studies to reveal sirtuin functions in cells.¹

Recently, we have developed compounds which mimic the binding of the N^ε-acetyl lysine containing substrates. We have reported a series of N^ε-thioacetyl lysine containing tri-, tetra- and pentapeptides, based on α -tubulin and human tumor suppressor protein p53 sequences, and demonstrated the effect of peptide length and selection of the side chains for the SIRT1 and SIRT2 inhibitory activity.² In the present studies we have found that primarily the main chain interactions at the -1 and +1 positions from the N^ε-thioacetyl lysine are important for the inhibitory activity. Several non-peptide replacements have been made on the peptidic backbone in order to find pseudopeptidic inhibitors for SIRT1 and SIRT2, which could retain the important main chain interactions.³ In addition, we have screened several potent N^ε-modified lysine containing inhibitors against SIRT1 and SIRT2. The studied N^ε-alkanoyl, N^ε- α,β -unsaturated carbonyl and N^ε-aroyl moieties showed that the acetyl binding pocket can accept rather large groups, but is sensitive to even small changes in electronic and steric properties of the N^ε-modification.⁴

Substrate based inhibitors may serve as pharmacological tools to study sirtuins. Potent pseudopeptides are applicable in the design of drug-like peptidomimetics, which no longer have enzymatically scissile peptide bonds. The peptidomimetic part of the inhibitor is primarily important for the recognition and sufficient affinity for SIRT1 and SIRT2, but it may also be chemically modified to improve the pharmacokinetic performance. N^ε-modified lysine part likely interferes with the deacetylation mechanism and therefore it may have influence on the inhibitory potency. In addition, the peptidomimetic part and the N^ε-modified lysine part may both have influence on the selectivity between sirtuins.

REFERENCES

1. Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G. *Cell Mol Life Sci* **2008**, 65, 4000.
2. Kiviranta, P. H.; Suuronen, T.; Wallen, E. A.; Leppanen, J.; Tervonen, J.; Kyrilenko, S.; Salminen, A.; Poso, A.; Jarho, E. M. *J Med Chem* **2009**, 52, 2153.
3. Huhtiniemi, T.; Salo, H.; Suuronen, T.; Lahtela-Kakkonen, M.; Poso, A.; Salminen, A.; Leppänen, J.; Jarho, E. *unpublished results* **2010**, manuscript.
4. Huhtiniemi, T.; Suuronen, T.; Lahtela-Kakkonen, M.; Bruijn, T.; Jääskeläinen, S.; Poso, A.; Salminen, A.; Leppänen, J.; Jarho, E. *unpublished results* **2010**, manuscript submitted.

POSTER COMMUNICATIONS – PC.437

DEVELOPMENT OF NOVEL ANDROGEN ANTAGONISTS BASED ON HYDROPHOBIC BORON CLUSTERS

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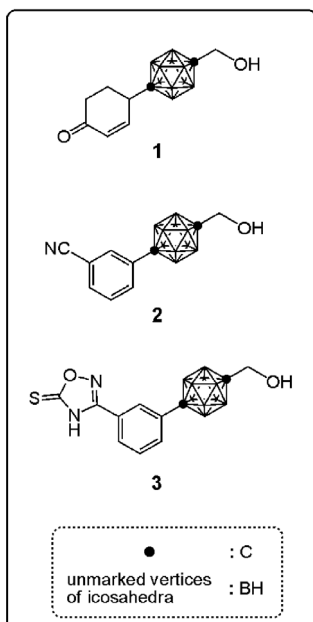
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Androgen receptor (AR), a member of the nuclear steroid hormone receptor, regulates various gene expressions with binding of specific hydrophobic ligands. Since AR is closely related to prostate cancer, AR antagonists such as flutamide and bicalutamide were developed and for clinical treatment of prostate cancer. Recently we developed AR antagonists bearing a carborane cage as a novel class of AR antagonists. Carborane is one of hydrophobic boron clusters that have unique properties such as spherical icosahedral geometry and remarkable chemical stability. Carborane moiety effectively interacts with the hydrophobic surface of the ligand-binding-domain of the nuclear receptor.

First, we designed and synthesized the compound **1** in which cyclohexenone ring and hydroxyl group are placed at the contralateral vertices of the carborane cage, by using endogenous ligand testosterone as a lead compound. Compound **1** exhibited significant binding affinity to human AR with similar potency of hydroxyflutamide. Reporter gene assay and cell growth inhibition assay using androgen dependent SC-3 cell revealed the androgen antagonistic activity of compound **1**. [1]

Then, based on the structures of compound **1** and known nonsteroidal AR antagonists, we designed phenylcarborane derivatives which have an electron-withdrawing group on the benzene ring. Structure-activity relationship studies revealed that compound **2** had remarkably high binding affinity to hAR. These compounds also strongly inhibited testosterone-promoted cell growth of SC-3 cell. The potency of the antagonistic activity of compound **2** was over 10-fold higher than that of hydroxyflutamide. [2]

By using carborane cage as the hydrophobic pharmacophore, we tried to develop novel hydrophilic pharmacophore of AR ligands. As a result, we found that compound **3** bearing an acidic heterocycle 1,2,4-oxadiazole-5-thione exhibited potent binding affinity to AR, which suggested that the heterocyclic functionality is potential bioisoster of the nitro and cyano groups. [3] These compounds would become the seeds of new AR antagonists and drug candidates for prostate cancer, and more detailed SAR studies are in progress.



REFERENCES

1. Fujii, S., Hashimoto, Y., Suzuki, T., Ohta, S., Endo, Y. *Bioorg. Med. Chem. Lett.*, **2005**, 15, 227-230.
2. Fujii, S., Goto, T., Ohta, K., Hashimoto, Y., Suzuki, T., Ohta, S., Endo, Y. *J. Med. Chem.*, **2005**, 48, 4654-4662.
3. Fujii, S., Ohta, K., Goto, T., Kagechika, H., Endo, Y. *Bioorg. Med. Chem.*, **2009**, 17, 344-350.

POSTER COMMUNICATIONS – PC.438

NEW IONIC LIQUIDS AND SALTS DERIVED FROM β -LACTAM ANTIBIOTICS

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In recent years ionic liquids (ILs) have been increasing the popularity and the number of applications. Ionic liquids were used mainly as solvent in organic synthesis, but in recent years they are also used in analytical chemistry, separation chemistry and material science. Additional to significant developments in their chemical properties and applications, ionic liquids are now

bringing unexpected opportunities at the interface of chemistry with the life sciences.

Ionic liquids (ILs) are currently defined as salts that are composed solely of cations and anions which melt below 100°C. Our goal in this work is to explore the dual activity of the ionic liquids, due to the presence of two different ions, an anion with bacterial activity as β -lactam antibiotics and different kinds of cations.

In this work the anions of ILs and salts were derived from three different antibiotics: ampicillin, penicillin and amoxicillin. The cations were derived from substituted ammonium, phosphonium pyridinium and methylimidazolium salts, such as: tetraethyl ammonium, trihexyltetradecylphosphonium, cetylpyridinium, choline (an essential nutrient), 1-ethyl-3-methylimidazolium, and 1-ethanol-3-methyl imidazolium structures.

Commercial ammonium and phosphonium halogen salts were first transformed into hydroxides on ionic exchange column (Amberlite IRA-400) in methanol. The prepared hydroxides were then neutralized with β -lactam antibiotics. After crystallization we obtained pure ILs and salts containing β -lactam antibiotics.

This work presents a novel method for preparation of new salts of antibiotics with low melting point and their chemistry and microbiological characterization.

Acknowledgment: The author would like to thank Escola Superior de Tecnologia da Saúde do Instituto Politécnico do Porto for financial support.

REFERENCES

1. Ionic Liquids in Synthesis (Green Chemistry (Wiley))(2 vol. set). Wiley-VCH: 2007; p 776.
2. Fukumoto, K.; Yoshizawa, M.; Ohno, H., Room Temperature Ionic Liquids from 20 Natural Amino Acids. *Journal of the American Chemical Society* 2005, 127 (8), 2398-2399.
3. Hough-Troutman, W. L.; Smiglak, M.; Griffin, S.; Reichert, W. M.; Mirska, I.; Jodynis-Liebert, J.; Adamska, T.; Nawrot, J.; Stasiewicz, M.; Rogers, R. D.; Pernak, J., Ionic liquids with dual biological function: sweet and anti-microbial, hydrophobic quaternary ammonium-based salts. *New Journal of Chemistry* 2009, 33 (1), 26-33.
4. Jain, N.; Kumar, A.; Chauhan, S.; Chauhan, S. M. S., Chemical and biochemical transformations in ionic liquids. *Tetrahedron* 2005, 61 (5), 1015-1060.
5. Pernak, J.; Syguda, A.; Mirska, I.; Pernak, A.; Nawrot, J.; Pradzynska, A.; Griffin, S. T.; Rogers, R. D., Choline-derivative-based ionic liquids. *Chemistry-a European Journal* 2007, 13, 6817-6827

POSTER COMMUNICATIONS – PC.439

DISCOVERY OF A NOVEL CLASS OF
METABOTROPIC GLUTAMATE RECEPTOR 2
POSITIVE ALLOSTERIC MODULATOR
(mGluR2 PAM):
STRUCTURE ACTIVITY RELATIONSHIP, *IN*
VITRO AND *IN VIVO* CHARACTERISATION OF
THIENO[2,3-D]PYRIMIDIN-4-AMINES

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mGluR2 is a G-protein coupled receptor (GPCR) that is expressed on presynaptic nerve terminals where it negatively modulates glutamate and GABA release. The physiological importance of the group II mGlu receptors, mGluR2 and mGluR3, has been shown by the efficacy of rigid glutamate analogs, such as the non-selective mGlu2/3 receptor agonist LY354740, in both preclinical animal models of anxiety and schizophrenia, as well as human clinical trials.

Since the discovery of sulfonamide derivatives, reported by Eli Lilly in early 2000's as the first mGluR2 PAM, a variety of structurally diverse chemotypes has been published.

We describe here the discovery of thieno[2,3-d]pyrimidin-4-amines as mGluR2 PAMs, and the identification of novel and potent derivatives from structural modifications of the initial hit **1**, identified via the proprietary Addex high throughput screening (HTS) platform. (Figure 1).

The synthesis, structure activity relationship (SAR), *in vitro* and *in vivo* characterizations of several analogs such as **2** and **3** are reported (Figure 2).

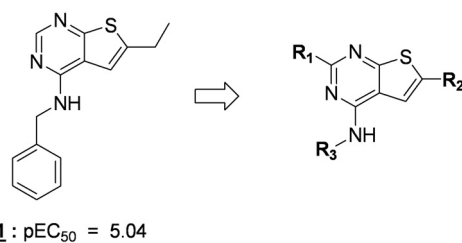


Figure 1

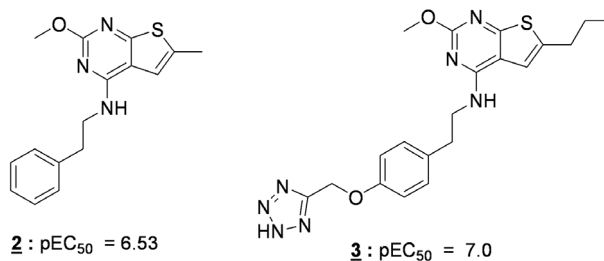


Figure 2

POSTER COMMUNICATIONS – PC.440

PILOT PHARMACOKINETIC EVALUATION OF A
NOVEL ANTICANCER DRUG

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A newly developed anti-cancer drug is currently under investigation in a phase II clinical study. Designed as an infusion solution, this new chemotherapeutic approach is meant to be used by end stage. Its combination consists of four active agents: alpha-ketoglutaric acid (KG), hydroxymethylfurfural (HMF), N-acetyl-L-methionine (NALM) and N-acetyl-L-selenomethionine (NASELM). The mechanism of action of the drug consists of different effects including angiogenesis suppression and disruption of the tumour cells energy metabolism. A pilot pharmacokinetic study is presented concerning the analytical data of KG in blood samples of patients affected by lungs tumour. Two different methods have been developed for its quantification. The first involved a SPE purification of the plasma samples by two different cartridges, an ion exchange cartridge for KG, NALM and NASELM and a polymeric hydrophobic cartridge for HMF. Afterwards, eluates containing KG were derivatised with dansyl-hydrazine leading to the formation of a hydrazone derivative analyzed via fluorescence detection. For the second method GS-MS was used. A derivatisation step was directly applied to plasma samples using O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PBFHA). The oxime derivative of KG was extracted with

hexane and further derivatised with (N-methyl-N-[tert-butyl-dimethylsilyl] trifluoroacetamide (MTBSTFA) as silylation agent. Both methods involve reaction with carbonyl groups and therefore they were developed for the contemporary analysis of KG and HMF. No free HMF was found in plasma samples thus necessitating further tests for the evaluation of its protein binding rate and metabolism.

POSTER COMMUNICATIONS – PC.441

APPLICATION OF CLINICALLY USED DPP-IV-INHIBITORS IN EXPERIMENTAL *IN VIVO* AND *IN VITRO* SETTINGS

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V. Matheeußen is a research assistant of the Fund for Scientific Research Flanders (FWO Vlaanderen).

Dipeptidyl peptidase IV/CD26 (DPP-IV) is a cell-surface protease that belongs to the prolyl oligopeptidase family of serine proteases. It cleaves off N-terminal dipeptides from peptides with proline or alanine at the penultimate position.¹ Sitagliptin² and vildagliptin³ are therapeutically available DPP-IV-inhibitors (IC₅₀ values of respectively 0.04 ± 0.001 µM and 0.12 ± 0.01 µM⁴) used for the treatment of type II diabetes because they increase the half-life of the incretin hormone GLP-1, a DPP-IV substrate.¹ These clinically used inhibitors are also a useful tool to clarify the function of DPP-IV in basic research settings. We investigated the *in vitro* and *in vivo* applicability in different types of laboratory animals, including mice, rat and piglets.

First the wash-out period of the inhibitor in rats was defined. Wistar rats were given a single IV injection of 1 mg/kg or 10 mg/kg vildagliptin. The 1 mg/kg vildagliptin is a dose comparable with the oral dose given in patients treated with this inhibitor for type II diabetes. In both groups the estimated percentage *in vivo* inhibition of the DPP-IV activity was > 90% 3 hours after the injection but then it dropped in the 1 mg/kg treated group. Thirteen hours after the injection of 10 mg/kg vildagliptin the DPP-IV activity was still inhibited for > 85% compared to 30% in the 1 mg/kg treated group.

The following experiments aimed to achieve a long-term *in vivo* inhibition of the DPP-IV activity, in mice and piglets respectively. Db/db mice received different doses of sitagliptin via the drinking water during weeks and piglets were treated with sitagliptin, once daily, mixed with their food, for 3 weeks. We conclude that an optimal dose of the inhibitor and administration interval should be studied separately for different species and disease

models. Particularly in mice it was difficult to obtain a long-lasting DPP-IV inhibition due to a quick elimination or inactivation of the inhibitor.

In addition we optimized the conditions of an *in vitro* enzyme activity assay to distinguish between DPP-IV and DPP-8/9 activity and we performed cell culture experiments to study the uptake of different inhibitors in the cell.

In summary, *in vivo* inhibition of the DPP-IV activity for a long period and *in vitro* cell culture settings make it possible to reveal other, biochemical and physiological, consequences of DPP-IV inhibition besides the favourable effect on the glucose tolerance in type II diabetes patients.

REFERENCES

1. Lambeir AM, Durinx C, Scharpé S, De Meester I. Crit Rev Clin Lab Sci. 2003; 40(3): 209-94.
2. Kim D, Wang L, Beconi M, et al. J Med Chem. 2005; 48(1): 141-51.
3. Villhauer EB, Brinkman JA, Naderi GB, et al. J Med Chem. 2003; 46(13): 2774-89.
4. Maes MB, Dubois V, Brandt I, et al. J Leukoc Biol. 2007; 81(5): 1252-7.

POSTER COMMUNICATIONS – PC.442

DISCOVERY AND DEVELOPMENT OF POTENT AND SELECTIVE mPGES-1 INHIBITORS

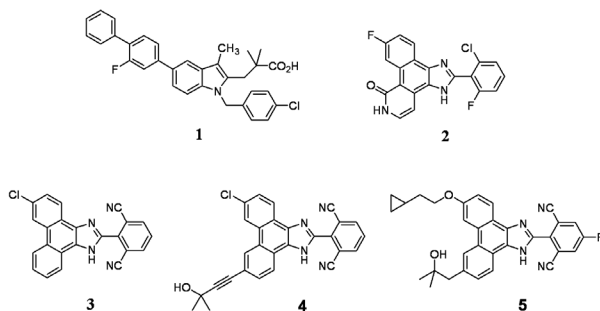
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Prostaglandin E₂ (PGE₂) is a common prostanoid with a variety of bioactivities and has been found to be a major mediator of pain, fever and inflammation. PGE₂ is produced by sequential conversion of arachidonic acid to PGH₂ by cyclooxygenase (COX) followed by the isomerization of PGH₂ to PGE₂ by a prostaglandin H synthase (PGES). Characterisation of the different PGES led to the identification of mPGES-1, a microsomal, glutathione-dependent, inducible enzyme responsible for PGE₂ production as the key component present in inflammation. Selective inhibition of mPGES-1 would preclude PGE₂ production but, unlike NSAIDs and coxibs, would not inhibit the biosynthesis of PGD₂, PGF_{2α}, PGI₂ and TXA₂.

As previously reported (*Biorg. Med. Chem. Lett.* **2007**, *17*, 6816), structure optimization from an azaphenanthrene **2** lead, led to the identification of the phenanthrene imidazole **3** as a potent and selective mPGES-1 inhibitor. Compound **3** is significantly more potent than the previously reported indole carboxylic acid **1** with a human whole blood IC₅₀ of 1.3 µM. This represents the first reported mPGES-1 inhibitor to demonstrate potency in a human whole blood (HWB) assay. Unfortunately, compound **3** did not have suitable pharmacokinetic to continue its development. Further SAR was undertaken and allowed the identification of

two disubstituted phenanthrene imidazole, **4** and **5**. These inhibitors are significantly more potent than the previously reported chlorophenanthrene imidazole **3** with a human whole blood IC₅₀ of 0.20 and 0.10 μ M, respectively. Furthermore, they exhibited analgesic effect in a guinea pig hyperalgesia model. Both inhibitors have excellent potencies, selectivities and a pharmacokinetic profile and therefore, were accepted as pre-clinical candidates. In this presentation, the overall profile for inhibitors **4** and **5** will be described including PhI data for compound **4**.



POSTER COMMUNICATIONS – PC.443

SYNTHESIS AND EVALUATION OF 1-(1H-INDOLE-3-YL)ETHANE-1,2-DIAMINE DERIVATIVES AS NEW ANTIBACTERIAL AGENTS

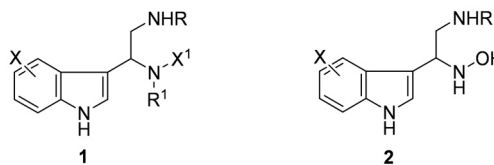
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Drug resistance to antibiotics, especially the multiple one (MDR), is nowadays an alarming and major public health concern worldwide.¹ Many strains of bacteria have developed resistances through the years following the first clinical use of antibiotics and the emergence and spreading of antibiotic-resistant bacteria is steady. Several strains responsible for infectious diseases that seemed to be under control are again causing deaths each year due to their evolution and the absence of an appropriate antibiotic. Thus there is still a need for new molecules that can be added to the pharmacopoeia in order to treat these infections.

During the screening of the chemical library of our laboratory on various biological targets, we discovered new families of antibacterial indole compounds **1** and **2** with the common 1-(1H-indole-3-yl)ethane-1,2-diamine scaffold. These indole derivatives have been prepared with the purpose of developing original synthetic methodologies based on the reaction between nitrones and indoles.² We found that the indole molecules **1** and **2** exhibit either a specific antibacterial activity against a sensible strain of *Staphylococcus aureus* or a selective inhibitory activity of the

NorA multidrug efflux pump in a resistant strain of the same species in which this efflux pump is over-expressed.³



Since our first discovery of new antibacterial agents with 1-(1H-indole-3-yl)ethane-1,2-diamine core, a new family of indolic molecules was designed using medicinal chemistry approaches. A SAR analysis was performed to improve both antibiotic and inhibitory activity of novel derivatives. For example, due to either already patented or recently developed chemical synthetic pathways, several sites of molecules **1** and **2** (X, X¹, R, R¹, etc.) were modified. New families of molecules were tested against nearly thirty strains of Gram-positive and Gram-negative bacteria. Extremely encouraging results open the way for our group to investigate the mechanisms of action and to develop in the same time new generations of analogs.

REFERENCES

1. Mitscher, L. A.; Pillai, S. P.; Gentry, E. J.; Shankel, D. M. *Med. Res. Rev.* **1999**, 19, 477.
2. (a) Denis, J.-N.; Mauger, H.; Vallée, Y. *Tetrahedron Lett.* **1997**, 38, 8515. (b) Chalaye-Mauger, H.; Denis, J.-N.; Averbuch-Pouchot, M.-T.; Vallée, Y. *Tetrahedron* **2000**, 56, 791.
3. (a) Denis, J.-N.; Guinchard, X.; Moreau, N.; Neuville, L.; Vallée, Y. *Synthesis of new indole derivatives, their preparation processes, and their antibacterial uses*, Fr demande (**2007**), FR 070717, 01/02/2007, (126pp). (b) Denis, J.-N.; Guinchard, X.; Moreau, N.; Neuville, L.; Vallée, Y. *Synthesis of new indole derivatives, their preparation processes, and their antibacterial uses*, PCT Int. Appl. (**2008**), 139pp. WO 2008110690 A2 20080918 CAN 149:356082 AN 2008:1130276.

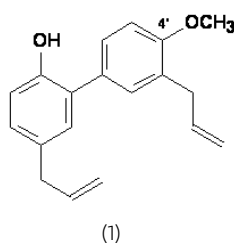
POSTER COMMUNICATIONS – PC.444

SYNTHESIS OF ANALOGS OF 4'-O-METHYLHONOKIOL AS GABA_A RECEPTOR MODULATORS

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The genus *Magnolia* plays a role in American as well as in Asian medicinal systems such as TCM or Japanese Kampo medicine [1,2]. Main lignan constituents in the seeds of the North American *Magnolia grandiflora* L. (Southern Magnolia) are 4'-O-methyl-honokiol (**1**), magnolol and honokiol.



Honokiol is well known for its GABA_A receptor-mediated anxiolytic effect [3]. For the evaluation of GABA_A receptor modulatory activity, chloride currents (I_{GABA}) were measured in GABA_A receptors expressed in *Xenopus laevis* oocytes using a fast perfusion system as described earlier [4].

In previous investigations, **1** was found to possess an even higher GABA_A receptor agonistic activity than honokiol (potentiation of I_{GABA} at 30 μM = 376% for honokiol and 822% for **1**, respectively) and hence was chosen as lead structure for further derivatisations. This enhanced activity found for the less polar compound **1** resulted in the synthesis of a set of derivatives of **1** targeting the polarity of key functionalities in **1**. Syntheses and results will be detailed.

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REFERENCES

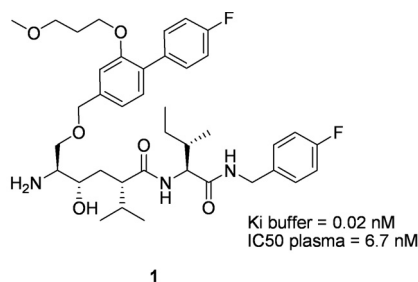
- Schühly W, Khan I, Fischer NH. The ethnomedicinal uses of Magnoliaceae from the southeastern United States as leads in drug discovery. *Pharm Biol* 2001; 39; 63-69.
- Sarker SD, Maruyama Y (Eds). The genus Magnolia: Medicinal and Aromatic Plants - Industrial Profiles. London: Taylor & Francis, 2002.
- Maruyama, Y.; Kuribara, H.; Morita, M.; Yuzurihara, M.; Weintraub, S. T. Identification of Magnolol and Honokiol as anxiolytic Agents in Extracts of Saiboku-to, an Oriental Herbal Medicine. *J. Nat. Prod.* 1998, 61, 135-138.
- Baburin I, Beyl S, Hering S. Automated fast perfusion of *Xenopus* oocytes for drug screening. *Pflugers Arch.* 2006, 453, 117-123.

POSTER COMMUNICATIONS – PC.445

DISCOVERY OF POTENT RENIN INHIBITORS: SAR OF PRIME SIDE MODIFICATIONS

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The renin-angiotensin system (RAS) mediates extracellular volume and arterial vasoconstriction regulating the mean arterial blood pressure. Renin is an aspartyl protease that participates in the first and rate limiting step of the RAS by cleaving its only known substrate angiotensinogen to angiotensin I. Renin is therefore an attractive target for the treatment of hypertension.

We have developed compound **1**, a fully reversible inhibitor of renin binding into the active site (K_i buffer = 0.02 nM, IC_{50} plasma = 6.7 nM). Herein, we will present the impact that modifications of the prime side substituent of **1** have on renin activity and membrane permeability.

POSTER COMMUNICATIONS – PC.446

TETRAHYDROISOQUINOLINE, TETRAHYDRO- β -CARBOLINE, BENZO- AND INDOLOAZEPINONE SCAFFOLDS IN THE DESIGN OF NOVEL NK1R ANTAGONISTS

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Non-peptidic NK1R (neurokinin 1 receptor) antagonists have been developed over the past decades for their therapeutic use in pathologies such as emesis, pain and peripheral inflammatory diseases.[1] Our research unit has developed and refined several versatile methodologies to provide conformationally constrained amino acids [2] in order to fix the biologically active conformation of different endogenous peptides (e.g. opioid peptides, bradykinin, melanocortin and somatostatin).[3] Moreover, these amino acids were used as central scaffolds for the preparation of low molecular weight receptor ligands of different G protein-coupled receptors, [4] and hence their use as 'privileged templates' was validated.

In this work four different types of constrained aromatic amino acids were derivatized to prepare new neurokinin 1 receptor (NK1R) antagonists. Tetrahydroisoquinoline 3-carboxylic acid **1**

(Tic), 4-amino-benzazepinone **2** (Aba), tetrahydro- β -carboline-3-carboxylic acid **3** and amino indoloazepinone **4** (Aia) templates were substituted at both the α -amino and carboxylic acid positions of the amino acids.

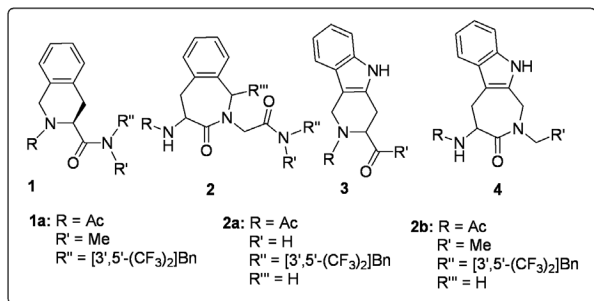


Figure 1

A series of 17 compounds (see general structures in Figure 1) were evaluated for NK1R agonism and antagonism via an assay measuring the agonist-induced calcium-dependent aequorine-luminescence of cells expressing NK1 receptors. The results obtained with this functional assay indicated that 3 of the synthesized compounds (**1a**, **2a** and **2b**) were potent NK1R antagonists. The initial *in vitro* evaluation of the active compounds, as well as molecular modeling results, will be discussed.

REFERENCES

1. a) Longmore et al. *Can. J. Physiol. Pharmacol.* **1997**, 75, 612; b) Saria, A. *Eur. J. Pharmacol.* **1999**, 375, 51; c) Leroy et al. *Exp. Opin. Invest. Drugs* **2000**, 9, 735.
2. a) Ballet et al. *Synlett.* **2005**, 18, 2791.; b) Tourwé et al. *Biopolymers* **1996**, 38, 1; Van Rompaey et al. *Tetrahedron* **2003** 59, 4421; Pulka et al. *Tetrahedron* **2007**, 63, 1459.
3. a) Ballet et al. *J. Pept. Res.* **2005**, 66, 222; b) Ballet, S. et al. *J. Pept. Sci.* **2007**, 13, 164; c) Ballet et al. *Bioorg. Med. Chem. Lett.* **2007**, 17, 2492; d) Feytens et al. *J. Med. Chem.* **2007**, 50, 3397.
4. a) Ballet, S. *Bioorg. Med. Chem. Lett.* **2010**, 20, 1610; b) Feytens J. *Med. Chem.* **2009**, 52, 95.

POSTER COMMUNICATIONS – **PC.447**

NEW STEROIDAL MINERALCORTICOID ANTAGONISTS

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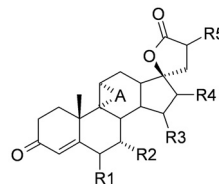
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The renin-angiotensin-aldosterone system (RAAS) has been identified as the key regulatory enzymatic cascade for cardiovascular homeostasis and is the current target for many therapeutics designed to ameliorate hypertension and to protect against congestive heart failure (CHF). Activation of RAAS leads to the synthesis of the mineralocorticoid aldosterone, the overproduction of which has been shown to be associated with such conditions as edema, CHF, nephrosis, essential hypertension, cirrhosis of the liver, and severe vascular injury¹.

Improved drug therapies for patients who do not respond well to conventional medicines to treat hypertension, heart failure and renal disease is a desirable goal. The development of aldosterone-receptor antagonists with improved selectivity for mineralocorticoid receptor over other steroid receptors may meet the requirements and reduce side effects.

The synthesis of new spiro lactones bearing different substitutions at carbon 6, 7, 9-11, 15-16 and 21 and *in vitro* and *in vivo* biological data for these compounds will be described.



REFERENCES

1. Alderman MH, Madhavan S, Ooi WL, Cohen H, Sealey JE, Laragh JH. *N. Engl. J. Med.* **1991**, 324, 1098-1104

POSTER COMMUNICATIONS – **PC.448**

PROPOLIS FROM AZORES (PORTUGAL): ANTIMICROBIAL AND ANTI-INFLAMMATORY PROPERTIES

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In a recent back to nature move, modern man is searching for natural products with medicinal properties. Propolis, the material used by bees to protect their hives, is a glue-like substance composed of plant resins, bee waxes and pollens. That has various biological activities such as antibacterial, antiviral, anti-inflammatory and anesthetic properties.

In order to evaluate some biological properties of portuguese propolis [1], we started studying and investigating some propolis

extracts as a potential sources of antimicrobial, anti-inflammatory and antioxidants compounds.

Propolis samples were collected as the crude materials and were extracted with ethanol at room temperature. The ethanol suspension was filtered and the filtrate was concentrated to give the ethanolic extract of propolis (EEP). The EEP was further fractionated with hexane and chloroform solvents. The fractions obtained, (HEX-Fr, CHLO-Fr) and the residual ethanolic extract (EtOH-Res) were concentrated. Total polyphenol and flavonoid contents were determined by using aluminum nitrate and Folin–Ciocalteu colorimetric methods. For evaluating antioxidant activity, the free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the scavenging activity on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation were used as assays systems.

There are only a few studies reporting on *in vivo* anti-inflammatory activity of propolis [2]. However, propolis is commonly used for the treatment of some skin inflammatory diseases. One enzyme that is involved in tissue remodeling during inflammation is hyaluronidase (EC 3.2.1.35), which degrades glycosaminoglycans, including hyaluronan, in human and animal tissues. Hyaluronan polymers are important constituents of the extracellular matrix of connective tissues, including cartilage, the synovial membrane, and synovial fluid of joints. If a certain compound is able to inhibit the activity of hyaluronidase, it will have a great potential as an anti-inflammatory agent. In this work, we tested the anti-hyaluronidase activity of propolis extracts from Azores and tried to correlate those activities with their phenolics content and antioxidant properties. Antimicrobial activity of propolis samples has been widely proved against a variety of bacteria and fungi and in both cases seems to be mainly associated with high polyphenol contents. As natural fungicides could present advantages over synthetic ones because of good efficacy and low toxicity, propolis could be a good source for such natural bioactive compounds. Propolis extracts from Azores were thus screened for antimicrobial properties against a panel of selected bacteria and yeasts.

REFERENCES

1. M Guimarães, MJ Carvalho, L Carvalho, A Cunha, C Aguiar e AM Ferreira (2009) "Antimicrobial and antioxidant properties of portuguese propolis" MicroBiotec09, 28-30 Novembro, Vilamoura, Portugal.
2. AFN Ramos e JL Miranda (2007) "Propolis: a review of its anti-inflammatory and healing actions" J. Venom. Anim. Toxins incl. Trop. Dis. v. 13, nº4, p697-710.

POSTER COMMUNICATIONS – PC.449

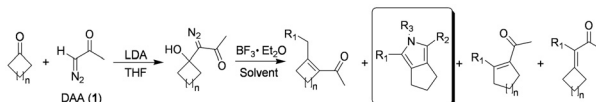
ON THE ROUTE OF BIOLOGICALLY ACTIVE HETEROCYCLES-CONTAINING COMPOUNDS: NOVEL EXPLORATIONS WITH CYCLIC α -DIAZO- β -HYDROXY KETONES

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Heterocycle-containing molecules are compounds of great chemical and biological significance since they represent the principal structural constituents of many bioactive products, medicinal important compounds, and organic materials.¹ Despite the advances realized, more flexible and general routes to a variety of heterocycles are needed. In particular, methods that can readily give access to heterocyclic cores with diverse and easily manipulated set of substituents are still of great importance. At this regards, efficient strategies based on diazo chemistry may constitute a nicely complement to the classical synthetic approaches.

In the frame of our continuing interest on the diazo chemistry,² here we report novel reactions of cyclic α -diazo- β -hydroxy ketones, prepared by aldol-type condensation between diazoacetone (DAA, **1**) and cyclic ketones, emphasizing their synthetic versatility as well as their application in the synthesis of compounds of biological and pharmacological interest. By using boron trifluoride etherate ($\text{BF}_3 \cdot \text{OEt}_2$) as catalyst in various solvents we achieved an array of synthetically useful and structurally-diverse products. The results obtained as well as the mechanistic insights of the reaction will be described.



REFERENCES

1. Bioactive Heterocycles VII, Topics in Heterocyclic Chemistry, Volume 16. ISBN 978-3-642-00335-6. Springer Berlin Heidelberg, 2009.
2. a. Pellicciari, R.; Natalini, B.; Sadeghpour, B. M.; Marinozzi, M.; Snyder, J. P.; Williamson, B. L.; Kueth, J. T.; Padwa, A. *J. Am. Chem. Soc.* **1996**, *118*, 1-12; b. Gioiello, A.; Khamidullina, A.; Fulco, M. C.; Venturoni, F.; Zlotsky, S.; Pellicciari, R. *Tetrahedron Lett.* **2009**, *54*, 5978-5980; c. Gioiello, A.; Venturoni, F.; Natalini, B.; Pellicciari, R. *J. Org. Chem.* **2009**, *74*, 3520-3523.

POSTER COMMUNICATIONS – PC.450

KEY INTERACTIONS OF ANTI-HIV DRUG CANDIDATE PF-232798 WITH CCR5 RECEPTOR

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CCR5 is a member of the chemokine receptor family and G protein-coupled receptors superfamily [1]. This protein is expressed by T cells and macrophages, and is known to be an important co-receptor for macrophage-tropic virus, including HIV, to enter host cells. Defective alleles of this gene have been associated with the HIV infection resistance. It has been recently shown that CCR5 antagonists can serve as HIV virus entry inhibitors. Insertion of these small molecules into the cavity located within the transmembrane region of the protein disrupts the geometry of a multi-point interaction between CCR5 and HIV-1 gp120 protein. This complex interaction is formed by binding of the second extracellular loop to elements of the gp120 V3 region and the tyrosine-sulfated N-terminus of CCR5 binding to a conserved bridging sheet that forms between the C1, C2, and C4 domains upon CD4 binding.

Unfortunately, due to the unknown structure and function of CCR5 protein only one compound, Maraviroc (Pfizer), has been synthesized and approved as drug both by European Commission and US Food and Drug Administration [2]. PF-232798 (Pfizer) is a 2nd generation molecule, that was shown to be a potent oral CCR5 antagonist with a primary and selectivity/safety pharmacology profile similar to Maraviroc [3]. It was obtained in a medicinal chemistry synthetic campaign guided by a biological screening cascade with Maraviroc as a starting point, with a goal to target Maraviroc-resistant strains of HIV. Pfizer reports that it is currently in Phase II of clinical trials, which makes it the most advanced candidate for anti-HIV therapy. Unfortunately the experimental data regarding this promising compound is very scarce. We decided to perform a combined experimental - computational study to investigate the binding site and binding mode of PF-232798 in CCR5. We hope that our study will help to establish key difference between Maraviroc and PF-232798 binding mode as well as shed more light onto the structure of CCR5, that will eventually help in more rational design of its inhibitors.

We have used GenSeMBLE, a new computational protocol, which allowed us to obtain a reliable CCR5 model and explain the experimental data and the differences between binding modes of PF-232798 and another CCR5 antagonist, Maraviroc. We postulate that the experimental data may be explained only by taking into account multiple possible CCR5 conformations with different ligands. We also show that W86 is one of the most important residues for PF-232798 binding to CCR5 and explain the 60-fold difference in the W86A mutation effect between PF-232798 and Maraviroc binding.

REFERENCES

1. Rossi D, Zlotnik A: The Biology of Chemokines and their Receptors. *Annu Rev Immunol* 2000, 18:217-242.
2. De Clercq E: 25 compounds approved within 25 years after the discovery of HIV. *Int J Antimicrob Ag* 2009, 33:307-320.
3. Dorr P, Westby M, McFadyen L, Mori J, Davis J, Perruccio F, Jones, R, Stuppel P, Middleton D: PF-232798, a Second Generation Pfizer Oral CCR5 Antagonist. Presented at the 15th Conference on Retroviruses and Opportunistic Infections. Boston, MA, February 3-6, 2008; Abstract 737.

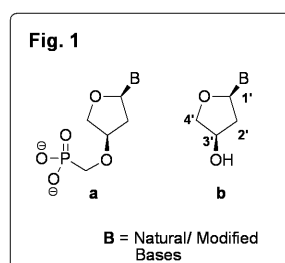
POSTER COMMUNICATIONS – PC.451

L-2-DEOXYTHREOSE NUCLEOSIDES: SYNTHESIS AND SCREENING AGAINST VIRAL STRAINS

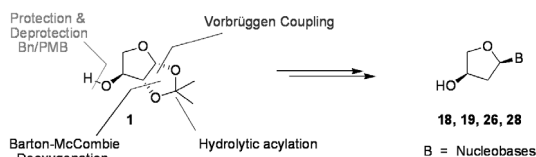
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Several nucleoside analogs are well-known to inhibit the reverse transcription (RT) process in HIV by terminating the synthesis of a proviral DNA strand. Recently, Herdewijn and coworkers showed that some L-2-deoxythreose nucleoside phosphonates (**a**; Figure 1) selectively inhibit HIV without affecting human DNA synthesis.¹ These nucleoside analogues require two additional phosphorylation steps by cellular kinases before they are incorporated in the viral genome and lead to chain termination. It is believed that a *cis* orientation of the base and the 3'-substituent is a structural requirement for optimal conversion to the diphosphate. Inspired by these findings, we decided to explore the antiviral properties of the threose-based nucleoside analogues (**b**; Figure 1), which are anticipated to have superior bioavailability compared to the parent phosphonates. Remarkably, such α -L-2'-deoxythreofuranosyl analogues (exhibiting a 1'R,3'R configuration) have not been reported before.



The synthesis of the desired T, U, C and A analogs (Scheme-1), were completed involving a Vorbrüggen coupling² and a Barton-McCombie deoxygenation³ as key steps. The synthetic course also revealed the importance of proper protecting groups.



Scheme-1

All the α -L-2'-deoxythreofuranosyl analogues synthesized were evaluated *in vitro* for cytotoxicity and for their activity against a variety of viruses, while their capacity to inhibit a panel of deoxyribonucleoside kinases was also assessed.

REFERENCES

- (a) Wu, T.; Froeyen, M.; Kempeneers, V.; Pannecouque, C.; Wang, J.; Busson, R.; De Clercq, E.; Herdewijn, P. *J. Am. Chem. Soc.* **2005**, 127, 5056-5065. (b) Vina, D.; Wu, T.; Renders, M.; Laflamme, G.; Herdewijn, P. *Tetrahedron*, **2007**, 63, 2634-2646.
- Vorbrüggen, H.; Krolkiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, 114, 1234 - 1255.
- Barton, D. H. R. and McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1*, **1975**, 1574 - 1585.

POSTER COMMUNICATIONS – PC.452

NCX 429: SYNTHESIS AND PHARMACOLOGICAL PROFILE

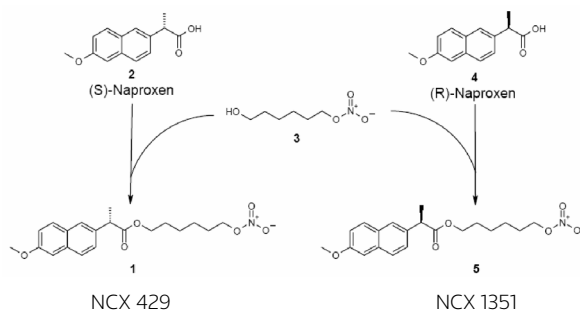
G. Ronsin, N. Dubois, E. Bastia, S. Casagrande, D. Miglietta, A. Poggi, C. Storini, K. Bendjama, S. Biondi, J. Padron, E. Ongini.

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Conventional non-steroidal anti-inflammatory drugs (NSAIDs) are effective drugs to treat pain by non selectively inhibiting cyclooxygenase enzymes (COX1 and COX2). Non selective COX inhibitors produce some detrimental effects on the gastrointestinal tract and cardiovascular system¹. Nitric oxide (NO) has various physiological functions that could contribute to reduce the side effects observed in NSAIDs by protecting the gastrointestinal wall and reducing blood pressure. Cyclooxygenase inhibitor-NO donor (CINOD) derivatives², like Naproxcinod, are dual acting drugs enabling the release of the parent NSAID and nitric oxide (NO) via a NO-donor.

NCX 429 **1** was synthesized from (S)-Naproxen **2** and its pharmacological profile evaluated both *in-vitro* and *in-vivo* to assess its efficacy to counteract the main adverse effects of **2**.

(R)-Naproxen **4** possesses a COX inhibition activity reduced compared to its (S) enantiomer. Coupled with the NO donor **3**, NCX 1351 **5** represents an interesting pharmacological tool to understand the role of NO with a reduced COX contribution.



Pharmacological experiments of these molecules will be described with the focus on showing the contribution of NO to the activity of NCX 429.

REFERENCES

- R. Jones, G. Rubin, F. Berenbaum, J. Scheiman, *Am. J. Cardiol.*, **2008**, 121, 6, 464
- J. L. Wallace, S. Viappiani, M. Bolla, *Trends in Pharm. Sci.*, **2009**, 30, 3, 112

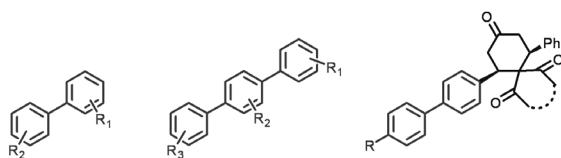
POSTER COMMUNICATIONS – PC.453

COMPARATIVE PROTEOMIC APPROACH TO INVESTIGATE THE MECHANISM OF ACTION OF BI-AND-TER-PHENYL-BASED CHEMICAL PROBES INTERFERING WITH THE CELL CYCLE PROGRESSION IN TUMOR CELL

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Given our interest in both medicinal chemistry and chemical biology, over the past few years, we have been engaged in a project aimed at identifying novel biologically active small molecules, which can be either innovative antitumor lead candidates or valuable chemical tools to study molecular pathways in cancer cells. In this context, the peculiar biological effects shown by some derivatives belonging to a small library of bi- and ter-phenyls (**1**) prompted us to continue our study on this class of compounds. With the aim of enhancing the structural complexity and diversity of the existing collection, we synthesized a series of new molecules that are hybrids of spirocyclic ketones as complexity-bearing cores with bi- and ter-phenyls as privileged fragments (**2**).



We studied some representative compounds on chronic myelogenous leukemia Bcr-Abl expressing K562 cells, and found that they caused a block in G_0 - G_1 phase for a long period of time (72 hours), and after five days of treatment they were able to induce morphological and functional differentiation. In contrast, other derivatives caused a block in G_0 - G_1 after 24 hours of treatment, and a massive apoptosis induction after 48 hours (3). Thus, we decided to investigate more in depth on the mechanism of action of some compounds, by comparing the whole protein expression profiles of treated and non-treated K562 cells. A comparative proteomic approach based on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and MALDI-TOF mass spectrometry was employed to define the protein expression profiles. For all the compounds tested, several protein spots resulted dysregulated in the same way respect to the untreated cell cultures, suggesting the alteration of common molecular pathways in cancer cells. However, pronounced differences were also detected among the proteomic patterns of K562 cells treated with the different molecules, providing evidence of derivative-specific mechanisms of action. The protein spots that showed at least two times enhanced/decreased expression were selected for identification.

REFERENCES

1. Pizzirani, D., et al., *ChemMedChem*, **3**, 345 (2008).
2. Pizzirani, D., et al., *Tetrahedron Lett.*, **48**, 7120 (2007).
3. Pizzirani, D., et al., *J. Med. Chem.*, **52**, 6936 (2009).

POSTER COMMUNICATIONS – PC.454

MASPIIT: TOWARDS THE IDENTIFICATION OF CELLULAR TARGETS OF SMALL-MOLECULE DRUGS

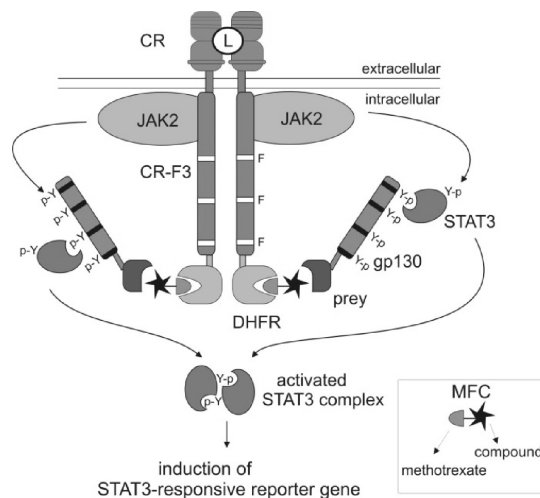
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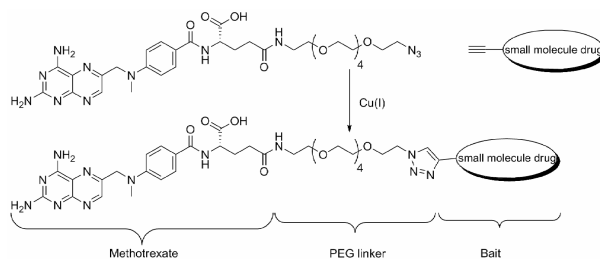
The identification of target proteins of small molecule drugs is essential to an understanding of the molecular basis of their mode of action.

A recently developed assay, Mammalian Small molecule-Protein Interaction Trap (MASPIIT)^[1] provides a new tool for the identification of intracellular targets. The principle of MASPIIT is based on the JAK/STAT pathway of the cytokine receptor. A small mol-

ecule, or bait, is tethered to methotrexate (MTX) via a PEG linker. The MTX moiety allows immobilization of the bait onto the DHFR unit which is fused to the cytokine receptor, thus allowing screening the bait against a collection of chimeric-prey proteins. Upon binding of the bait to a prey protein, the JAK/STAT signal cascade is activated, resulting in the expression of luciferase allowing a facile read out.



The MTX-bait conjugates are synthesized from two pieces using the copper(I) catalyzed azide/alkyne cycloaddition. The first of these pieces is identical for all conjugates to be evaluated and is responsible for the immobilization of the bait molecules. It comprises a methotrexate tag regioselectively condensed to a PEG linker that is equipped with an azide. The second piece concerns the actual bait and generally consists of a small molecule of interest that is equipped with a primary alkyne. The point of attachment of the alkyne is judiciously chosen as to minimize the effect on the biological activity of the small molecule.



REFERENCES

1. M. Caligiuri, et al. *Chem. Biol.* **2006**, *13*, 711–722

POSTER COMMUNICATIONS – **PC.455**

HYPER-SENSITIVE KINASE ASSAY BASED ON RADIO-PHOSPHORYLATION DETECTION ON A BIOCHIP

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Phosphorylation is a ubiquitous cellular regulatory mechanism found in all cells of the body. It occurs through the addition of a phosphate group to an amino acid residue via transfer of a terminal phosphate from an adenosine triphosphate (ATP) molecule catalyzed by enzymes, namely, kinases or phosphatases.¹ In general, kinases and phosphatases are of considerable interest to researchers involved in a drug discovery and development, because of their role in a wide variety of diseases.² In this study, we investigated the feasibility of applying a radio-phosphorylation detection technique to a protein kinase assay for the development of a diagnostic biochip. It was found that the RI detection technique is highly sensitive and time saving when compared with other conventional methods. We cloned protein-fused substrates for protein kinases using an elevating protein. Results of the study indicate that the protein-fused substrates can be used effectively as substrates for detecting the activity of protein kinases.^{3,4} Further it can be a useful tool for a high throughput screening and for studying enzyme-substrate interactions.

REFERENCES

1. S.J. Lee, S.Y. Lee, Anal. Biochem. **2004**, 330, 311-316.
2. S.H. Park, K.C. Ko, H.J. Gwon, J. Label. Compd. Radiopharm. **2007**, 50, 724-728.
3. K.C. Ko, M.H. Choi, S.H. Park, J. Label. Compd. Radiopharm. **2009**, 52, 123-127.
4. S.H. Park, K.C. Ko, M.H. Choi, J. Label. Compd. Radiopharm. **2009**, 54, 422-426.

POSTER COMMUNICATIONS – **PC.456**

DISCOVERY OF TAK-875: A POTENT, SELECTIVE, AND ORALLY BIOAVAILABLE GPR40 AGONIST

Nobuyuki Negoro, Shinobu Sasaki, Satoshi Mikami, Masahiro Ito, Masami Suzuki, Yoshiyuki Tsujihata, Ryo Ito, Ayako Harada, Koji Takeuchi, Nobuhiro Suzuki, Junichi Miyazaki, Takashi Santou, Tomoyuki Odani, Naoyuki Kanzaki, Miyuki Funami, Toshimasa Tanaka, Akifumi Kogame, Shinichiro Matsunaga, Tsuneo Yasuma, and Yu Momose

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GPR40, a G protein-coupled receptor predominantly expressed in pancreatic β -cells, mediates enhancement of glucose-stimulated insulin secretion by free fatty acids. A potent and selective GPR40 agonist may prove to be a safe and effective anti-diabetic drug with little or no risk of hypoglycemia. Initial efforts based on ligand-based drug design identified benzyloxyphenylpropanoic acids as a promising lead series. Cyclization of the phenylpropanoic acid moiety of the lead compound produced fused phenylalkanoic acids with favorable in vitro agonist activities and pharmacokinetic profiles. Further optimization led to the discovery of TAK-875, a dihydrobenzofuran derivative, as a potent, selective, and orally bioavailable GPR40 agonist. The design, structure-activity relationship, in vivo animal studies, and a molecular modeling study will be presented.

POSTER COMMUNICATIONS – **PC.457**HYPER-SENSITIVE Lp-PLA₂ ASSAY BASED ON RADIO-DEACETYLATION DETECTION ON A BIOCHIP*Sang Hyun Park¹, Mi Hee Choi^{1,2}, Jung Ae Kang^{1,3} and Kyong-Cheol Ko⁴*¹Radiation Research Division for Biotechnology, Korea Atomic Energy Research Institute, Jeongseup, Jeonbuk 580-185, Republic of Korea,²Department of Food Science and Technology, ³Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Republic of Korea, ⁴Molecular Bioprocess Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongseup, Jeonbuk 580-185, Republic of Korea

Microarrays have recently become a powerful research tool for proteomics and clinical investigations.^{1,2} They are of major importance in the detection of diseases and the drug discovery. The use of radioisotope is conventionally regarded as one of the most sensitive detection methods.³ The rapid increase of heart and vascular diseases was caused by increase of meat consump-

tion, excessive intake of fat and complex social life stress. Atherosclerosis is a common disorder affecting arterial blood vessels. It occurs when cholesterol, fat, etc. made in the arterial blood vessels form hard substances called a plaque. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is used as a marker which is independent risk factor for coronary artery diseases. The hyper-sensitive detection of Lp-PLA₂ was accomplished by using radioactive [³H-acetyl]PAF as a substrate and inhibitive activity of native plant extracts was determined by using the biochip.⁴ It was found to be applicable to a high-throughput screening of inhibitors for developing atherosclerosis therapeutic agents.

REFERENCES

1. S.J. Lee, S.Y. Lee, *Anal. Biochem.* **2004**, 330, 311-316.
2. K.C. Ko, M.H. Choi, S.H. Park, *J. Label. Compd. Radiopharm.* **2009**, 52, 123-127.
3. S.H. Park, K.C. Ko, M.H. Choi, *J. Label. Compd. Radiopharm.* **2009**, 54, 422-426.
4. K.C. Ko, M.H. Choi, S.H. Park, K.H. Cho, K.T. Lee, *J. Radiat. Ind.* **2009**, 3(1), 25-29.

POSTER COMMUNICATIONS – PC.458

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL INDENO [1,2-c]ISOQUINOLIN-5,11-DIONES AS ANTICANCER AGENTS

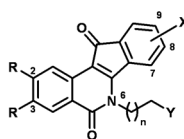
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Indeno[1,2-c]isoquinolin-5,11-diones are a new class of compounds displaying potent antitumor properties. Although the cytotoxic mechanism of these drugs has not been clearly elucidated, they probably act through the inhibition of DNA religation by topoisomerases I and/or II and are considered as topoisomerase "poisons" with the stabilisation of covalent enzyme-DNA complexes. Most active indeno[1,2-c]isoquinolin-5,11-diones such as NSC706744, indimitecan (NSC725776) or indotecan (NSC724998) were recently selected for therapeutic development.¹

A variety of new and constitutionally diverse indeno[1,2-c]isoquinolin-5,11-dione derivatives that have been structurally modified at the N-6, C-7, C-8 or C-9 positions in order to increase sol-

ubility, DNA-affinity and to enlarge structure-activity relationships in this class of compounds has been designed. Two series of compounds were assembled for this purpose. In Series 1, the new derivatives were equipped with aminoethyl or aminopropyl chains at the N-6 and C-7, C-8 or C-9 positions.² Regarding analogues of Series 2, the N-6 position of the indenoisoquinoline-diones was substituted with basic aminoacid moieties. The amino-acid was linked to the tetracyclic scaffold through a spacer of optimal length. Our compounds were evaluated for DNA interaction, topoisomerases I and II inhibition and cytotoxicity against HL60 cell line.



Lead compound: NSC706744
R = OCH₃, X = O-CH₂-O, Y = NH(CH₂)₂-OH, n = 2

New derivatives
R = H, OCH₃
X = H, OH, O-(CH₂)_n-N(CH₃)₂, NHCO-(CH₂)_n-N(CH₃)₂
Y = N(CH₃)₂ (Series 1), amino acid (Series 2)
n = 0, 1, 2

REFERENCES

1. Y. Pommier, M. Cushman, *Mol. Cancer Ther.* **2009**, 8, 1008
2. A. Ryckebusch, D. Garcin, A. Lansiaux, J. F. Goossens, B. Baldeyrou, R. Houssin, C. Bailly, J. P. Hénichart, *J. Med Chem.* **2008**, 51, 361

POSTER COMMUNICATIONS – PC.459

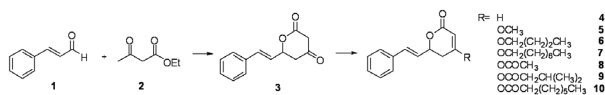
SYNTHESIS AND ANTIPROLIFERATIVE ASSAY OF NOVEL KAVALACTONE DERIVATIVES

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Kava-kava is a traditional beverage in the South Pacific Island prepared from the root of the kava plant *Piper methysticum* which has been reported to display anxiolytic, analgesic, antifungal and muscle-relaxing properties. The kavalactone family, including kavain (**5**), corresponds to a big part of the rootstock, and the low incidence of cancer in natives of this region and evidences of the Michael addition between kavain and glutathione suggest an anticancer activity for kavalactones.¹

Herein, we describe the synthesis and *in vitro* cytotoxicity of novel kavalactone derivatives in order to evaluate: 1) their role as a Michael acceptor for biological nucleophiles such as glutathione and, 2) the effect of the lipophilic nature of these derivatives in the cytotoxicity activity against cancer cell lines. These derivatives were synthesised from cinnamaldehyde (**1**) and ethyl acetate (**2**) in 2 steps: an aldol reaction followed by *in situ* lactonization leading to intermediate **3**, followed by etherification or esterification, providing **5** and derivatives **6-10**.



These compounds were evaluated *in vitro* against six human tumor cells lines: breast (MCF-7), kidney (786-0), ovary expressing resistance phenotype (NCI-ADR/RES), lung (NCI-H460), prostate (PC-03) and colon (HT-29).

Table 1: TGI values ($\mu\text{g/mL}$) for kavalactone (**3**), goniotalamin (**4**), kavain (**5**) and derivatives (**6-10**).

	DOX	3	4	5	6	7	8	9	10
MCF-7	2.73	39.03	11.29	>250	31.70	27.37	31.79	29.97	28.72
NCI-ADR	2.47	39.95	7.76	>250	34.85	26.80	40.21	30.37	>250
786-0	1.71	50.40	2.24	>250	36.43	33.82	39.25	30.36	>250
NCI-H460	2.13	>250	9.25	>250	>250	41.98	>250	54.58	>250
PC-03	2.49	40.19	2.07	>250	30.24	32.84	36.02	30.06	37.34
HT-29	1.81	41.57	12.73	>250	33.45	31.02	101.39	29.74	46.63
VERO	2.84	59.53	9.72	>250	34.25	31.53	47.77	37.81	51.38

DOX: Positive control: doxorubicin; VERO: green monkey kidney cell: non-tumor cell

The total growth inhibition (TGI) values above indicate that: 1) all the kavain derivatives were less cytotoxic than doxorubicin; 2) poor Michael acceptors such as **5-10** are less cytotoxic than goniotalamin (**4**) and, 3) kavain (**5**) was less cytotoxic than all the investigated compounds.

REFERENCES

1. a) Whitton, P. A.; *et al. Phytochemistry*, **2003**, 64, 673; b) Smith, T. E.; *et al. Org. Lett.*, **2004**, 6, 2317 and related reference; c) Pollastri, M. P.; *et al. Chem. Biol. Drug Des.*, **2009**, 74, 121.

POSTER COMMUNICATIONS – PC.460

NOVEL TRIAZINEDIONE DERIVATIVES AS GABA-B RECEPTOR POSITIVE ALLOSTERIC MODULATORS

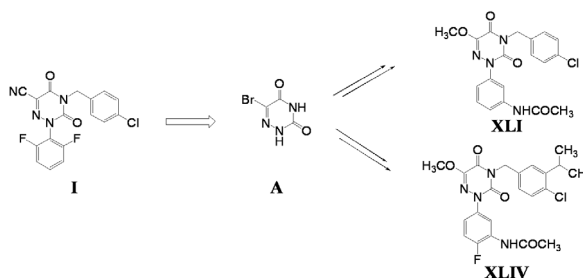
B. Campo¹, F. Girard², S. Poli¹, A. Gibelin¹, K. Mallah¹, M. Galby¹, E. Le Poul¹, J.P. Rocher¹, V. Mutel¹ and E. Riquet¹

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GABAB receptor (GABABR) represents one of the attractive targets for drug discovery efforts, and has received attention for the potential benefit of those suffering from anxiety, nociceptive pain and gastro-intestinal reflux disease (GERD), where GABAB activation has been shown to provide clinical benefit. The only marketed GABAB agonist is [R]-Baclofen, but side-effects such as sedation, muscle relaxation, hypothermia and the development of tolerance have limited its use to the treatment of spasticity in multiple sclerosis patients, and other disorders with similar symptoms of muscle rigidity, despite that it has potential in many other indications. Positive allosteric modulators (PAM)

may offer a way to avoid tolerance and dose limiting side effects in a variety of indications. PAM with affinity in the micro molar range, such as CGP7930 and GS39783, have shown better side-effect profiles compared to Baclofen.

In our drug discovery program, using functional assays, such as GTP γ ³⁵S assay on rat cortical membranes, FLIPR assay on rat and human recombinant GABABR in heterologous HEK-cell lines, we have identified dioxotriazine derivatives (Cpd Nber **I**: EC₅₀ GTP γ ³⁵S=1356nM / Efficacy 218%) that act through an allosteric mechanism, that is modulating the receptor by binding to a site different from the highly conserved orthosteric binding site. We were able to rapidly identify more potent compounds with a high efficacies in native biological assays (Cpd Nber **XLI**: EC₅₀ GTP γ ³⁵S=150nM / Efficacy 232% and Cpd Nber **XLIV**: EC₅₀ GTP γ ³⁵S=0.8nM / Efficacy 291%). We report here the synthesis starting from **A**, the structure-activity/properties relationship of brain penetrant and selective GABABR PAM compounds of this series. The pharmacological characterization, pharmacokinetic properties and *in vivo* activity in rodent model of anxiety of compound **XLI** are also given.



POSTER COMMUNICATIONS – PC.461

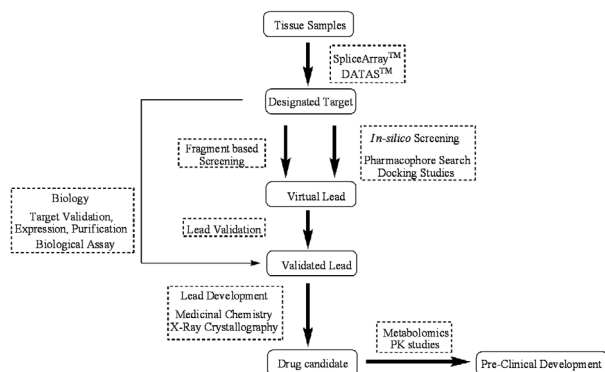
FROM GENE TO LEAD: EXONHIT'S DISCOVERY PLATFORM

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With the completion of the human genome sequencing in 2001, several thousands of genes have emerged as potential targets for disease treatment. To date, it is estimated that less than 500 targets are involved in the mechanisms of marketed drugs, leaving a huge space for innovative programs. In light of this abundance of potential targets, high-throughput filtering methods are required for rapid target selection and validation. Among these, transcriptomic analysis using microarray technologies has already proved successful to highlight, for instance, the importance of kinases (PIPK1)¹, GPCR (RAI3)² or splice variants such as osteopontin³ in breast cancer. Having developed a strong expertise in transcriptomic analysis using our proprietary SpliceArrayTM and DATASTM technologies in addition to our

bioinformatics and medicinal chemistry resources, we wish to report on a new discovery platform implemented at ExonHit to rapidly identify innovative targets and subsequent lead compounds ready for development.



REFERENCES

1. Sun et al. Breast Cancer Research **2010**, 12:R6.
2. Nagahata et al. Endocrine-Related Cancer **2005**, 12, 65–73.
3. He et al. Oncogene **2006**, 25 (15), 2192–2202.

POSTER COMMUNICATIONS – PC.462

FIRST LINE DRUG ISONIAZID BOOSTERS: A NEW STRATEGY TO FIGHT TUBERCULOSIS

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With one third of the world population infected, more than 9 million people developing the active disease and 2 million deaths each year, Tuberculosis (TB) remains a worldwide health threat.¹ Attempts to treat TB are hindered by the increasing spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* leading to less effective and more toxic drugs, longer periods of treatment and a decreasing compliance of the therapy.

Current tuberculosis therapies include a large number of prodrugs that must be metabolically activated to manifest their activity. This is the case for ethionamide (ETH) that requires activation by the mycobacterial monooxygenase EthA to be effective. This phenomenon is limited by the mycobacterial transcriptional regulator EthR and we recently showed that appropriate inhibitors of EthR can enhance *M. tuberculosis* sensitivity to ETH *in vitro* and *in vivo*.²

As ETH, isoniazid (INH) is a prodrug whose bioactivation is provided by the mycobacterial catalase-peroxidase KatG.³ KatG expression is down-regulated by FurA, a ferric uptake regulator and several groups showed that inactivation of *furA* resulted in derepression of KatG leading to an increased sensitivity of *Mycobacterium tuberculosis* to INH.³ Our strategy relies on the development of FurA inhibitors in order to boost INH bioactivation *in vivo*, to lower doses and minimize side effects, making the therapeutic regimen more efficient, improving compliance and reducing the occurrence of resistant strains of mycobacteria.

Since no crystallographic data were available at the start of the project, we developed an *in vitro* high-throughput fluorescence-based thermal shift assay (TSA) to screen our library of drug-like molecules (45 000). In parallel a functional assay was used to confirm hits. Data will be presented. Additionally, the protein has been crystallized to support hit to lead optimization.

REFERENCES

1. Global tuberculosis control: epidemiology, strategy, financing: **WHO report 2009**, WHO/HTM/TB/2009.411.
2. **Synthetic EthR inhibitors boost antituberculous activity of ethionamide** Willand N, Dirie B, Carette X, Bifani P, Singhal A, Desroses M, Leroux F, Willery E, Mathys V, Déprez-Poulain R, Delcroix G, Frénois F, Aumercier M, Loch C, Villeret V, Déprez B, Baulard AR *Nature Medicine* **2009**, 15, 537–544.
3. **Regulation of catalase-peroxidase (KatG) expression, isoniazid sensitivity and virulence by FurA of Mycobacterium tuberculosis** Molecular Microbiology Pym A, Domenech P, Honoré N, Deretic V, Cole S **2001**, 40, 879–889.

POSTER COMMUNICATIONS – PC.463

LOW MOLECULAR WEIGHT COMPOUNDS FOR THE CONTROL OF REGENERATIVE BIOLOGY AT THE INNER EAR

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Restrictions in the ability of hearing and thus to communicate affect profoundly the quality of life in nearly all vocational and social fields. Therefore defective hearing, affecting ten percent of the population of developed nations, is one of the main prob-

lems of health care in societies which are dependent on communication. The costs for the national economies caused by untreated defective hearing are presently estimated to amount to more than 75 billion Euros per year in Europe. In many cases, the reasons for defective hearing are related to the loss of sensory cells in the inner ear through ageing or loud noise. Currently, the only treatment offered is the use of hearing aids with often unsatisfactory results for the user. No curative therapy is available.

The aim of this project was the identification of low molecular weight substances which effect in the Corti's Organ a dedifferentiation to multipotent progenitor cells of the inner ear and lead thereby to restoration of hearing through de novo formation of sensory cells.

A set of 50 compounds from EMC's proprietary collection of 20,000 low molecular weight heterocyclic compounds has been selected by computational methods and has been screened in in-vitro model systems. A hit compound has been structurally optimized and tested in in-vivo models. The elucidation of the biological targets is still ongoing.

POSTER COMMUNICATIONS – PC.464

HIGH QUALITY PROTEIN DATASET FOR FRAGMENT-BASED DRUG DESIGN

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Fragment-based (FB) approaches are firmly established tools in drug discovery campaigns in which low molecular weight molecules (100-250 Da) are screened against biological targets of pharmaceutical interest¹. If compared to drug-like molecules, fragments are simpler and, as a consequence, they are ready to be functionalised in order to grow them into effective drugs. The success of FB methods can be quantified by the great number of compounds under clinical trials or in pre-clinical development that originated from them². On the downside, because of their low binding affinities for macromolecules, fragments can be assayed only by highly sensitive biophysical techniques that are expensive and quite hard to fully automatize³. To this purpose it is then tempting to prioritize fragments with well established computational techniques, such as molecular docking, before experimental testing. However, since current algorithms are typically tuned for bigger molecules with drug-like features, some issues emerge about their prompt applicability in the fragment-based field.

The main objective of this study is to provide a set of protein-fragment complexes to test the performance of standard docking softwares when dealing with fragments. To this aim, a selected dataset of diverse high quality protein complexes of pharma-

ceutical interest was retrieved from the worldwide Protein Data Bank (wwPDB)⁴ taking into account global structural features (e.g. resolution, residue completeness etc.) along with the mandatory presence of a fragment-like molecule bound. Eventually the dataset was challenged by means of self- and cross-docking procedures to address several issues such as: a) can molecular docking reproduce correctly the experimentally solved structures? b) How thorough must be the sampling in order to reproduce the experimental data? c) Are standard scoring functions able to discriminate between the native pose and the other energy minima? d) Are there classes of fragments more likely to get high or low scores regardless of the protein structure?

The dataset in a ready-to-dock format is to be made freely available to the scientific community to assess the reliability of in silico screening protocols before starting time-demanding efforts and to offer computational scientists worldwide a reliable test set to work with to develop new docking protocols purposely designed for fragment-based drug discovery.

REFERENCES

1. Chessari, G.; Woodhead, A. J. From fragment to clinical candidate—a historical perspective. *Drug Discov Today* **2009**, 14, 668.
2. de Kloe, G. E.; Bailey, D.; Leurs, R.; de Esch, I. J. Transforming fragments into candidates: small becomes big in medicinal chemistry. *Drug Discov Today* **2009**, 14, 630.
3. Carr, R. A.; Congreve, M.; Murray, C. W.; Rees, D. C. Fragment-based lead discovery: leads by design. *Drug Discov Today* **2005**, 10, 987.
4. Berman, H.; Henrick, K.; Nakamura, H. Announcing the worldwide Protein Data Bank. *Nat Struct Biol* **2003**, 10, 980.

POSTER COMMUNICATIONS – PC.465

PLEUROMUTILINS AS NEW ANTITUBERCULAR AGENTS

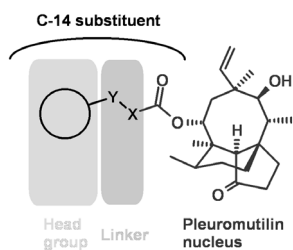
Esther Fernández, David Barros

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Pleuromutilins are inhibitors of bacterial protein synthesis having a therapeutically relevant spectrum of antimicrobial activity. They interact with the 50S bacterial ribosome subunit in a unique way and therefore there should not be pre-existing resistance mechanisms to them. The major obstacle for developing a semi-synthetic pleuromutilin for oral treatment of bacterial infections is their quick metabolism in the liver. GlaxoSmithKline has worked for several years on this family of antibiotics and now has been able to make pleuromutilins with enhanced metabolic stability. These derivatives are highly effective *in vivo* when given orally in different animal models of bacterial infections, particularly in respiratory tract infections with multi-resistant strains.

The anti-tubercular screening of GSK antimicrobial collection revealed a high rate of active compounds and this prompted us to start a Lead Optimization project. Our objective was to identify a pleuromutilin derivative active against *Mycobacterium tuberculosis*, including multidrug-resistant strains (MDR), which enable reduction of the duration of treatment. A clean CYP450 inhibition profile could be of special interest to avoid potential interactions with other anti-tuberculars used in combination therapies. This novel agent should be orally administered, well tolerated, and with low propensity to generate resistances.

We focused our chemistry effort in a new family of C14-alkoxy-carbonyl carbamates. SAR around this family led us to identify new derivatives active in the bacterial ribosome (tenths of nanomolar) and active *in vitro* against *Mycobacterium tuberculosis* (MIC < 1 µM). They have shown *in vitro* metabolic stability, good oral absorption and presented *in vivo* efficacy in animal models close to that showed by Linezolid as well as tropism to lungs. All these data will be described in detail in the poster.



POSTER COMMUNICATIONS – PC.466

DEVELOPMENT OF COGNITIVE ENHANCERS ACTING THROUGH AMPA POTENTIATION: DESIGN OF NOVEL THIENOTHIADIAZINE 1,1-DIOXIDES

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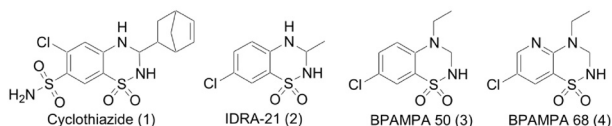
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Alzheimer's disease (AD) represents one of the greatest health problems in our industrialized countries, in view of the ageing population. Only four drugs are currently approved against AD; as these drugs have a limited time of efficacy, the need is great for additional innovative AD treatments. Besides some disease-modifying strategies currently investigated to avoid AD progress, numerous symptomatic approaches are studied, such as the cholinergic agents or the AMPA potentiators.

The AMPA receptors represent an interesting target to develop cognitive enhancers, since this subtype of ionotropic glutamate receptors (iGluRs) is involved in the expression and the maintenance of long-term potentiation, a phenomenon closely linked to

learning and memory processes. AMPA potentiators may also be of interest for the management of schizophrenia or depression.

Starting from the structure of cyclothiazide (1) and IDRA-21 (2), our team previously synthesized *in vitro* active 2,3-dihydro-4H-1,2,4-pyrido and benzothiadiazine 1,1-dioxides. BPAMPA 50 (3)¹ and BPAMPA 68 (4)², bearing a short alkyl chain in the 4-position were found to be the most interesting compounds during *in vitro* testings. Unfortunately, the *in vivo* activity of these derivatives was poor maybe due to bad pharmacokinetic properties.



In attempt to enhance the *in vivo* activity as well as to optimize the pharmacokinetic parameters, our present work focalised on the bioisosteric replacement of the benzene ring found in BPAMPA 50 by the thiophene ring. The modulation of the alkyl chain is based on the previously established structure-activity relationships. Biological evaluations are realized *in vitro* on *Xenopus* oocytes expressing AMPA receptors. The *in vitro* screening permitted to point several compounds for *in vivo* studies.

REFERENCES

1. Francotte et al., J. Med. Chem. 2007, 50, 3153-3157.
2. Francotte et al., Bioorg. Med. Chem., 2008, 16, 9948-9956.

POSTER COMMUNICATIONS – PC.467

MICROWAVE ENABLED IMPROVED SYNTHESIS OF THERAPEUTICALLY IMPORTANT NITROIMIDAZOLE DERIVATIVES

Ravi Hegde, Chandan Atreya, Vijaya Raj KK and Sudeshna Adak

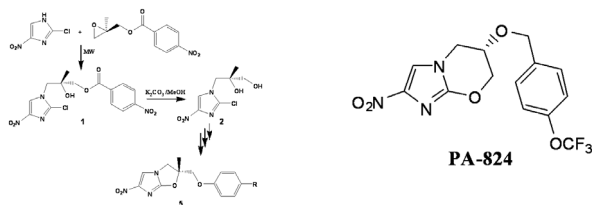
GE Healthcare MDx Research, John F. Welch Technology Centre, Bangalore, India.

Tuberculosis (TB), an infection caused by *Mycobacterium tuberculosis*, is the leading cause of death in the world. Due to pathogenic synergy with HIV and the emergence of drug-resistant and multidrug-resistant TB, the disease has been spreading at a steady rate over the last decade. There are 8 million new TB cases with 3 million deaths per year worldwide.¹ The rapid spread of TB has intensified the need for new and more effective chemotherapeutic agents with novel mechanisms of action to combat the emergence of drug resistance and shorten the duration of treatment.

The nitroimidazole series of compounds are remarkable in two aspects. First, for its spectrum of activity against Gram positive and Gram negative bacteria, protozoa, certain helminthes and *Mycobacterium tuberculosis* and second, the relatively low incidence of resistance in anaerobes. No other group of drugs seems to display such a range of action, nor the relative lack of resist-

ance.² One of the nitroimidazole derivative, PA-824 is in clinical trial for the treatment of TB.³ In another study, a series of nitroimidazole compounds were synthesized, screened and found a potent, orally active compound that is a promising candidate for the treatment of TB.⁴ Our aim was to study the effect of halo functionality at the para position of the 6 membered aromatic ring on the antimycobacterial activity. Synthetic challenges faced during the synthesis of this novel molecule and its antimycobacterial activity is discussed in this study.

The synthetic scheme for the desired set of compounds (general structure, **5**) is as shown below. As per the literature procedure,⁴ compound **1** was obtained by heating at 60-65°C for 6 hours in the presence of triethylamine. We attempted this reaction in a microwave reactor (Biotage). Surprisingly after 4 hours at 120° C we obtained compound **2** directly without isolating compound **1** thereby reducing one step in the synthesis, which could not be achieved by the conventional methods as described in literature. Initial antimycobacterial activity screening data showed similar potency as that of parent molecule.



REFERENCES

1. Science, 1992, 257, 1055.
2. J. Antimicrob. Chemother., 1993, 31, 9.
3. Nature, 2000, 405, 962. 4) J. Med. Chem., 2006, 49, 7854.

POSTER COMMUNICATIONS – PC.468

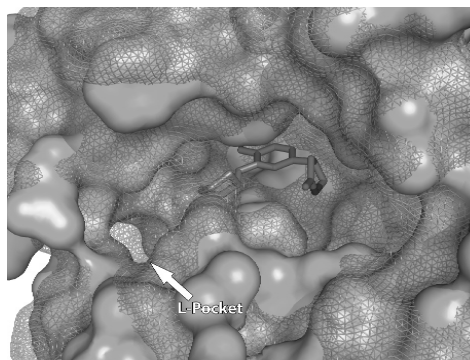
CHARACTERISATION OF PHOSPHODIESTERASES FOUND IN PARASITIC PROTOZOA

Chimed J. W. Jansen, Chris de Graaf, Rob Leurs, Iwan J. P. de Esch

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The parasitic protozoa *Trypanosoma brucei* and *Leishmania major* are endemic to the tropics and northern subtropics. Infection by these protozoa may be debilitating, or even deadly. The current treatment options are inadequate. Inhibition of the phosphodiesterases (PDEs) B1 and B2 in the protozoa appears to be a promising method by which to control the parasites.¹ Unfortunately, all tested compounds have higher affinities for human PDEs than for the parasite PDEs. In contrast a selectivity of 100 fold for the parasite PDEs, over human PDEs, has been proposed as a minimum to avoid unacceptable side effects.

To develop highly selective drugs, a thorough understanding of the structural differences between the active sites of the PDEs must be developed. To this end the sequences of the parasite and human PDEs were aligned. The crystal structures of the PDEs were compared to find structural differences. Interactions between the ligand and active sites of LmjPDEB1 and human PDE4s were assessed using Interaction FingerPrint (IFP) software. A homology model of the TbrPDEB1 sequence was built using the LmjPDEB1 crystal structure as a reference. Together these tools may be used to improve future structure based design and *in silico* screening studies aimed at finding high affinity, selective compounds for the parasite PDEs.



An overlay of crystal structure surfaces of PDE4B containing rolipram (PDB Code: 1XMY, purple) over LmjPDEB1 (PDB Code: 2R8Q, green).

REFERENCES

1. Oberholzer, M., Marti, G., Baresic, M., Kunz, S., Hemphill, A., and Seebeck, T., The *Trypanosoma brucei* cAMP phosphodiesterases TbrPDEB1 and TbrPDEB2: flagellar enzymes that are essential for parasite virulence. FASEB **2007**.

POSTER COMMUNICATIONS – PC.469

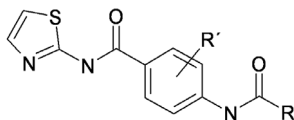
HIT TO LEAD OPTIMIZATION OF A SERIES OF N-THIAZOL-2-YL-BENZAMIDE DERIVATIVES IDENTIFIED AS A2A ANTAGONISTS

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The neuromodulator adenosine acts on a family of G protein-coupled receptors termed A1, A2A, A2B and A3. The A2A receptor is predominantly expressed in the striatum, closely co-localized with dopamine D2 receptors. The A2A receptors interact with D2 receptors, causing a decrease in affinity of D2 receptors for dopamine, upon stimulation. Thus, A2A antagonists are capable of enhancing the effect of dopamine or dopamine agonists, making A2A receptor antagonists interesting as drugs for

the treatment of Parkinson's Disease. A HTS campaign identified a series of hits. Herein, the result of a hit to lead optimization is presented. This optimization resulted in the identification of A2A antagonist lead compds., possessing good PK properties, acceptable selectivity towards A1 and with a demonstrated effect in a mechanistic in vivo model.



POSTER COMMUNICATIONS – PC.470

RATIONAL DESIGN OF POTENT CXCR1 (INTERLEUKIN-8 TYPE 1 RECEPTOR) AND C5AR (C5A RECEPTOR) INHIBITORS TARGETING NEUTROPHILS IN A RAT MODEL OF HEPATIC POSTISCHAEMIA REPERFUSION INJURY

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C5a and CXCL8 are potent chemotactic factors whose biological activities are specifically mediated by C5aR and CXCR1/2 respectively, both belong to the GPCR family and widely expressed in non-inflammatory and inflammatory cells, mainly neutrophils (PMN). PMN are implicated in many inflammatory and autoimmune diseases, including psoriasis, postischaemia reperfusion injury in organ transplantation, bullous pemphigoid and COPD. We previously described Reparixin as a new candidate drug in a novel class of small molecular weight (SMW) non competitive inhibitors that binds to CXCR1, freezing it in a inactive conformation state. Despite the low overall homology between CXCR1 and C5aR, we discovered that the allosteric site of Reparixin on CXCR1 is functionally conserved on C5aR. Combining molecular modelling techniques and single point mutagenesis experiments we rationally developed a novel class of potent SMW non competitive modulators that potently inhibited both C5a (IC₅₀ 8.0 ± 0.1 nM) and CXCL8-induced (IC₅₀ of 2.0 ± 0.4 nM) human PMN migration. The efficacy of dual inhibitors in a rat model of hepatic postischaemia reperfusion injury has been proven. In conclusion, our data highlight that dual targeting represents a promising therapeutic strategy to provide benefit in treating complex autoimmune diseases that involve C5a and CXCL8 activation.

REFERENCES

1. Allegretti M et al. Curr. Med. Chem. 2005, 12, 217.
2. Allegretti M. et al. J. Med. Chem. 2005, 48, 4312.
3. Bertini R. et. al P.N.A.S. 2004, 101, 11791.

POSTER COMMUNICATIONS – PC.471

DESIGN AND SYNTHESIS OF SUBSTRATES FOR A FLUORESCENCE POLARIZATION ASSAY FOR THE DprE1 ENZYME OF *M. TUBERCULOSIS*

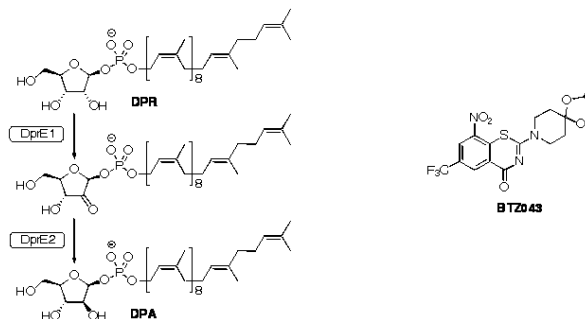
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Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE) has been recently identified as a target for 8-nitro-4*H*-benzo[e][1,3]thiazin-4-ones (BTZs), a family of compounds with potent anti-tubercular activity [1]. BTZ043 had an impressive MIC₉₉ of 1 ng/mL. Inhibition of this enzymatic activity abolishes the conversion of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA), a key precursor that is required for the synthesis of the cell-wall arabinans, thus causing bacterial death.

The assay currently used for assessing DprE activity is complex (radioactively-labeled substrates and TLC-monitoring of the formation of ¹⁴C-DPA), thus unsuitable for screening of large compound libraries. Given the chemical validation of DprE as a suitable target to kill *M. tuberculosis*, it became essential to develop an alternative assay for this enzyme, suitable for High-Throughput Screening (HTS). HTS of large compound libraries could lead to the discovery of alternative inhibitor scaffolds, which would be crucial in case BTZ043 fails in later stages of drug development.

Fluorescence Polarization (FP) assays have been increasingly used for HTS due to their simplicity and robustness. We are developing a novel FP assay for DprE, and the synthesis of two types of fluorescent ligands (to be used as substrates for the assay) is in progress, namely fluorescently-labeled BTZs and DPR analogues. We will report the synthesis of these fluorescent probes and preliminary results regarding their use in a DprE FP assay.



REFERENCES

1. Makarov, V. *et al.* - Benzothiazinones Kill Mycobacterium tuberculosis by Blocking Arabinan Synthesis, *Science* 2009, 324: 801-804.

POSTER COMMUNICATIONS – PC.472

NOVEL CFTR CHANNEL ACTIVATORS, EFFECT ON $\Delta F508$ MUTATION RESPONSIBLE FOR CYSTIC FIBROSIS

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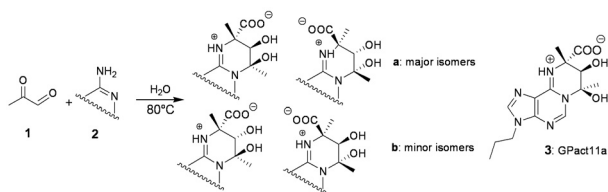
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The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein is an ABC transporter mediating Cl⁻ transepithelial transport through the apical membrane. Defective function of CFTR is responsible for Cystic Fibrosis (CF), one of the most common lethal autosomal recessive human diseases in Caucasians.¹ CFTR most common mutation $\Delta F508$ induces a trafficking- and gating-defect leading to a lack of channel activity.² Correctors such as Miglustat are able to rescue the trafficking-deficient $\Delta F508$ -CFTR to the plasma membrane.³ A bitherapy involving a corrector and an activator appears as a promising strategy for the treatment of $\Delta F508$ -CF.

In the search for molecules increasing the activity of CFTR, we describe new compounds able to selectively modulate CFTR activity. These compounds result from the condensation of methylglyoxal **1** and different 2-aminoazaheterocycles **2**.^{4,5}



During *in vitro* (iodide efflux, fluorescence imaging), *ex vivo* (short circuit current measurements) and *in vivo* (mice salivary secretion) experiments, compound GPact11a **3** appeared to be a potent activator of CFTR.⁶ This non-toxic and water soluble agent is a good candidate alone or in combination with a F508del-CFTR corrector for the development of a CFTR modulator in cystic fibrosis.

REFERENCES

1. Ratjen F., Doring G., *Lancet*, **2003**, 361, 681-689.
2. Dalemans W., Barbry P., Champigny G., Jallat S., Dott K., Dreyer D., Crystal R.G., Pavirani A., Lecocq J.-P. Lazdunski M., *Nature*, **1991**, 354, 526-528.

3. Noel S., Wilke M., Bot A.G.M., De Jonge H.R., Becq F., *J. Pharm. Exp. Ther.*, **2008**, 325, 1016-1023.
4. Routaboul C., Dumas L., Gautier-Luneau I., Vergne J., Maurel M.-C., Décout J.-L., *Chem. Comm.*, **2002**, 10, 1114-1115.
5. Décout J.-L., Routaboul C., Becq F., Norez C., 23/10/03, FR 2861304, WO 2005039589, US 20060258612. UJF/CNRS/Université de Poitiers
6. Bertrand J., Boucherle B., billet A., Melin-Heschel P., Dannhoffer L., Vandebrout C., Jayle C., Routaboul C., Molina M.-C., Décout J.-L., Becq F., Norez C., *Eur. Respir. J.*, in press.

POSTER COMMUNICATIONS – PC.473

LIGAND SELECTIVITY ON MUTATED HUMAN PROTEIN KINASES: A LITERATURE KNOWLEDGE-BASED APPROACH

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Protein kinases are important therapeutic targets for today's drug discovery as they are implicated in many diseases including cancer and metabolic disorders. Kinase mutations are often found in certain types of cancers and may explain the sensitivity of certain tumors to inhibitors molecules. Moreover, one of the main causes of treatment failure is the development of drug resistance by cancer cells due to mutations. AurSCOPE Kinase is one of Aureus Pharma's knowledgebases developed to manage chemical and biological knowledge for this target family. Chemical structures of ligands as well as precise descriptions of their associated targets and all reported *in vitro* and *in vivo* pharmacological responses have been collected from literature (articles and patents). By mining the Aureus Kinase knowledgebase, an extensive survey of the ligand activity for different wild type and mutated kinases has been performed. This study focuses on eight kinases of particular interest for cancer therapy: ABL, KIT, EGFR, ErbB2, RET, PDGFR, BRAF and PI3K.

POSTER COMMUNICATIONS – PC.474

DRUG REPURPOSING OPPORTUNITIES USING IN SILICO COMPOUND PROFILING

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Repositioning of drugs has existed since the beginning of the pharmaceutical industry through clinical observations and side-effects that could have utility in other diseases. In addition, recent advances in pharmacology and biochemistry are enabling better understanding of molecular mechanisms of diseases and

the potential to screen against a large panel of therapeutic targets. Therefore, new opportunities for drug repositioning of existing drugs, off-patent drugs or for rescue potential candidates have increased rapidly.

Aureus Pharma has developed a unique knowledge management system consisting of exhaustive databases annotated for major therapeutic drug targets, G-Protein Coupled Receptors, Ion channels, Kinases, Nuclear receptors and Proteases. Ligand chemical structures, associated activity data points as well as precise description of experimental protocols collected from published articles and patents are included in every knowledge database.

Taking benefit of the vast amount of literature data already available, *in silico* profiling of several marketed drugs (methadone, rapamycin, saquinavir and telmisartan) was performed in order to find new potential targets and therefore identify some opportunities of new therapeutic indications.

Based on chemical and 2D pharmacophoric similarity searches, *in silico* drug profiles were generated and visualized within an interactive heat map with a color code representing activity values. Detailed analysis was performed associated with the accessible current knowledge. New potential targets have been identified and discussed.

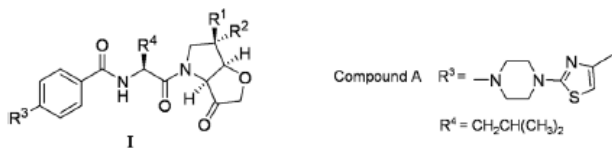
POSTER COMMUNICATIONS – PC.475

DESIGN OF CATHEPSIN K INHIBITORS WITH IMPROVED STABILITY

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In this study we report on the development of a series of cathepsin K inhibitors with the general structure I shown below.



Compound A, R^1 & $R^2 = H$ in structure I, although displaying high potency against cathepsin K and good selectivity over related enzymes (K_i : Cath K = 1.7 nM; Cath L, S, B, & H > 1000 fold selectivity for the human enzymes), was found to be reductively metabolized when subjected to whole blood stability tests. In contrast, structurally related compounds, e.g. Compound A, $R^1 = O^iPr$, $R^2 = H$, were found to be stable in the same assays and fortuitously still maintained excellent potency against cathepsin K

and selectivity against related enzymes (K_i : Cath K = 2.1 nM; Cath L, S, > 1000 fold selectivity for the human enzymes).

Herein, we describe our efforts at developing the appropriate assays and the SAR for the metabolic transformation of template I. Ultimately in this series, compounds of high stability were identified through modifications at the C-6 position of the bicycle. Whilst the overall DMPK properties of these particular compounds were unsuitable for further progression, the SAR work ultimately allowed us to design compounds with favourable overall DMPK properties both *in vitro* and *in vivo*, one of which is currently being progressed in preclinical development studies.

POSTER COMMUNICATIONS – PC.476

DEVELOPMENT OF MULTI-TASK MODELS QSAR FOR RESEARCH OF TYROSINASE INHIBITORS

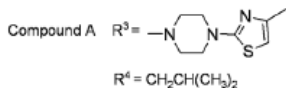
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Quantitative Structure Activity Relationship (QSAR) is a well known tool with a very large application range^[1,2]. For instance QSAR approaches are used to predict protein function^[3], antimicrobial compounds^[4], toxicity^[5] and so on. Developments of new compounds for the modulation^[7] of tyrosine hydroxylase is a major goal for the medicinal chemistry, because the product of the reaction catalyzed by the enzyme is the tyrosine, an amino acid involved in several biological processes. In fact, it is not only precursor of a wide range of hormones and catecholamines such as dopamine, noradrenaline and adrenaline, but also pigments such as melanine. In human beings, its synthesis comes from another essential amino acid, phenylalanine, which is acquired through the diet. In the suprarenal gland, tyrosine is transformed by the enzyme tyrosine in L-dopa, an intermediate in the biosynthetic pathway of dopamine, which is involved in the Parkinson. Thus, any disorder of this enzyme provokes several heavy diseases such as vitiligo, pityriasis or melanoma. In this work we develop the first multi-target (mt) QSAR to optimize the synthesis of new molecules for the modulation of the activity of the tyrosine hydroxylase^[6]. A large database of 700 compounds tested against the mushroom tyrosinase and L-DOPA has been selected from the literature. Then a mt-QSAR model was obtained that correctly classified the 80% of the compounds presents in the dataset. In conclusion, the final goal of the present model is predict the activity and direct the synthesis of new compounds with inhibitor activity against one or both enzymes.



REFERENCES

1. H. Gonzalez-Diaz, *Current topics in medicinal chemistry* **2008**, 8(18), 1554.
2. H. Gonzalez-Diaz, F. Prado-Prado, F. M. Ubeira, *Current topics in medicinal chemistry* **2008**, 8(18), 1676-1690.
3. R. Concu, M. A. Dea-Ayuela, L. G. Perez-Montoto, F. Bolas-Fernandez, F. J. Prado-Prado, G. Podda, E. Uriarte, F. M. Ubeira, H. Gonzalez-Diaz, *Journal of proteome research* **2009**, 8(9), 4372-4382.
4. H. Gonzalez-Diaz, F. J. Prado-Prado, *Journal of computational chemistry* **2008**, 29(4), 656-667.
5. H. Zhu, T. M. Martin, L. Ye, A. Sedykh, D. M. Young, A. Tropsha, *Chemical research in toxicology* **2009**, 22(12), 1913-1921.
6. S. Jawaid, T. H. Khan, H. M. Osborn, N. A. Williams, *Anti-cancer agents in medicinal chemistry* **2009**, 9(7), 717-727.
7. K. U. Schallreuter, S. Kothari, B. Chavan, J. D. Spencer, *Experimental dermatology* **2008**, 17(5), 395-404.

POSTER COMMUNICATIONS – PC.477

GROUP-III METABOTROPIC GLUTAMATE RECEPTORS: FROM A VIRTUAL SCREENING HIT TO SELECTIVE AGONISTS

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Metabotropic glutamate receptors (mGluRs) are promising therapeutic targets for several CNS disorders because of their modulatory role in glutamatergic synaptic transmission. In particular activation of presynaptic group III receptors showed beneficial effects in animal models of Parkinson's disease¹ and neuropathic pain². Large efforts were made to discover allosteric modulators of these receptors, however orthosteric agonists may also be of therapeutic value if they hold the required ADMET and selectivity properties. We thus initiated a program aiming at the discovery of such agonists. In a virtual high throughput screening of the mGlu4 receptor binding site, we identified (R)-PCEP a hit that was optimized in a series of derivatives³. Importantly the virtual hit was found to reach a new binding pocket next the glutamate binding pocket³. Thus, while it had been difficult in the past to find new orthosteric ligands of group III mGlu receptors, a large number of these new compounds were able to activate these receptors. Among them LSP1-2111 revealed a preference for subtype 4 versus 8, the two most similar group III mGluR subtypes. LSP1-2111 was then further evaluated at native receptors and in an animal model of Parkinson disease⁴. Most interestingly, it produced antiparkinsonian effects at lower doses than with

previously known agonists (e.g. L-AP4, ACPT-I¹ and (1S,2R)-APCPr⁵) and was systemically active⁴. Further optimization of the virtual hit led to LSP4-2022 that was more selective than LSP1-2111 regarding mGlu8, but also more potent at mGlu7 (Table 1). LSP4-2022 was tested in neuropathic and inflammatory pain models and abolished hyperalgesia.

Table 1. EC₅₀ values of group III mGluR agonists (μM).

	mGlu4	mGlu6	mGlu7	mGlu8
Glu	3.4	24	>500	2.4
L-AP4	0.2	1	249	0.3
ACPT-I	1.7*	10.6*	280*	5*
DCPG	3.2	3.2	>500	0.04
LSP1-1080 (S-PCEP)	6	6.7	89	6.5
LSP1-2111	2.5	3.2	101	43
LSP4-2022	0.1	2.5	4.7	38

EC50 values as determined by intracellular calcium release measurement except for * inositol phosphate accumulation. ACPT-I, 1-aminocyclopentane 1,3,4-tricarboxylic acid; DCPG, 3,4-dicarboxyphenylglycine.

REFERENCES

1. Lopez, S. et al. *J. Neurosci.* **2007**, 27, 6701-6711.
2. Goudet, C. et al. *Pain* **2008**, 137, 112-124.
3. Selvam, C. et al. *J. Med. Chem.* **2010**, 53, 2797-2813.
4. Beurrier, C. et al. *FASEB J.* **2009**, 23, 3619-3628.
5. Sibille, P. et al. *J. Med. Chem.* **2007**, 50, 3585-3595.

POSTER COMMUNICATIONS – PC.478

3-AMINOPIPERIDINE BASED PEPTIDE ANALOGUES AS CYSTEINE PROTEASE INHIBITORS

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We have synthesized a series of compounds as potential inhibitors of the cysteine protease IdeS. IdeS inhibitors are of interest for treatment of diseases such as scarlatina, sepsis and necrotizing fasciitis [1,2]. IdeS hydrolyzes the peptide bond between two glycine residues, Gly₂₃₆-Gly₂₃₇, in the antibody IgG¹. Short peptides based on the amino acid sequence of IgG covering the IdeS cleavage site are not hydrolysed. Thus, in this study such peptides have instead been used as templates for synthesis of analogues which could be tested for their inhibitory activity. The structures of the peptide analogues were based on a 3-aminopiperidine moiety see Figure 1, which was synthesized by

reductive amination using 3-piperidone and glycine. The enantiomers were separated by preparative HPLC equipped with a chiral column. The stereoselectivity of this reaction has also been studied. The core structure has been extended in both directions in order to vary the amino acid sequence for different peptide analogues. The analogues were tested for their ability to inhibit IdeS using both surface plasmon resonance (Biacore) and gel electrophoresis techniques. Interestingly, several of the synthesized compounds showed inhibitory activities.

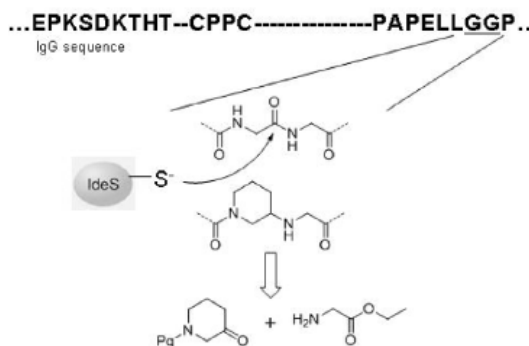


Figure 1. The sequence of the antibody IgG was used as a template for the design of novel peptide analogues. The middle core of the analogues consists of a piperidine ring and the scissile amide bond has been omitted. The synthesis of the core structure was performed via reductive amination using 3-piperidone and amino acids.

REFERENCES

1. Vincents et al., *Biochemistry*, **43**, 15540, (2004).
2. Wenig, K., *Proc. Natl. Acad. Sci. USA*, **101**, 17371, (2004).

POSTER COMMUNICATIONS – PC.479

CLASSIFICATION MODELS FOR THE IDENTIFICATION OF P-GP INHIBITORS

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P-glycoprotein (P-gp) is an efflux pump involved in the protection of tissues of several critical districts by influencing the xenobiotics disposition. Experimental evidences showed that P-gp plays a key role both in the progression of many neurodegenerative diseases and in the onset of drug resistance. On this basis, the development of new and more efficacious therapeutics targeting P-gp pump represents an intriguing challenge in drug discovery. P-gp may be considered as a valid approach to improve drug bioavailability as well as to overcome the drug resistance of many kind of tumors characterized by an over-expression of such protein. This study aimed at the development of classification models of new potential P-gp inhibitors. These models has been developed using *in vitro* data of molecules already synthesized by us and evaluated for their activity

through three systems: (i) [^{3} H]-vinblastine transport inhibition, (ii) ATP-ase activation, and (iii) apparent permeability. The batteries of statistically validated models thus obtained were addressed to the identification of “true” P-gp inhibitors, i.e. compounds able to inhibit [^{3} H]-vinblastine transport inhibition ($IC_{50} < 2$), unable to activate ATPase function and with a good permeability (BA/AB ratio) at BBB level.

POSTER COMMUNICATIONS – PC.480

SYNTHESIS AND THERMOANALYSIS OF PEPTIDE NUCLEIC ACID-CONJUGATES

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Peptide nucleic acids (PNA) (Figure 1) are DNA/mRNA analogues with considerable potential as therapeutic agents in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS).¹ However, their use is currently limited due to poor cellular uptake.

This project aims to explore a novel method of improving PNA solubility through the addition of a lipophilic carrier such as α -tocopherol, the most biologically active form of lipid soluble antioxidant Vitamin E. Various α -tocopherol analogues have been designed, synthesised and coupled to a specific PNA sequence. The PNA sequence targeted is the ALS-linked gene superoxide dismutase 1 (SOD1G93A) which has been shown as having neuroprotective properties both *in vivo* and *in vitro*.²

The current course of this work centers on the thermal analysis of PNA binding to a complimentary strand of PNA or DNA, utilising ITC and UV-Vis spectroscopy as a direct and indirect method, respectively, of determining enthalpy.

Exploring the effectiveness of a PNA-Vitamin E conjugate to improve the cellular uptake of PNA may facilitate the development of a treatment for ALS, where currently none exists.

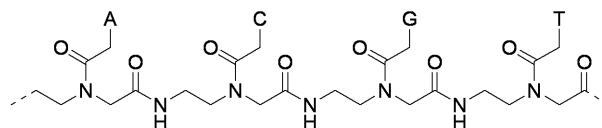


Figure 1: Schematic view of PNA backbone.

REFERENCES

1. Braasch, D. A., Corey, D. R. (2002), *Biochem.* 41, 4503-4510.
2. Turner, B. J., Cheah, I. K., Macfarlane, K. J., Lopes, E. C., Petratos, S., Langford, S. J., Cheema, S. S. (2003) *J. Neurochem.* 87, 752-763.

POSTER COMMUNICATIONS – PC.481

SYNTHESIS AND EVALUATION OF THE BIOLOGICAL ACTIVITY OF NEW MACROCYCLIC LIGANDS IN A7R5 CELL LINE

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Natural molecules have played a crucial role in the development of novel antiproliferative therapeutic drugs.¹ Amongst natural products, macrocyclic compounds clearly stand out. The chemical diversity of macrocyclic compounds is vast and provides an invaluable source of inspiration for innovative drug design.²

A recent idea for anticancer drug design is to use macrocycles for stabilized the G-quadruplexes in the human telomeric sequence resulting in inhibition of the activity of the telomerase enzyme, which act as a reverse transcriptase and catalyses, involved in the synthesis of telomeric DNA repeats, and is preferentially expressed in cancer cells.³ Usually, the quadruplex-stabilization occurs via π - π and electrostatic interactions resulting in the stack of the ligand towards the terminal G quartets.⁴ Selectivity for human telomeric quadruplexes over duplex DNA is a key requirement, which has been challenging to achieve for such polycyclic ligands, beyond minimized undesired toxicity. In our ongoing research aimed on G-quadruplex ligands, we synthesized three new macrocyclic compounds constituted of two intercalative-type moieties bridged by polyammonium linkers and were originally designed on the basis of supramolecular concepts for molecular recognition of nucleotides. These compounds have two semi-rigid scaffolds (pyrazino[2,3-f][1,10]phenanthroline and dipyrrodo[3,2-a:20,30-c] phenazine) that have some of the favorable binding properties of rigid molecules (entropy) and two amino-alkyl side chains to provide some flexibility to adapt their conformations during the binding event. Also, these aromatic moieties have the useful fluorescence properties to perform the *in vivo* fluorescence microscopic studies.

Preliminary *in vivo* studies on A7R5 rat aortic smooth-muscle cells by live-cell imaging studies revealed no signs of cytotoxicity in broad range of concentrations (10nM–100 μ M). The compounds easily cross the plasma membrane in the time scale of minutes and, by inspection of the micrographs, the compounds possible act as mitochondrial biomarkers. Subsequent STD-NMR experiments will be done to verify if the compounds bind to the mtDNA. The mtDNA will be obtained by several extractions from the cells.

REFERENCES

1. D. Obrecht, J.A. Robinson, F. Bernardini, C. Bisang, S.J. DeMarco, K. Moehle, F.O. Gombert, *Current Medicinal Chemistry*, 2009, 16, 42-65
2. A. Arola, R. Vilar, *Current Topics in Medicinal Chemistry*, 2008, 8, 1405-1415.
3. D. Monchaud, M.-P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, 6, 627–636.
4. S. Neidle, *Current Opinion in Structural Biology* 2009, 19:239–250.

POSTER COMMUNICATIONS – PC.482

STUDY OF THE BIOLOGICAL EFFECTS OF HETEROCYCLIC COMPOUNDS IN LIVING CELLS: *IN VITRO* CYTOTOXICITY ASSAY AND LIVE-CELL IMAGING MICROSCOPY

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There is a pressing need for the development of more effective and less toxic drugs for the treatment of cancer diseases. Novel and promising anti-cancer strategies have emerged over the past decade by targeting the G-quadruplex-DNA using small molecules called macrocycles.¹ The efficiency of these molecules is due to their well-known preorganization comparatively to the acyclic ligands, resulting in a perfect shape adaptation to its target.² In order to have bioactivity at cellular level essential parameters need to be achieved, such as, the ability to cross crucial membranes, the *in vitro* and *in vivo* toxicity screens, the metabolic stability, the specificity, and the strength of the binding.³ Herein we present our last results of a comparative study regarding the cytotoxicity and the ability to penetrate into the cells of four synthetic poliazamacrocycles, one cryptand and one acyclic compound. The four cationic macrocycles incorporate one or two aromatic units of pyridine and phenanthroline linked by one or two polyammonium chains, and the cryptand has three different cavities and an additional aromatic fragment. The macrocycles were synthesised by [2+2] cyclo-condensation of the respective dialdehydes and the appropriate diamines or triamines followed by reduction of the Schiff bases. The cryptand was obtained by macrocyclization in dilute conditions followed by recrystallization. The binding ability of these compounds towards ssDNA vs quadruplex was determined by Surface Plasmon Resonance experiments. They exhibit high affinity with ssDNA over quadruplex.

The cytotoxicity of the compounds was measured by MTT assay in normal human dermal fibroblasts and tumor MCF-7 cells in a range of concentrations (10nM–100 μ M) in the respective culture medium and incubation for a period of 48 h. The compounds presented low cytotoxicity when added over 48h at concentrations between 10 nM to 100 μ M, except for the acyclic compound and the macrocycle with the pyridine ring at concentration 100

μM . The entry of the compounds into the cells and their cellular localization was supported by fluorescence microscopic imaging. Overall inspection of the fluorescence images provided convincing evidence that the compounds cross the plasma membrane and are incorporated the mitochondria. To confirm the colocalization, a membrane potential-dependent mitochondrial probe (MitoTracker Red) and the compounds were incubated during 1h. The live-cell images also evidenced an intact mitochondria structure.

Although these compounds are weak G-quadruplex ligands, relatively low cytotoxicity combined with the colocalization results obtained from fluorescence images, suggest that these heterocycles can act as potential mitochondrial biomarkers.

REFERENCES

1. J. L. Huppert, Chem. Soc. Rev., 2008, 37, 1375–1384.
2. M.-P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J.-L. Mergny, A. David, J.-M. Lehn, W. D. Wilson, J. Am. Chem. Soc. 2003, 125, 4732–4740.
3. D. Monchaud, M.-P. Teulade-Fichou, Org. Biomol. Chem., 2008, 6, 627–636.

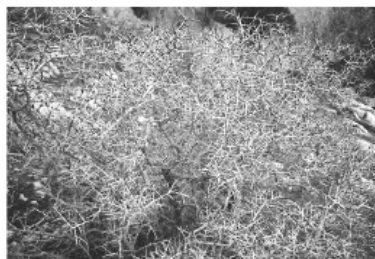
POSTER COMMUNICATIONS – PC.483

FLAVANONES FROM AMYGDALUS LYCIOIDES SPACH: ISOLATION, CHARACTERIZATION AND BIOLOGICAL EVALUATION

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As part of our research in the field of the drug discovery from plants, we recently addressed our attention to *Badam Talkh kuhi*, an endemic species extending into south Anatolia. It is commonly used by native people of Iran as folk remedy in the treatment of various diseases since ancient time. In detail, its stem and branchlets decoction have been used for managing pain and inflammatory conditions, inflammatory skin disorders and infectious diseases.¹ At first, our efforts were directed towards the investigation of the folk claims of *Badam Talkh kuhi*, which was classified by us as *Amygdalus lycioides* Spach. Different extracts were prepared using various extraction procedures combined with solvents of different polarity and their biological activity, in terms of antimicrobial activity, free radical scavenging effect and anti-TNF α property, evaluated.² Basing on biological results, the extract obtained employing Microwave-Assisted Solvent Extraction (MASE) procedure combined with *n*-hexane-acetone (*n*-Hex-Ac) as extraction mixture was selected to be further investigated.



Herein we report our progress on the phytochemical composition study of the MASE *n*-Hex-Ac extract. Its phytochemical fingerprint (HPLC-UV-PAD) will be presented and the isolation of the main phytochemicals described. Finally, the structures of isolated compounds will be elucidated by 1D-, 2D-NMR and MS spectra in comparison with literature data.

REFERENCES

1. Parsa A. Medicinal plants and drugs of plant origin in Iran IV. *Plant Foods Hum Nutr*, 1960, 7: 65–136.
2. Gaggeri R, Rossi D, Hajikarimian N, Martino E, Bracco F, Grisoli P, Dacarro C, Leoni F, Mascheroni G, Collina S, Azzolina O. *The Open Natural Products Journal*, 2010, 3: 20–25.

POSTER COMMUNICATIONS – PC.484

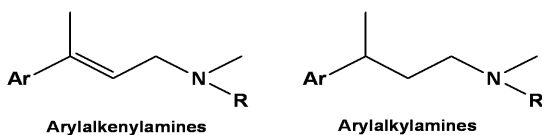
DISCOVERY OF NEW AND SELECTIVE SIGMA 1 LIGANDS POTENTIATING NERVE GROWTH FACTOR INDUCED NEURITE OUTGROWTH IN PC12 CELLS

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Sigma 1 ($\sigma 1$) receptors have been studied mostly with respect to their neuroprotective functions within the CNS. $\sigma 1$ receptor agonists reduce neuronal death associated to glutamate toxicity and promote the neurite elongation in spinal cord motor neurons.¹ Additional evidences suggest that $\sigma 1$ receptor agonists potentiate nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells.² Accordingly, our research in the field of neuroprotection has been recently focused on the design, synthesis and pharmacological evaluation of new $\sigma 1$ ligands based on arylalkenyl- and arylalkylaminic scaffolds.³ In order to extend the structure activity relationships (SAR) study of our $\sigma 1$ ligands,

novel derivatives, characterized by various aromatic nucleus combined with different aminic moieties, were prepared and their affinities towards σ , κ , μ -opioid and NMDA receptors were investigated. The drug-likeness properties of novel compounds were then assessed on the basis of their structural features by applying both the Lipinski's 'rule of five' and additional molecular descriptors. Finally, for the most interesting compounds, the potentiating effect on neurite sprouting and elongation induced by low concentration of NGF was evaluated in PC12 cells.



Synthetic procedures applied for the preparation of target compounds and biological activity investigation results will be presented and discussed.

REFERENCES

1. Guzmán-Lenis MS, Navarro X, Casas C. *Neuroscience*, 2009, 162: 31-38.
2. Takebayashi M, Hayashi TP, Su TP. *J. Pharmacol. Exp. Ther.*, 2002, 303: 1227-1237.
3. Collina S, Loddo G, Urbano M, Linati L, Callegari A, Ortuso F, Alcaro S, Laggner C, Langer T, Prezzavento O, Ronsisvalle G, Azzolina O. *Bioorg. Med. Chem.*, 2007, 15: 771-783.

POSTER COMMUNICATIONS – PC.485

CXCR3 LIGANDS: FROM SCREENING HIT TO ANTAGONISTS

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Small-molecule ligands for the CXCR3 chemokine receptor receive considerable attention as a means to interrogate the roles of CXCR3 in vitro and in vivo and/or to potentially treat various conditions (inflammatory diseases, cancer). We have developed a novel class of small-molecule antagonists for CXCR3. A medium-throughput screen revealed an adamantane-guanidine (IPAG) as a hit. The guanidine unit was replaced by a small quaternary ammonium group, leading to ca. 0.7 log unit increase in affinity. Substitution of the adamantane group by a myrtenyl moiety further increased affinity, while the benzyl group was decorated with an additional (substituted) aryl ring. This led to the identification of several bisaryl-based antagonists with CXCR3 affinities of around 100 nM.

POSTER COMMUNICATIONS – PC.486

MALDI IMAGING: A NEW TECHNIQUE OF UNLABELLED DRUGS AND METABOLITES IMAGING

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Since its inception, Matrix Assisted Laser Desorption Ionization (MALDI) imaging technique is a powerful tool for the detection directly on-tissue of many different natures of molecules such as peptides, lipids and drugs. This *in vitro* molecular imaging technique allows different studies in biomedical and pharmaceutical fields, by offering the capabilities to detect biomarker and drugs/metabolites. The added value of MALDI imaging is the quickness and sensitivity of a technique which does not need any labeling. In consequence, this technique is very fast compared to classical autoradiography technique which is used to validate the distribution of targeted drugs and metabolites.

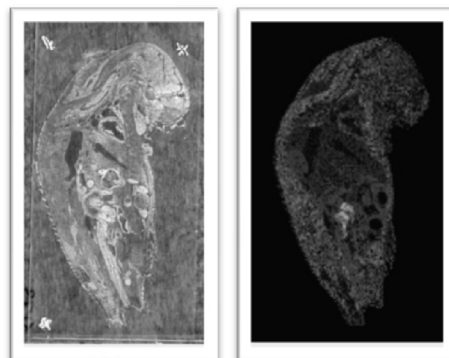


Figure 1. Mouse whole body imaging by MALDI Mass Spectrometry which shows drug distribution in green

Since few years, this technique of MALDI imaging is used to assess the absorption, the distribution, the metabolism and the elimination of drugs and metabolites (ADME). It is also a great technique for the pharmacokinetics study.

In terms of equipment, even if the main source of MALDI is used, many different analyzers are used to increase the capabilities of targeting molecules in order to image drugs and metabolites in preclinical phase of research.

In this work, we evaluate the capabilities of MALDI-TOF instruments to follow different drugs and their metabolites in whole body and specific organs like kidney and brain.

POSTER COMMUNICATIONS – PC.487

CONTROLLED RELEASE OF A NEW NITRIC OXIDE DONOR COMPOUNDS BASED ON THEOPHYLLINE AND PARACETAMOL FROM CELLULOSE/CHONDROITIN SULFATE HYDROGELS

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Nitric oxide synthesized in endothelial cells that line blood vessels has a wide range of functions vital for maintaining a healthy cardiovascular, nervous and immune systems. The most studied actions of NO are in the cardiovascular system where reduced nitric oxide availability is implicated in the initiation and progression of many cardiovascular diseases. Delivery of exogenous NO is an attractive therapeutic option, particularly with a view to slowing progression of atherosclerosis and reducing the risk of thrombosis. New compounds, in which NO donor groups are linked to the classical parent molecules have been synthesized with an improved pharmacological profile in terms of the increase of the therapeutically efficiency and of the reduction of the side effects. In our previous works, a novel nitric oxide donor, where two parent molecules namely theophylline and paracetamol linked by a nitric oxide donor chain have been synthesized.

Among polymers proposed for hydrogels preparation, polysaccharides presents some advantages over synthetic ones, being used mainly in pharmaceutical and medical fields due to the lack of toxicity, high biocompatibility and biodegradation ability¹.

Polysaccharides-based hydrogels present biomedical applications because of their tissue compatibility, mainly caused by their high water content and their soft, rubbery consistency giving them a certain resemblance to living tissue².

Hydrogels based on natural, biodegradable and biocompatible polysaccharides, such as cellulose (C) and chondroitin sulfate (CS) with various ratios between the two polysaccharides were prepared by a crosslinking technique. The swelling behaviour of the crosslinked hydrogels was followed in 90:10 water:ethanol medium, at 37 °C, where the studied drug presents high solubility.

These hydrogels have been loaded with a novel nitric oxide donor derivative (NO-donors) of 7-[2-nitroxyacetyl-oxy-3-(4-acetyl-amino-phenoxy)-propyl]-8-1,3-dimethyl-xanthine compound and its controlled release was evaluated also in 90:10 water:ethanol medium at 37 °C.

The swelling and drug release studies showed that an increase of CS content in hydrogels composition leads to a higher swelling ratio and a decrease of drug percent released and very small release rate.

The relationships between the release profiles, kinetics and matrix characteristics and/or drug properties have been established which assessed the potential of the cellulose/chondroitin sulphate as sustained release carriers.

Keywords: cellulose, chondroitin sulphate, hydrogels, nitric oxide donor compound, drug delivery

ACKNOWLEDGMENTS

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REFERENCES

1. O. Wichterle and D. Lim, *Nature*, **18**, 117 (1960)
2. L.E. Nita, A.P. Chiriac and C. Vasile, *RSCC Magazine*, **4(1)** (2004)

POSTER COMMUNICATIONS – PC.488

NEW NITRIC OXIDE RELEASING THEOPHYLLINE DERIVATIVES WITH IMPROVED PHARMACOLOGICAL PROFILE

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During the 1980s, the free radical, nitric oxide (NO), was discovered to be a crucial signalling molecule, with wide-ranging functions in the cardiovascular, nervous and immune systems. Furthermore, NO would be expected to inhibit inflammatory cell activation, preventing infiltration into the plaque and also the propagation of proinflammatory signals^{1,4}. Exogenous NO also has the ability to exert bronchodilatory effects in bronchial asthma and NO is used in the treatment of preterm children to improve lung capacity³. Organic nitrates were first used to relieve the symptoms of angina over a century ago, long before the identification of NO as an endogenous messenger and, despite limitations, they remain the most commonly used NO donor drugs in cardiovascular medicine. Recently, a range of novel NO donor drugs have been evolved as potential alternatives to conventional nitrates. New compounds, in which NO donor groups are linked to the classical parent molecules, have been synthesized². This strategy has as purpose to identify new molecules with an improved pharmacological profile in terms of the increase of the therapeutically efficiency and of the reduction of the side effects.

The aim of this study is synthesis and pharmacotoxicological evaluation of new nitric oxide releasing theophylline derivatives.

The synthesis of the NO-donors has performed in two steps. In the first step by reaction of theophylline and 8-R-theophylline with 4-(2,3-epoxy-propyl)-acetaminophen the 7-[2-hydroxy-3-(4-acetyl-amino-phenoxy)-propyl]-8-R-1,3-dimethyl-xanthine derivatives were obtained. In the second step these intermediary derivatives by reaction with chloroacetyl chloride, in tetrahydrofuran (THF) and triethylamine (TEA) were turned at corresponding chloroacetyl derivatives, that by reaction with silver nitrate in dimethylformamide medium lead to some new nitric oxide donors, 7-[2-nitroxyacetyl-oxy-3-(4-acetyl-amino-phenoxy)-propyl]-8-R-1,3-dimethyl-xanthine.

The structure of the intermediary and nitric oxide donors was confirmed by elemental analysis, spectroscopic (FT-IR and ¹H-NMR) and thermal methods. The intermediary and finally compounds were also evaluated for toxicity degree, bronchodilatory and antiinflammatory effects. The synthesized compounds are less toxic, and more active than theophylline for reduction of the induced bronchoconstriction and subacute inflammatory oedema.

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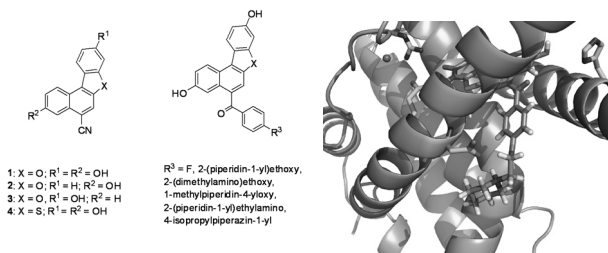
REFERENCES

1. Hamza, M., Dionne, R.A., *Curr. Mol. Pharmacol.*, **2**(1) (2009).
2. Miller, M.R., Megson, I.L., *Br. J. Pharmacol.*, **151**(3) (2007).
3. Rottier, B.L., Duiverman, E., *J. Paediatr. Respir. Rev.*, **10**(4) (2009).
4. Simpson, J.L., Phipps, S., Gibson, P.G., *Pharmacol. Ther.*, **124**(1) (2009).

(osteoporosis, HRT, hot flushes, prostate cancer) or their antagonist activity (hormone-sensitive breast cancer)¹. Recently, we have reported the design, synthesis and evaluation of their affinity for both receptors ER α and ER β of new scaffolds for the synthesis of potential SERMs (compounds **1**, **2** and **4**).²

In the present work we report the study of biological activity of these compounds and some derivatives where a basic side chain (R³) has been introduced in order to potentiate their antagonist character. The binding affinity of the synthesized compounds for both ER α and ER β was determined in a competitive binding assay using human ERs α and β expressed in baculovirus-infected insect cells. Their capacity to inhibit cell proliferation in estrogen-positive human breast cancer MCF-7 cells was also evaluated. All of them displayed interesting anti-proliferative activity.

Compounds with the highest binding affinity were assayed to determine their agonist/antagonist potency. The quantification of their functional activities was performed by using non-mammalian reporter cells that contained a resident luciferase reporter gene, and expressed ER α and ER β . The rationalization of the results as a whole, together with computational studies, will lead us in the design of improved SERMs.



REFERENCES

1. Jordan, V. C. *Nat. Rev. Cancer* **2007**, 7, 46–53.
2. Martín-Santamaría, S.; Rodríguez, J. J.; de Pascual-Teresa, S.; Gordon, S.; Bengtsson, M.; Garrido-Laguna, I.; Rubio-Viqueira, B.; López-Casas, P. P.; Hidalgo, M.; de Pascual-Teresa, B.; Ramos, A. *Organic and Biomolecular Chemistry* **2008**, 6, 3486–3496.

POSTER COMMUNICATIONS – PC.490

BIOLOGICAL ACTIVITY OF NEW ESTROGEN RECEPTOR LIGANDS: FROM SELECTIVE ER β AGONISTS TO ANTAGONISTS

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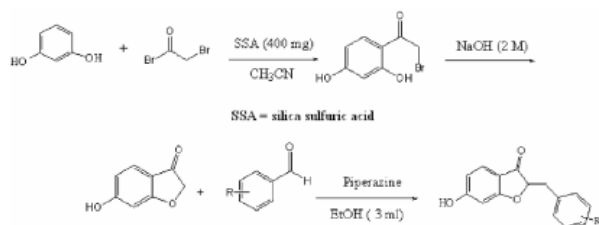
Selective estrogen receptor modulators (SERMs) are a class of compounds that interact with subsets of estrogen receptors alpha (ER α) and beta (ER β). These compounds are distinguished from estrogens by their capacity to function as both agonists and antagonists in different cell types.¹ They are used in estrogen-relative therapies, based on their agonist properties

SYNTHESIS AND INVESTIGATION OF ANTIOXIDANT ACTIVITIES OF 2-BENZYLIDENE-3-COUMARANONES

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Free radicals are produced in the normal or pathological cell metabolism. Oxidation is one of the essential processes to many living organisms to produce energy for biological processes. On the other hand, generation and uncontrolled production of reactive oxygen species (ROS) and free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis inflammation, ischemic heart disease, neurodegenerative such as Alzheimer and Parkinson diseases [1]. Flavonoids play an important role in the defense against free radicals. These compounds also show antibacterial, antiviral, anti-inflammatory, antiatherogenic properties, and preventing role in cancer, Alzheimer's, Parkinson's and cardiovascular diseases. Some of these activities are at least partially related to their antioxidant properties [2]. Aurones are very similar to flavonoids in structure and a series of aurone derivatives have been used for in vivo imaging of β -amyloid plaques in the brain of Alzheimer's disease [3]. We report our efforts towards a new heterogeneous procedure for the synthesis of 6-hydroxy-2-benzylidene-3-coumaranones and evaluate their in vitro antioxidant activities. In this project we used solid silica sulfuric acid (SSA) as a heterogeneous catalyst for the synthesis of 6-hydroxy-3-coumaranone from resorcinol and bromoacetyl bromide. The antioxidant activity was assessed using two methods, including, 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and reducing power assays. Some of the benzylidene coumaranones showed antioxidant activity more than Trolox as reference antioxidant.



REFERENCES

1. Elmastas M, Isildak O, Turkecul I, Temur N. *J. Food Compos. Anal.* **2007**, 20, 337–345.
2. Arnous A, Makris DP, Kefalas P. *J. Agri. Food Chem.* **2001**, 49, 5736–5742.
3. Ono M, Maya Y, Haratak M, Ito K, Mori H, Nakayama M. *Biochem. Biophys. Res. Commun.* **2007**, 361, 116–121.

POSTER COMMUNICATIONS – PC.492

SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND CARBONIC ANHYDRASE II INHIBITORY ACTIVITY OF BIS-SULFONAMIDES

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Sulfonamides are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases. The synergetic action of sulfonamides with trimethoprim has brought about enormous resurgence of sulfonamide usage everywhere over the last decade. We now wish to report that sulfamic acid catalyzes efficiently the reaction of arylsulfonyl chlorides with alkyl diamines under solvent-free and heterogeneous conditions and bis-sulfonamides were obtained at room temperature with high yields and purity via easy work-up procedure. These compounds were also investigated for antimicrobial (including Gram-positive cocci, Gram-negative bacilli and the yeast by disk diffusion and MICs methods), and carbonic anhydrase II inhibitory activities. The test compounds were evaluated for their antibacterial activity against three Gram-positive cocci (*Staphylococcus aureus* ATCC 25922, clinical strains of *Staphylococcus aureus* VISA (Vancomycin Intermediate *Staphylococcus aureus*) and *Enterococcus faecium*), three Gram-negative bacilli (*Pseudomonas aeruginosa* ATCC 27853, clinical strains of *Klebsiella pneumonia* and *Escherichia coli*), and the yeast (clinical strains of *Candida albicans*) using microbroth-dilution method. The obtained MICs (minimum inhibitory concentrations) values were compared to gentamicin, ciprofloxacin, sulfamethoxazole, ceftriaxone, and fluconazole as the reference drugs. Inhibition zones were determined by disc diffusion method and compared with fluconazole (25 μ g), gentamicin (10 μ g), co-trimoxazole (1.25:23.75 μ g), ceftioxin (30 μ g), imipenem (10 μ g), tetracycline (30 μ g), and azithromycin (15 μ g) as the reference drugs. The potency of test compounds for inhibition of CA II has been studied in vitro and compared to the reference drug dorzolamide (with known IC₅₀).

REFERENCES

1. Hasegawa T, Yamamoto H, *Bull. Chem. Soc. Jpn.* **2000**, 73, 423.
2. Banwell MG, Crasto CF, Easton CJ, Forrest AK, Karoli T, March DR, Mensah L, Nairn MR, O'Hanlon P J, Oldham MD, Yue W. *Bioorg. Med. Chem. Lett.* **2000**, 10, 2263.

POSTER COMMUNICATIONS – PC.493

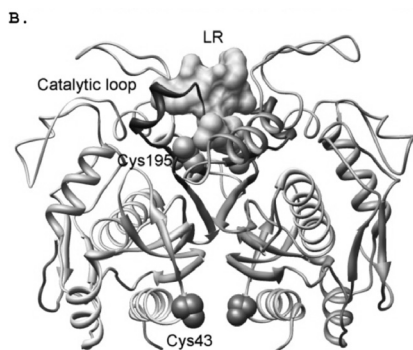
UNUSUAL TARGETING OF HUMAN THYMIDYLATE SYNTHASE INTERFACE TO ACHIEVE CIS-PLATIN RESISTANT OVARIAN CANCER CELL GROWTH INHIBITION

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Thymidylate synthase (TS) plays a key role in the biosynthetic supply of thymidylate, an essential precursor for DNA replication and repair. Indeed, in human cells, downregulation of this pathway halts cellular replication and leads to apoptosis of rapidly dividing cells, such as tumoural ones; so, hTS active-site inhibitors are largely used in cancer chemotherapy. On the other hand, resistance frequently sets in during treatment with these drugs. While the molecular bases of this phenomenon are complex, there is growing evidence that it is correlated with protein overexpression presumably connected with the loss of RNA-binding capacity when the protein is bound to its inhibitors. It is therefore desirable to identify inhibitors that act through new mechanisms, such that RNA regulation is not altered.



The design of peptides that mimic portions of the monomer-monomer interface of multimeric proteins has been shown to be a useful approach for the discovery of inhibitors that bind at such interfaces. With this approach, we have designed and synthesized several octapeptides following the antipeptide strategy. In this contribution, we wish to show how these peptides interact with hTS in vitro: the main thermodynamic and structural aspects of this interaction, as well as its consequences on the enzyme catalytic efficiency, have been determined through a multidisciplinary approach: computational design, synthesis. X-ray crystallography, fluorescence and CD spectroscopies,

calorimetry and kinetic analysis have been set to work with the common goal of identify compounds able to inhibit hTS with an uncommon mechanism. These findings suggest a new mechanism of action and provide useful information for testing against ovarian cancer cells.

We have tested the most potent peptides against ovarian cancer cells sensitive and resistant to cis-platin. The peptides were able to inhibit cancer cell growth and do not show protein over expression.

The project is supported by FP6 european grant (LIGHTS, Small ligands to interfere with Thymidylate synthase dimer formation as new tools for development of anticancer agents against ovarian carcinoma. www.light-eu.org), LSH 038752.

REFERENCES

1. Chu, E. et al. Identification of an RNA binding site for human thymidylate synthase. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 517-521 (1993).
2. Cardinale D, Salo-Ahen OM, Ferrari S, Ponterini G, Cruciani G, Carosati E, Tochowicz AM, Mangani S, Wade RC, Costi MP. Homodimeric enzymes as drug targets. *Curr Med Chem.* 2010;17(9):826-46

POSTER COMMUNICATIONS – PC.494

SYNTHESIS OF NOVEL DESIGNED UREIDOQUINOLINS AS CANDIDATE FOR ANTICANCER AGENTS

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Since selective treatment of cancer is still one of the concerning topics, continuous studies on the previously reported cytotoxic quinolines (1) led us to design novel Ureidoquinolines, (N(azolythioalkyl)-N'-(2-methyl-quinolin-4-yl) urea, as candidates for less toxic anticancer agent using computational chemistry and molecular modeling (2).

The cell culture results confirmed that their cytotoxicity against a type of breast cancer cell line (T47D) is almost comparable to Adriamycin, Etoposide and Vincristine as routine anticancer agents. Also using virtual facilities a good correlation ($r^2 = 0.64$) was resulted between the primarily designed quinolines and the model of DNA-intercalators which include groove binding plus intercalation (3). Furthermore the Ureidoquinolines' behavior against two sources of DNA was analyzed using two various spectroscopic instruments in considerable dilutions.

The obtained results encourage us to follow studying this series of compounds to discover lead quinolinic cytotoxic agents.

REFERENCES

1. Mark D.Abel, Hiep T.Luu, Ronald G.Micetich, Dai Q.Nguyen, A.Bernadette Oreski, Mark L.tempest and Mohsen Daneshlab. *Journal of .Heterocyclic Chemistry* 2006; 33,415-420.
2. S. Rasoul Amini; A. Khalaj; A. Shafiee; M. Daneshlab; A. Madadkar Sobhani; S. Fouladdel; E. Azizi, Antitumor activity of new quinoline derivatives in human breast cancer T47D cells. *International Journal of Cancer Research*. 2006; 2(2):102-08.
3. A. Madadkar Sobhani; S. Rasoul Amini ; J.D. A.Tyndall; E. Azizi; M. Daneshlab; A. Khalaj, A Theory of Mode of Action of Azolylalkylquinolines as DNA Binding Agents Using Automated Flexible Ligand Docking. *Journal of Molecular Modeling and Graphics*. 2006; 25:259-69.

POSTER COMMUNICATIONS – PC.495

DESIGN, SYNTHESIS AND ANTIBACTERIAL EVALUATION OF NOVEL BIS-AZOLE STRUCTURES

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Discovering novel antibiotics is one of still considerable topics in medicinal chemistry because of reporting multidrug resistant bacteria in clinic.

Hence three groups of bis-azole possessing structures were designed during studies on DNA intercalating compounds as antibacterial agents on the base of the primary molecular modeling studies (1). They can be categorized in (a) terazolyl; (b) triazolyl and (c) benzothiazolyl with aromatic or aliphatic substitutes.

The designed structures were synthesized from the appropriate starting materials in high yield. The synthesized compounds were characterized by IR, NMR and Mass.

Then their antibacterial efficacy was evaluated against a couple of Gram-negative (such as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Proteus vulgaris*, *Shigella sonnei*) and Gram-positive (such as *Staphylococcus aureus* and *Streptococcus epidermis*, *Bacillus subtilis*, *Bacillus hemophillous*) comparing to reference drugs by disk diffusion and MIC method.

The obtained biological results confirmed that the designed bis-azole structures have antibacterial activity and in some cases

more powerful against gram-negative bacteria rather than gram-positive bacteria. However they are still weak agents in comparison to the clinic antibiotics.

Three novel groups of bis-azole possessing structures were designed according to the primary molecular modeling. It is believed that they have suitable portions in their structures to cooperate with DNA. According to their biological results this project has already been a preliminary forwarding step in designing potent and selective bis-azole antibacterial agents.

REFERENCES

1. A. Madadkar Sobhani; S. Rasoul Amini; J.D. A.Tyndall; E. Azizi; M. Daneshlab; A. Khalaj, A Theory of Mode of Action of Azolylalkylquinolines as DNA Binding Agents Using Automated Flexible Ligand Docking. *Journal of Molecular Modeling and Graphics*. 2006; 25:259-69.

POSTER COMMUNICATIONS – PC.496

SYNTHESIS OF S-ALKYL DERIVATIVES OF 4,6-DIAMINOPYRIMIDINES AS POTENTIAL DUAL DIHYDROFOLATE REDUCTASE AND THYMIDYLATE SYNTHASE INHIBITORS AND EVALUATION OF THEIR CYTOTOXIC ACTIVITIES

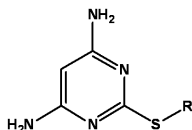
Shohreh Mohebbi, Farzad Kobarfard, Farshad H. Shirazi

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Dihydrofolate reductase (DHFR) is one of the important enzymes in both eukaryotic and prokaryotic cells. It is responsible for catalyzing the reduction of dihydrofolate (DHF) to tetrahydrofolate which is crucial for the DNA, RNA and protein synthesis. Thymidylate synthase (TS) is another vital enzyme in the process of DNA synthesis in cells. Classical DHFR inhibitors such as methotrexate contain a polar L-glutamic acid in their structures which help them to be transferred into cells by a carrier-mediated transport mechanism while non classical DHFR inhibitors need lipophilic moieties to enter into cells by passive transport mechanism. Unfortunately the nonclassical DHFR inhibitors often suffer from low potency and require co-administration of other medications to provide synergy.

In the present study a group of 4,6-diaminopyrimidine derivatives has been designed and synthesized based on the existing SAR for DHFR and TS inhibitors as potential dual TS and DHFR inhibitors and as antitumor agents. A diverse group of lipophilic side chains has been linked to the 4,6-diaminopyrimidine ring via a sulfur atom at position 2. The key intermediate in their synthesis was 4,6-diaminopyrimidine-2-thione which was conveniently attached to the various side chains through its sulfur atom using a nucleophilic substitution reaction with different alkyl halides.

Cytotoxic activities of the prepared compounds were evaluated using colonogenic and MTT bioassay tests. Significant and interesting trends of activities were observed by comparing the IC_{50} s for the homolog and analog series.



POSTER COMMUNICATIONS – PC.497

SYNTHESIS OF MONOAMINOPYRIMIDINE DERIVATIVES AS CYTOTOXIC LEAD CANDIDATES: INTRODUCING “ROUTE-BASED HIT IDENTIFICATION” APPROACH

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Different methods of hit identification have been introduced to the field of lead discovery in medicinal chemistry during the past few decades such as combinatorial chemistry (high throughput screening, HTS), virtual screening (VS), fragment-based drug discovery (FBDD). On the other hand computational and computer-aided tools have been well exploited in medicinal chemistry. The ultimate goal of all these methods is shortening the time and cutting the high cost of drug discovery process.

The main objective of this study was introducing a new approach for hit identification and SAR determination based on the synthesis route which is used for the preparation of a series of biologically active compounds.

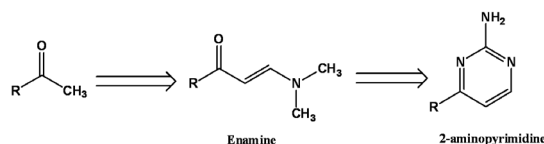
New lead candidates can be considered as chemical structures which have been synthesized from smaller building blocks using different chemical reactions. Small and simple molecules become larger and more complex during a multi-step synthesis route and the desired structure is finally constructed. Evaluation of biological activity of the starting and intermediate compounds which are used for the synthesis of a lead candidate could provide an insight regarding the structural features which are responsible for the activity in the final structure which in turn could be used for better understanding of SAR and determining the pharmacophore groups.

In the present study a three step synthesis route was used for the synthesis of a group of monoaminopyrimidines. The process involved converting a methylketone to enamine followed by cyclization of the enamine in reaction with guanidine HCl to monoaminopyrimidine derivative.

Cytotoxic activities of the intermediate enamines as well as the activities of the corresponding desired aminopyrimidines were evaluated using colonogenic assay method. The plots obtained

for determination of IC_{50} s for both enamines and aminopyrimidines groups were compared and a significant difference in their patterns indicated the emergence of cytotoxic activity after conversion of enamines to aminopyrimidines. This observation re-emphasizes the key role of aminopyrimidine ring in imparting cytotoxic activity in this group of compounds. Further modification of aminopyrimidines and tracking the pattern on change in the cytotoxic activity of them would provide more insights to the SAR for these compounds.

Based on the results obtained in this study, it could be concluded that “synthesis route-based hit identification” could be introduced as a new approach along with HTS and FBDD to make the process of hit identification shorter, less costly and more productive.



POSTER COMMUNICATIONS – PC.500

ANTIMICROBIAL ACTIVITY OF NOVEL PYRIMIDINOPHANES

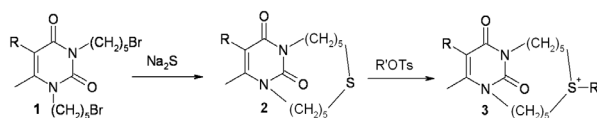
A.E. Nikolaev, V.E. Semenov, R.Kh. Giniyatullin, A.D. Voloshina, V.S. Reznik

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Hundreds and thousands of biologically active compounds contain pyrimidine units. Thus, we consider pyrimidine containing macrocycles – pyrimidinophanes – to be promising compounds: the ability of the pyrimidine unit to bind with the specific sites of almost all substrates (DNA, RNA, peptides) through a variety of non-covalent interactions can result in a unique biological activity.

Earlier we've shown that pyrimidinophanes do possess high biological activity, comparable with that of reference drugs, and demonstrate a wide spectrum of antimicrobial activity [1].

Here we present the synthesis of another type of pyrimidinophanes and the initial studies of the biological activity. Reaction of 1,3-bis(ω -halogenoalkyl)uracils **1** with a slight excess of Na_2S in DMF at 100-110°C afforded a series of pyrimidinophanes **2**. S atoms in bridges were converted to sulfonium ions by the reaction with alkyl tosylates.

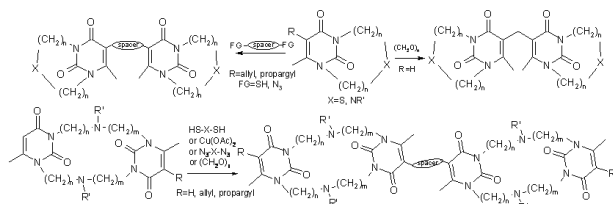


We discovered that some of the macrocycles **3** show high antimicrobial activity and selectivity (MIC for the *S. aureus* is almost 1000 times lower than for the others). Acyclic analogues are

much less active which confirms the basic hypothesis: macrocyclic structure has greatly affected the activity.

We wanted to increase the activity further and tried to obtain nanosized structures containing various pyrimidinophanes with high activity. We supposed that a compound, consisting of several "monomeric" pyrimidinophanes linked together in some way, would demonstrate higher activity and/or selectivity.

We've developed several routes towards such structures. We used paraform to cross-link the macrocycles containing 6-methyluracil and a variety of routines (coupling of acetylenes with $\text{Cu}(\text{OAc})_2$, azide-alkyne cycloaddition, thiol addition, etc.) was used to cross-link allyl or propargyl substituents.



Synthesized dimeric pyrimidinophanes were alkylated and the resulting onium salts were tested towards a series of bacteria and fungi, obtained interesting results will be discussed.

Financial support of the Russian Foundation for Basic Research (project #10-03-00365-a) and the President's grant for young Russian researchers (project MK-64480.2010.3) is acknowledged.

REFERENCES

1. V.E. Semenov *et al.* // Eur. J. Med. Chem. 2006. Vol. 41. P.1093-1101.

POSTER COMMUNICATIONS – PC.501

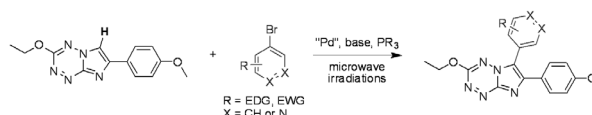
IMIDAZOTETRAZINES : SYNTHESIS, REACTIVITY, PHARMACOMODULATIONS AND FIRST BIOLOGICAL RESULTS

L. Pellegatti,¹ E. Vedrenne,¹ J. M. Leger,² C. Jarry,² S. Routier^{1*}

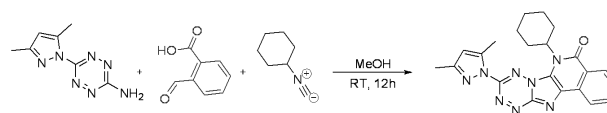
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Nitrogen-bridgehead heterocycles containing an imidazole ring are scaffolds which are widely used to design bioactive molecules. Indeed motifs, such as imidazo[1,2-*a*]pyridines, imidazo[1,2-*a*]pyrimidines, are interesting cores found in compounds showing a wide range of biological activities.^[1] Among these heterocycles, imidazo[1,2-*b*][1,2,4,5]tetrazines raised, surprisingly, little attention: only a few reports on this core were published in the literature. Based on the interests of our group in the synthesis of biologically active compounds, we thought that imidazo[1,2-*b*][1,2,4,5]tetrazines could be considered as aza-bioisosters of imidazopyridines or imidazopyrimidines.

We thus envisioned the development of a new methodology giving access to a library containing such functionalized imidazo[1,2-*b*][1,2,4,5]tetrazines, through a regioselective palladium-catalyzed (het)arylation as a key step.^[2]



Quinolines-Based Tetracycles, bearing an imidazo[1,2-*b*][1,2,4,5]tetrazine core, could also be directly synthesized through a Groebke-Blackburn type multicomponent reaction. Those compounds were obtained in excellent yields.



Those methodologies should be suitable for the design of more complex heterocyclic structures, such as bioactive derivatives containing an imidazo[1,2-*b*][1,2,4,5]tetrazine core.

REFERENCES

1. (a) Gudmundsson, K. S.; Williams, J. D.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **2003**, 46, 1449-1455; (b) Kaminsky, J. J.; Doweiko, A. M. *J. Med. Chem.* **1997**, 40, 427-436; (c) Rival, Y.; Grassy, G.; Michael, G. *Chem. Pharm. Bull.* **1992**, 40, 1170-1176; (d) Abiegente, E. *Actual. Chim. Ther.* **1991**, 18, 1253-1257; (e) Byth, K. F.; Culshaw, J. D.; Green, S.; Oakes, S.; Thomas, A. P. *Bioorg. Med. Chem. Lett.* **2004**, 14, 2245-2248.
2. Jensen, M. S.; Hoerrner R. S.; Li, W.; Nelson D. P.; Javadi G. J.; Dormer P. G.; Cai D.; Larsen R. D. *J. Org. Chem.* **2005**, 70, 6034-6039.

POSTER COMMUNICATIONS – PC.502

PREPARATION OF (HET)ARYLTETRAHYDROINDOLIZINONES DERIVATIVES AS PROTEIN KINASE INHIBITORS

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Protein kinases, and in particular Cyclin Dependant Kinases (CDKs), play vital roles in the regulation of the cell division cycle (G1 to S phase) by controlling the transition between the different phases of the process.¹ Deregulation of the cell cycle causes an uncontrolled cell proliferation that has been strongly linked to the molecular pathology of cancer.² One consequence is the

contribution of research groups to design small-molecule inhibitors of CDKs. In this area tetrahydropyrrolo[2,1-*a*]isoindol-5-one framework **I** (Figure 1) is described to bind to the enzymes and fully inactivate them in the nanomolar range.³

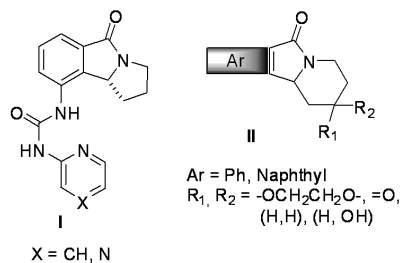


Figure 1. CDK inhibitors **I** and tetrahydropyrrolo[1,2-*a*]isoindolone **II**.

Here in, we describe the preparation of fused (Het)Aryltetrahydroindolizinones *via* *N*-acyl iminium intermediates. Two different routes were explored to achieve the synthesis⁴. The first one consists in the intramolecular reaction of β -hydroxylactams, whereas the second route is an intermolecular condensation between a 2-formylester or β -alcoylactone and an appropriate primary amine **1**. We also developed two heterocyclic strategies to obtain the adequate unavailable starting materials in phenyl, naphthyl, pyridine, pyrazine, quinoline and quinaxoline series and then performed all inter or intramolecular reactions. Scope and limitations are given.

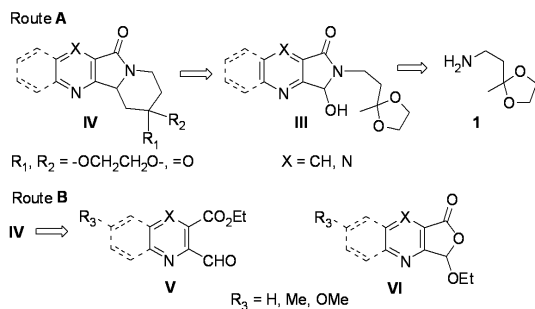


Figure 2. Retrosynthetic Scheme.

REFERENCES

- Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. in: *Molecular Cell Biology*, Garland, New York, 4th ed., **2002**, chapter 17.
- (a) Toogood, P. L. *Med. Res. Rev.* **2001**, *21*, 487-498; (b) Huwe, A.; Mazitschek, R.; Giannis, A. *Angew. Chem. Int. Ed.*, **2003**, *42*, 2122-2138
- Chiurato, M.; Routier, S.; Troin, Y.; Guillaumet, G. *Eur. J. Org. Chem.* **2009**, *18*, 3011-3021.
- Chiurato, M.; Boulahjar, R.; Routier, S.; Troin, Y.; Guillaumet, G. *Tetrahedron*. 2010

POSTER COMMUNICATIONS – PC.503

NEW APPLICATIONS IN CANCER THERAPY: DESIGN OF LIGANDS FOR CATHEPSIN L

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During the last decade cathepsins (in particular cysteine proteases) were shown to be involved in a variety of physiological and pathological processes, such as maturation of the MHC class II complex, bone remodeling, keratinocyte differentiation, atherosclerosis. Moreover there is a strong evidence for participation in cancer, tumor invasion and metastasis (1). Recent studies showed that cathepsin L, in particular, is involved in these last processes. Joyce (2) used inhibitors consisting of small molecule as probes to show that cathepsin activity (including the one of cathepsin L) is increased during tumorigenesis and the inhibition of the cathepsin function, instead, decreases tumor development.

Therefore cathepsin L was recently suggested to be an attractive target for the development of anticancer agents (3,4). In this work we describe the design of cathepsin L inhibitors for potential applications in cancer therapy.

Several compounds giving rise to a virtual library were designed on the basis of SAR data obtained from the literature. The compounds were screened by means of molecular docking onto the cathepsinL binding site. Some of them, particularly promising, were further optimized through MD simulations. Among them a compound was found to be able of engaging a covalent bond with the Cys25 residue of CathepsinL, as required for a relevant inhibitory activity. As future development of this work, the predicted interactions with the biomolecular target will be validated by using NMR techniques.

REFERENCES

- Kuester D., Lippert H., Roessner A., Krueger S. The cathepsin family and their role in colorectal cancer. *Pathology research and practice* **2008**; 204: 491-500.
- Joyce J., Baruch A., Chehade K., Meyer-Morse N., Giraudo E., Tsai F., Greenbaum D., Hager J., Bogoy M., Hanahan D. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell* **2004**; 5: 443-453.
- Beavers M., Myers M., Shah P., Purvis J., Diamond S., Cooperman B., Huryn D., Smith III A. Molecular docking of Cathepsin L inhibitors in the binding site of Papain *J. Chem. Inf. Model* **2008**; 48: 1464-1472.
- Vicik R., Busemann M., Gelhaus C., Stiefl N., Scheiber J., Schmitz W., Schulz F., Mladenovic M., Engels B., Leippe M., Baumann K., Schirmeister T. Aziridine-Based Inhibitors of Cathepsin L: Synthesis, Inhibition Activity, and Docking Studies. *Chem Med Chem* **2006**

POSTER COMMUNICATIONS – PC.504

ALLOSTERIC MODULATIONS IN
CONDITIONALLY PERMISSIVE NUCLEAR
RECEPTOR COMPLEXES: THE CASE OF
FXR/RXR HETERODIMER*Andrea Carotti, Antonio Macchiarulo, Daniele Bellocchi,
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In the fine regulation of several biological systems, a pivotal role is played by the allosteric communication of macromolecules, and in particular by the allosteric modulation across protein complexes.¹

Well known examples include the trans-cellular signal mediation operated by G-protein coupled receptors, the phosphorylation dependent activity of tyrosine-kinase, the cooperative binding of oxygen molecules to haemoglobin subunits, and the transcriptional effects of nuclear receptors.²

As far as it concerns nuclear receptors, the Farnesoid X Receptor (FXR, NR1H4) represents an interesting case study. FXR belongs to the class of metabolic nuclear receptors and is endogenously activated by bile acids (BAs). Upon BAs activation, FXR binds a set of target genes either as monomer or heterodimer, using the Retinoid X Receptor (RXR, NR2B1) as binding partner. This interaction leads to the regulation of a number of physiological functions, including BA homeostasis, lipid and glucose metabolism.³

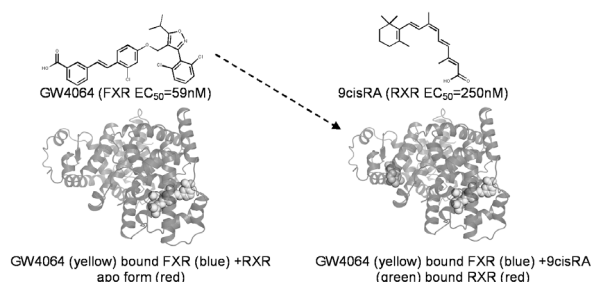
Two major classes of ligands have so far been reported as FXR modulators, comprising steroidal and non-steroidal compounds. While GW4064 (**1**) represents one of the most active non-steroidal compounds,⁴ 6ECDCA (**2**, INT-747) is a potent steroidal FXR agonist, now moving into Phase III clinical studies for primary biliary cirrhosis (PBC).⁵

As part of our ongoing research project aimed at understanding the molecular basis of FXR modulation,^{6,7} we were interested in gaining insights into the role of the heterodimer partner, RXR, in affecting the ligand dependent activation of FXR.

Indeed, while the activity of FXR-RXR heterodimer was formerly described as permissive,⁸ few papers have shown that the ligand dependent activity of FXR can be antagonized by RXR agonists, leading to define FXR-RXR complex more properly as conditionally permissive.⁹

Accordingly, in this communication we report molecular dynamic simulations of two different FXR-RXR heterodimeric systems aimed at elucidating the cross-talk between FXR and RXR.

While the results are instrumental to further understand the molecular basis of agonist-induced FXR activation, the investigation of specific conformations of the heterodimer will prove valuable to design and identify novel potent and selective FXR modulators.



REFERENCES

1. Del Sol A. et al. *Structure*. 2009, 8,1042-50.
2. Shulman A. I. et al. *Cell* 2004, 116, 417-29.
3. Wang Y. D. et al. *Histol. Histopathol.* 2008, 23, 621-7.
4. Akwabi-Ameyaw A. et al. *Bioorg. Med. Chem. Lett.* 2008, 15, 4339-43.
5. Costantino G. et al. *Bioorg. Med. Chem. Lett.* 2003, 11, 1865-8.
6. Costantino G. et al. *J. Med. Chem.* 2005, 9, 3251-9.
7. Meyer U. et al. *J. Med. Chem.* 2005, 22, 6948-55.
8. Repa J. J. et al. *Science* 2000, 289, 1524-9.
9. Kassam A. et al. *J. Biol. Chem.* 2003, 278, 10028-32.

POSTER COMMUNICATIONS – PC.505

SYNTHESIS AND DOCKING STUDIES
OF SOME NOVEL 1,4-DIHYDROPYRIDINE
DICARBOXAMIDES AS POTENTIAL
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Multidrug resistance (MDR) is a problem in cancer therapy. Anticancer drugs are transported out of the cells by trans-membrane ATP-dependent efflux pumps which mediate the cellular efflux of structurally diverse compounds across the cell membranes. This prevents the drug to reach the therapeutic intracellular concentrations and diminishes the effectiveness of drugs. It was shown that 1,4-dihydropyridines (DHPs) have reversing effects on MDR. Because of the cardiovascular activity, common DHPs didn't have the chance to be introduced into clinic as MDR reversing agents¹. Here we report the synthesis and docking studies of some 1,4-dihydropyridine dicarboxamides as potential MDR reversing agents. The synthesized compounds contain different aromatic rings, fuaryl, thienyl and substituted imidazolyl, at the C-4 position and aryl carboxamide groups at C-3 and C-5 positions of the 1,4-dihydropyridine ring. Lacking structural requirements for cardiovascular activity makes these compounds good candidates for this study.

1,4-dihydropyridine dicarboxamide derivatives were prepared by a multi-component single step reaction between one mole of aldehyde, two moles of N-arylacetoacetamide derivatives and one mole of ammonium acetate according to our previous reports². Substituted imidazole aldehydes which provide the C-4 substituent of the dihydropyridine ring were synthesized through a three-step reaction. N-arylacetoacetamides were prepared by the condensation of diketene acetone adduct (2,2,6-trimethyl-1,3-dioxane-4-one) with appropriate aryl amines. In the last part of synthesis procedure, the desired dihydropyridines were prepared by the one pot Hantzsch reaction. All molecules were drawn and optimized with Hyperchem 7.0 software using MM+ and PM3 calculation methods. Autodock 4.0 was used to investigate binding site and binding energy of ligands on MDR1 molecule. PDB code (2hyd) for MDR1 protein was taken from www.PDB.org. Kollman charges for protein and Gasteiger-Marsili partial charges for ligands were calculated by Autodock tools 4.0. Grid maps were calculated using Autogrid 4.0. Lamarckian genetic algorithm (LGA) with step sizes of 0.2Å⁹ for translation and 5.0 degree for orientation was preferred. Torsions were chosen and the maximum number of energy evaluation was set to 1.5×10⁶. For each of 200 independent runs, a maximum number of 27000 GA operators were generated on a single population of 150 individuals. Operator weights for crossover, mutation, and elitism were default parameters (0.8, 0.02, 1 respectively). Synthesis of all of the compounds was confirmed after structural elucidation by ¹HNMR, FT-IR and mass spectral data. A ligand that had the lowest binding energy and the best binding site was selected. Its lowest binding energy was -10.14 Kcal/mol and the molecule had the most hydrogen binding with active site. It belonged to run 90, and the Van der Waals and electrostatic energies of the molecule were -12.56 and 0.06 Kcal/mol, respectively. According to the obtained results, Van der Waals energy is more negative than electrostatic energy. So Van der Waals forces are more important than electrostatic forces in ligand binding to receptor. It is in accordance with hydrophobic properties of active site pocket and our experimental results.

REFERENCES

1. Voigt B., Coburger C., Monar J., Hilgeroth A. Structure–activity relationships of novel N-acyloxy-1,4-dihydropyridines as P-glycoprotein inhibitors. *Bioorg. Med. Chem.* 2007; 15: 5110–5113
2. Fassihi A., Azadpour Z., Delbari N., Saghaie L., Memarian H.R., Sabet R., Alborzi A., Miri R., Pourabbas B., Mardaneh J., Mousavi P., Moenifard B., Sadeghi H. Synthesis and Antitubercular activity of Novel 4-Substituted imidazolyl-2,6-dimethyl-N³,N⁵-bisaryl-1,4-dihydropyridine-3,5-dicarboxamides. *Eur. J. Med. Chem.* 2009; 44: 3253–3258.

POSTER COMMUNICATIONS – PC.509

DESIGN AND SYNTHESIS OF NEW TYROSINE KINASE INHIBITORS

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Angiogenesis is a critical process that is implicated in growth, metastasis of tumor cells and many other pathological conditions. It has been demonstrated that the vascular endothelial growth factor (VEGF) and its receptors, especially receptor 2 (VEGFR2) play key role in the process of neo vascularisation. Then the inhibition of the VEGFR2 kinase activity will result in the reduction of angiogenesis and suppression of tumor growth. Several classes of small molecule-based VEGFR-2 kinase inhibitors merged as promising anti-angiogenic agents for the treatment against a wide variety of cancers. Recently, a small molecule drug that inhibit VEGFR-2 kinase, Sorafenib (BAY-43-9006) and Sunitinib (SU-11248) were approved by the FDA for the treatment of renal and gastrointestinal cancer. These molecules bind reversibly to the ATP site of their targets (tyrosine kinase receptors) and inhibit their catalytic domain. To understand their binding mode with the VEGFR2, we have docked the molecules into the binding pocket, and the complex obtained with each molecule was used to determine the main protein-ligand interactions.

Based on known kinase inhibitors and the molecular modeling, we have developed new series of tyrosine kinase inhibitors.

Key words: Angiogenesis, molecular modeling, tyrosine kinase, docking, 3D-QSAR.

POSTER COMMUNICATIONS – PC.510

DISSOLUTION OF MAGNESIUM FLUORIDE, TECHNICAL AND CRYSTALS, IN ACID SOLUTION

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The study of a compound's solubility is of paramount importance in pharmacology, especially in therapeutics and materia medica. The lower solubility of the compound would be an obstacle for testing biological or physiological effects in animals or humans and maybe other applications. Both fluoride and magnesium play a dynamic role in many biological reactions and

processes. In fact, in respiratory physiology, both had relaxing effects on bronchial smooth muscle [1, 2]. So, it is interesting to study the combination of the two elements (to form magnesium fluoride (MgF_2)) despite the fact that MgF_2 is less soluble. The solubility was estimated to $10^{-5} \text{ mol/l}^{-1}$ [3].

The aim of this study was to prove that the MgF_2 could be more soluble in acid environment.

Dissolution of magnesium fluoride (MgF_2) in its two forms technical and crystals had been studied in the presence of acetic acid and under certain conditions. The solutions obtained were filtered through a Millipore filter diameter = $0.22 \mu\text{m}$ and the pH of the filtrate obtained adjusted a value of 6.5. A determination acid residue has been done to accurately determine the acidity of the solution obtained. The magnesium concentration was quantified by ionometry and the fluoride by a specific fluoride ion electrode. A solution containing MgF_2 dissolved in NaCl 9% was used as control.

The results indicated that the dissolution of MgF_2 (technical and crystals) was markedly improved ($p < 0.05$) with the presence of acetic acid compared to control solution (MgF_2 dissolved in NaCl 9%). The total molar concentration of magnesium and fluoride increased by adding the acetic acid. The solubility of MgF_2 crystals was improved by 5 and the solubility of MgF_2 technical was improved by 6 compared to the control solution.

Acetic acid is a good product solubilization of MgF_2 . These results would be useful for understanding properties of magnesium fluoride and for perhaps therapeutical applications in the field of smooth muscle pharmacology.

REFERENCES

1. K. L. Gourgoulisian, G. Catziparasidis, A. Chatziefthimiou, P.A. Mlyvdas. Magnesium as a relaxing factor of airway smooth muscle. *J Aerosol Med.* 2001;14: 301-307.
2. W. Zhao, H. Guénard. The inhibitory effect of fluoride on carbachol-induced bovine bronchial contraction. *Respir Physiol.* 1997;108: 171-179.
3. Y. Fovet and J. Y. Gal. Formation constants b2 of calcium and magnesium fluoride at 25°C. *Talanta.* 2000; 53: 617-626.

POSTER COMMUNICATIONS – PC.512

SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF NOVEL BIGINELLI PYRIMIDINES

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Biginelli pyrimidines, 4-aryl substituted 3,4-dihydropyrimidine-5-carboxylate derivatives, are important molecules in drug discovery and medicinal chemistry research. They are easily pre-

pared in a one pot reaction which is catalyzed by a Lewis acid catalyst¹. Biginelli pyrimidines have a diverse range of pharmacological properties such as anti-inflammatory, analgesic, and calcium channel modulating activity. Some derivatives have been screened as antitumor agents. They have also been studied as potential antiviral agents. Nitracin, a 3,4-dihydropyrimidine derivative, has exhibited modest antibacterial activity². Some other derivatives have been reported to be appropriate antifungal agents against *C. albicans*, *A. fumigatus* and *Penicillium marneffe*. In this study it was decided to prepare and determine the antimicrobial activity of some novel Biginelli pyrimidines.

A series of 20 novel 3,4-dihydropyrimidine-2-ones with thienyl or substituted imidazolyl groups at C-4 and different carboxamide or ester derivatives at C-5 were prepared by Biginelli reaction: 1.0 mol of the proper aldehyde, 1.3 mol of urea or thiourea and 1.0 mol of appropriate acetoacetate ester or acetoacetamide derivatives were refluxed in absolute ethanol using ferric chloride as a Lewis acid catalyst. Substituted imidazole aldehydes which provide the C-4 substituent of the 3,4-dihydropyrimidine ring were synthesized through three reactions: Preparation of the properly substituted imidazole ring, then methylation of mercapto group at the C-2 position of the imidazole ring, and finally oxidation of the hydroxymethyl to aldehyde group. N-aryl-acetoacetamides were prepared by the condensation of diketene acetone adduct (2,2,6-trimethyl-1,3-dioxine-4-one) with appropriate aryl amines³. Condensation of diketene acetone adduct with appropriate alcohols resulted in the desired acetoacetate esters.

Synthesis of all of the compounds was confirmed after structural elucidation by ¹HNMR, FT-IR and mass spectral data. Minimum inhibitory concentrations (MICs) were determined by Micro plate Alamar Blue® Assay method against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*. The synthesized compounds showed mild activity against gram negative bacteria. They were moderate to strong antifungal agents.

REFERENCES

1. O'Reilly B.C., Atwal K.S. Synthesis of substituted 1,2,3,4-tetrahydro-6-methyl-2-oxo-5-pyrimidinecarboxylic acid esters: The Biginelli condensation revisited. *Heterocycles* 1987; 26:1185-1192
2. Kappe CO. 100 years of the Biginelli dihydropyrimidine synthesis. *Tetrahedron* 1993; 49: 6937-6963.
3. Fassihi A., Azadpour Z., Delbari N. Saghaie L., Memarian H.R., Sabet R., Alborzi A., Miri R., Pourabbas B., Mardaneh J., Mousavi P. Moeinifard B., Sadeghi H. Synthesis and Antitubercular activity of Novel 4-Substituted imidazolyl-2,6-dimethyl-N³,N⁵-bisaryl-1,4-dihydropyrimidine-3,5-dicarboxamides. *Eur. J. Med. Chem.* 2009; 44: 3253-3258.

POSTER COMMUNICATIONS – PC.515

FROM MODELLING TO ACTIVITY - NOVEL SIRTUIN INHIBITORS WITH A THIOBARBITURATE STRUCTURE

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Sirtuins are NAD⁺ dependent class III histone deacetylases which cleave off acetyl groups from acetyl-lysines in histones and other non-histone proteins like e.g. p53¹, tubulin, FOXO proteins and HIV tat.² Seven members (Sirt1-7) with different cell-localization are known to catalyze deacetylase reactions in humans regulating different physiological processes like apoptosis, cell differentiation, DNA recombination and HIV transactivation but are also involved in neurodegenerative diseases. Besides nicotinamide, sirtinol³ and cambinol only a limited number of sirtuin inhibitors is known. Cambinol was discovered from random screening and for the first time anticancer activity in an animal model could be demonstrated using this compound.⁴ We have identified already new sirtuin inhibitors from various structural classes, for example the bisindolylmaleinimide Ro31-8220⁵, suramins⁶ and splitomicins.⁷ We present here the results from a combined approach using a pharmacophore based screening of a large commercial database (Chembridge, 330,000 compounds) and a subsequent protein-based docking procedure of the preselected hits. The combination of docking and in vitro experimental testing resulted in the identification of novel sirtuin inhibitors with a thiobarbiturate structure with activity in the low micromolar region.⁸ The in vitro assays are based on a fluorescent assay that detects aminomethylcumarin (AMC), a fluorophore containing product formed by a first deacetylation step by human sirtuins followed by a second enzymatic cleavage by trypsin. For inhibitor testing, we used E. coli expressed recombinant human GST-tag Sirt1, His-tag Sirt2 and His-tag Sirt3. Starting out from the most potent compounds we synthesized analogues via Knoevenagel condensation and, in some cases, we did this after prior Suzuki cross-coupling. We could then verify low micromolar inhibitory potency. Further tests in inhibitor treated HeLa cells also showed local tubulin hyperacetylation.

REFERENCES

1. Vaziri et al. (2001) Cell 107:149-59
2. Pagans et al. (2005) PLOS Biol. 3:e41
3. Grozinger et al. (2001) J.Biol.Chem. 276:38837-43
4. Heltweg et al. (2006) Cancer Res. 66:4368-4377
5. Trapp et al. (2006) J.Med.Chem. 49:7307-16
6. Trapp et al. (2007) ChemMedChem 10:1419-1431

7. Neugebauer et al. (2008) J.Med.Chem. 51:1203-1213

8. Uciechowska, Schemies et al. (2008) ChemMedChem 12:1965-1976

POSTER COMMUNICATIONS – PC.516

CDC7 KINASE INHIBITORS: 5-HETEROARYL-3-CARBOXAMIDO-2-ARYL PYRROLES AS POTENTIAL ANTICANCER AGENTS

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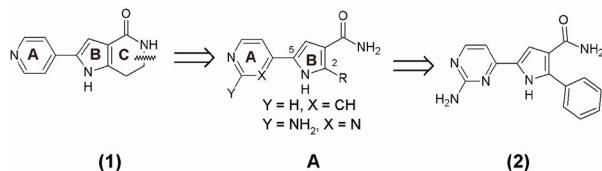
The Cell Division Cycle 7 (Cdc7) serine/threonine kinase is a key player in the regulation of DNA replication in eukaryotic organisms. It promotes DNA replication origin firing by phosphorylating one or more subunits of the MCM DNA helicase complex (Mcm2-7), thus leading to the unwinding of double-stranded DNA at replication origins.^{1,2} Cdc7 inhibition through siRNAs or prototype small molecule inhibitors causes p53 independent tumor cell death, while simply reversibly blocks cell cycle progression in primary fibroblasts,^{3,4} therefore implying that Cdc7 kinase could be considered an interesting target for anti-cancer therapy.

We previously reported on the pyrrolopyridinone chemical class,^{5,6} a source of potent and selective Cdc7 kinase inhibitors (as compound **(1)**, IC₅₀ = 10 nM in a Cdc7/Dbf4 kinase assay and greater than 20-fold selectivity vs a panel of other kinases).

In this poster we report on further evolution of the previous series, through the opening of the lactamic ring, thus yielding a new chemical class of 5-heteroaryl-3-carboxamido-2-substituted pyrrole derivatives (general structure **A**), that offers clear advantages in term of chemistry diversification and synthetic simplification. We present the synthesis, structure-activity relationships (SAR), and biological characterization of a series of compounds belonging to this new chemical class.

In particular this work led to the identification of an interesting lead compound **(2)**, with biochemical data and ADME profile similar to those of compound **(1)**, but characterized by superior

efficacy in *in vivo* models. Compound (**2**) when evaluated in a s.c. A2780 xenograft model by oral administration (90 mg/Kg TID for 5 days) showed a TGI = 86% with demonstration of *in-vivo* target inhibition.



REFERENCES

1. Jiang, W. *et al. EMBO J.* **1999**, *18*, 5703-5713.
2. Montagnoli, A. *et al. J. Biol. Chem.* **2006**, *281*, 10281-10290.
3. Montagnoli, A. *et al. Cancer Res.* **2004**, *64*, 7110-7116.
4. Montagnoli, A. *et al. Nature Chem. Biol.* **2008**, *4*, 357-365.
5. Vanotti, E. *et al. J. Med. Chem.* **2008**, *51*, 487-501.
6. Menichincheri, M. *et al. J. Med. Chem.* **2009**, *52*, 293-307.

POSTER COMMUNICATIONS – PC.518

IN SILICO METHODS FOR DEVELOPMENTAL TOXICITY: IMPROVING ACCURACY BY USING MULTIPLE PREDICTIONS

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Reproductive and developmental toxicity testing will potentially account for a large proportion of the testing costs associated with drug development. Therefore, the application of non-testing *in silico* approaches is hugely attractive in terms of savings in both money and animal usage. At present there are a number of computational methods which can be used to predict reproductive toxicity potential, ranging from traditional (Q)SAR models to category formation and read-across approaches. However, given the limited mechanistic knowledge in this area, coupled with the complex nature of reproductive toxicity endpoints, performance of these models is often poor. As a result, the use of a Weight-of-Evidence (WoE) approach is both warranted and beneficial in

terms of increasing prediction confidence. Using a publicly available dataset of 290 compounds, the current study explores the performance of a number of methods to predict developmental toxicity, consisting of (Q)SAR models, read-across predictions and predictions from rule-based expert systems. In addition the implementation of a WoE strategy is also explored, for which strengths and weaknesses are clearly identified. As expected, this study demonstrates the limited success of current modelling methods when used in isolation. However, the study also indicates that when used in a WoE setting, better use may be made of the limited toxicity data available and overall predictivity can be increased. The funding of the European Union 6th Framework OSIRIS Integrated Project (GOCE-037017-OSIRIS) and the ReProText Integrated Project (LSHB-CT-2004-503257) are gratefully acknowledged.

POSTER COMMUNICATIONS – PC.519

THE DERIVATION OF A NOVEL SET OF CHEMISTRY BASED ALERTS FOR DNA BINDING

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The aim of this study was to develop a mechanistically based profiler to account for covalent DNA binding. The profiler has been developed using mechanistic chemistry principles based firmly on deriving the chemistry associated with DNA binding. The analysis reviewed five structural alert compilations related to mutagenicity and genotoxic carcinogenicity in order to define the electrophilic reaction chemistry domains. In addition, mechanistic information was gathered from data related to idiosyncratic drug toxicity. This resulted in a DNA profiler featuring 57 structural alerts corresponding to a wider coverage of the mechanistic chemistry related to covalent DNA binding than has been previously published. The resulting mechanistic chemistry has been subjected to expert review and incorporated into the OECD QSAR Application Toolbox. The key use of the profiler will be in the development of chemical categories to aid in data gap filling when toxicological information is missing. The funding of the European Chemicals Agency (ECHA) Service Contract No. ECHA/2008/20/ECA.203 is gratefully acknowledged.