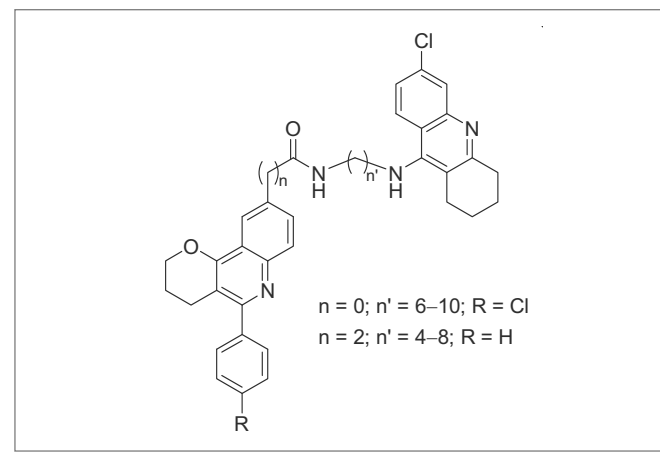
SYNTHESIS, PHARMACOLOGICAL EVALUATION, AND MOLECULAR MODELING OF A NOVEL FAMILY OF 6-CHLOROTACRINE-BASED DUAL BINDING SITE ACETYLCHOLINESTERASE INHIBITORS

Carles Galdeano (1), Pelayo Camps (1), Xavier Formosa (1), Diego Muñoz-Torrero (1), Lorena Ramírez (1), Elena Gómez (2), Nicolás Isambert (2), Rodolfo Lavilla (2), Albert Badia (3), M. Victòria Clos (3), Manuela Bartolini (4), Francesca Mancini (4), Vincenza Andrisano (4), Mariana P. Arce (5), M. Isabel Rodríguez-Franco (5), Óscar Huertas (6), Thomai Dafni (6), and F. Javier Luque (6)

(1) Laboratori de Química Farmacèutica (Unitat Associada al CSIC), Facultat de Farmàcia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, (2) Institute for Research in Biomedicine, Barcelona Science Park, and Laboratori de Química Orgànica, Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain, (3) Departament de Farmacologia, Terapèutica i Toxicologia, Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Spain, (4) Department of Pharmaceutical Sciences, Alma Mater Studiorum, Bologna University, Bologna, Italy, (5) Instituto de Química Médica (CSIC), Madrid, Spain, (6) Departament de Fisicoquímica, Facultat de Farmàcia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain

Two isomeric series of dual binding site acetylcholinesterase (AChE) inhibitors have been designed, synthesized, and tested for their ability to inhibit AChE, butyrylcholinesterase, AChE-induced and self-induced β-amyloid (Aβ) aggregation and β-secretase (BACE-1). The new hybrids consist of a unit of 6-chlorotacrine and a multicomponent reaction-derived pyrano[3,2-c]quinoline scaffold as the active-site and peripheral-site interacting moieties, respectively, connected through an oligomethylene linker containing an amido group at variable position. Molecular modeling studies have confirmed the dual site binding of these hybrids, which retain the potent and selective human AChE inhibitory activity of the parent 6-chlorotacrine, while exhibiting a significant *in vitro* Aβ anti-aggregating effect and BACE-1 inhibitory activity, thus constituting promising anti-Alzheimer drug candidates.



The research was supported by Dirección General de Investigación of Ministerio de Ciencia y Tecnología and FEDER (projects CTQ2008-03768/PPQ, BQU2006-03794, SAF2008-05595, SAF2006-04339, and SAF2006-01249), Comissionat per a Universitats i Recerca of the Generalitat de Catalunya (projects 2005-SGR00180 and 2005-SGR00662), MIUR (PRIN 2007), Rome (Italy) and EU's 7FP funding (NMP-2007-1.1-1; GA n. 214538) and by fellowships for Carles Galdeano from Institut de Biomedicina (IBUB) of the University of Barcelona and Óscar Huertas from Ministerio de Ciencia y Tecnología.

POSTER COMMUNICATIONS – PC.02

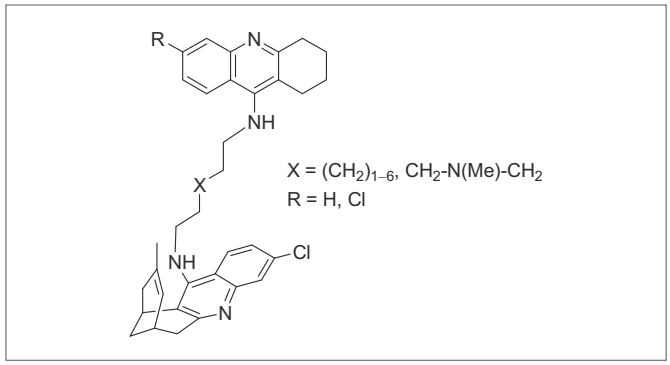
HUPRIN–TACRINE HYBRIDS AS A NOVEL FAMILY OF MULTI-TARGET DRUG CANDIDATES AGAINST ALZHEIMER'S AND PRION DISEASES

Diego Muñoz-Torrero (1), Pelayo Camps (1), Xavier Formosa (1), Carles Galdeano (1), Elisabet Viayna (1), Albert Badia (2), M. Victòria Clos (2), Marta Pera (2), Míriam Ratia (2), Belén Pérez Fernández (3), Manuela Bartolini (4), Francesca Mancini (4), Vincenza Andrisano (4), Gema C. González-Muñoz (5), M. Isabel Rodríguez-Franco (5), Lucie Rivail (6), and F. Javier Luque (6)

(1) Laboratori de Química Farmacèutica (Unitat Associada al CSIC), Facultat de Farmàcia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, (2) Departament de Farmacologia, Terapèutica i Toxicologia, Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Spain, (3) Departament de Farmacologia, Terapèutica i Toxicologia, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain, (4) Department of Pharmaceutical Sciences, Alma Mater Studiorum, Bologna University, Bologna, Italy, (5) Instituto de Química Médica (CSIC), Madrid, Spain, (6) Departament de Fisicoquímica, Facultat de Farmàcia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain

Several years ago, a short series of racemic huprine–tacrine hybrids was developed as a new class of dual binding site acetylcholinesterase (AChE) inhibitors[1]. These compounds consisted of: i) a unit of huprine Y, a compound with one of the highest affinities for the active site of AChE yet reported, ii) a unit of tacrine, a compound with known affinity for the peripheral site of AChE, and iii) a linker of appropriate length to allow simultaneous binding to both the active and peripheral sites of the enzyme.

Recently, the series of huprine–tacrine hybrids has been extended with the synthesis of shorter and longer homologues. Also, the two enantiomers of two of the most potent racemic huprine–tacrine hybrids have been independently synthesized from readily available (–)- and (+)-huprine Y, and their binding mode has been studied by molecular dynamics simulations. Moreover, additional pharmacological and pharmacokinetic studies have been undertaken. Among other properties, some huprine–tacrine hybrids have been shown to be able to significantly inhibit the AChE-induced Aß and prion peptide aggregation, two key pathogenic processes involved in AD and in prion diseases, and seem to be able to cross the blood–brain barrier.



The research was supported by Dirección General de Investigación of Ministerio de Ciencia y Tecnología and FEDER (projects CTQ2008-03768/PPQ, SAF2008-05595, SAF2006-04339, and SAF2006-01249), Comissionat per a Universitats i Recerca of the Generalitat de Catalunya (project 2005-SGR00180), MIUR (PRIN 2007), Rome (Italy) and EU's 7FP funding (NMP-2007-1.1-1; GA n. 214538) and by a fellowship for Carles Galdeano from Institut de Biomedicina (IBUB) of the University of Barcelona.

1. Camps, P.; Formosa, X.; Muñoz-Torrero, D.; Petrignet, J.; Badia, A.; Clos, M.V. J. Med. Chem. 2005, 48, 1701-1704.

NOVEL DONEPEZIL-BASED INHIBITORS OF CHOLINESTERASES AND ACETYLCHOLINESTERASE-INDUCED β-AMYLOID AGGREGATION

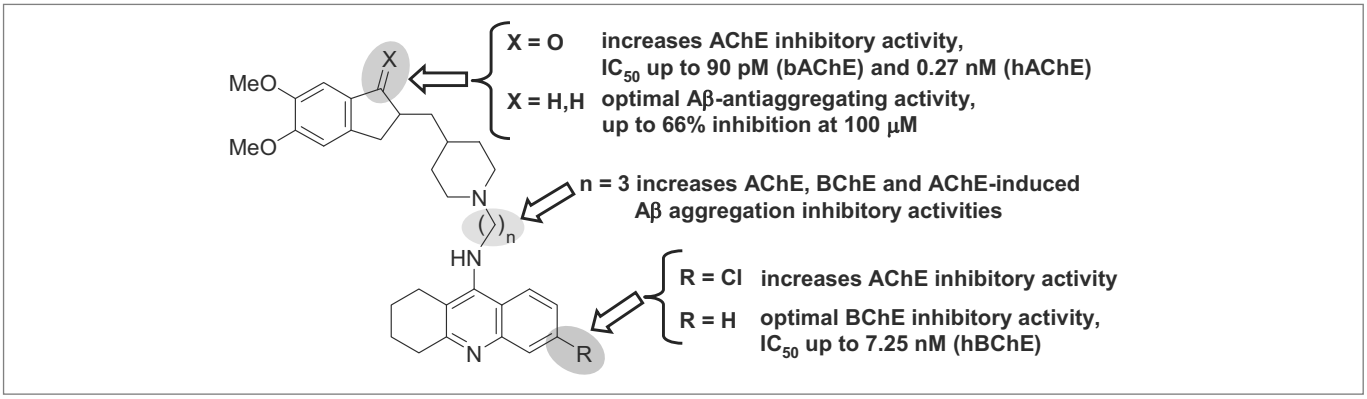
Elisabet Viayna (1), Pelayo Camps (1), Xavier Formosa (1), Carles Galdeano (1), Tània Gómez (1), Diego Muñoz-Torrero (1), Michele Scarpellini (1), Albert Badia (2), M. Victòria Clos (2), Antoni Camins (3), Mercè Pallàs (3), Manuela Bartolini (4), Francesca Mancini (4), Vincenza Andrisano (4), Joan Estelrich (5), Mònica Lizondo (5), Axel Bidon-Chanal (5), and F. Javier Luque (5)

(1) Laboratori de Química Farmacèutica (Unitat Associada al CSIC), Facultat de Farmàcia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, (2) Departament de Farmacologia, Terapèutica i Toxicologia, Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Spain, (3) Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain, (4) Department of Pharmaceutical Sciences, Alma Mater Studiorum, Bologna University, Bologna, Italy, (5) Departament de Fisicoquímica, Facultat de Farmàcia, Institut de Nanociència i Nanotecnologia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain

A novel series of donepezil–tacrine hybrids designed to simultaneously interact with the active, peripheral and midgorge binding sites of acetylcholinesterase (AChE) have been synthesized and tested for their ability to inhibit AChE, butyrylcholinesterase (BChE), and AChE-induced β-amyloid (Aβ) aggregation. These compounds consist of a unit of tacrine or 6-chlorotacrine, which occupies the same position than tacrine at the AChE active site, and the 5,6-dimethoxy-2-[(4-piperidinyl)methyl]-1-indanone moiety of donepezil (or the indane derivative thereof), whose position along the enzyme gorge and the peripheral site can be modulated by a suitable tether which connects tacrine and donepezil fragments. All of the new compounds are highly potent inhibitors of bovine and human AChE and BChE, exhibiting IC_{50} values in the subnanomolar or low nanomolar range in most cases. Moreover, six out of the eight hybrids of the series, particularly those bearing an indane moiety, exhibit a significant Aβ antiaggregating activity, which makes them promising anti-Alzheimer drug candidates[1].

The research was supported by Dirección General de Investigación of Ministerio de Ciencia y Tecnología and FEDER (projects CTQ2005-02192/BQU, CTQ2008-03768/PPQ, SAF2006-04339, CTQ2005-09365, and SAF2005-01604), Comissionat per a Universitats i Recerca of the Generalitat de Catalunya (projects 2005-SGR00180, 2001-SGR00216, and 2005-SGR00893), and by a fellowship for Carles Galdeano from Institut de Biomedicina (IBUB) of the University of Barcelona.

1. Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia, A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3588-3598.



POSTER COMMUNICATIONS – PC.04

COMBINING MOLECULAR DYNAMICS SIMULATION AND DOCKING: AN ALTERNATIVE APPROACH FOR THE VIRTUAL SCREENING OF DNA GYRASE B INHIBITORS

Liane Saíz-Urra (1,2), Matheus Froeyen (2), Eveline Lescrinier (2), Miguel Ángel Cabrera Pérez (1)

(1) Chemical Bioactive Center, Central University of Las Villas, Santa Clara, Villa Clara, C.P. 54830, Cuba. (2) Laboratory for Medicinal Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

Currently, infectious diseases cause a death toll around 2 million people a year encouraging the search for new antimicrobial agents.

DNA gyrase is a well-established antibacterial target consisting of two subunits, GyrA and GyrB, in a heterodimer A2B2. The first one is involved in DNA breakage and reunion and the second one catalyzes the hydrolysis of ATP.

Here, is reported the exploration of the conformational space of this enzyme, specifically of the GryB subunit. Molecular dynamics (MD) simulations were carried out on two complexes expressed in E. coli, 1EI1 (PDB code) with the inhibitor ADPNP and 1KZN with clorobiocin (CBN), as well as the unliganded systems. The main difference between the complexes is the conformation of the flexible loop (residues 97-119) which is closed with ADPNP and open with CBN. AMBER package version 10 was used for the MD simulations.

Clustering based on the average-linkage algorithm provided a reduced number of structures from the trajectories to be analyzed. Based on the calculation of the rms of these structures we compared the unliganded systems and the complexes. This analysis showed how the inhibitors stabilize the active site including the flexible loop.

Thus, we focused on the average structure from the MD of the 1KZN-CBN system to be used as docking template with Autodock4 software. CBN, novobiocin (NOV) and the cyclothialidine GR122222X (GRX) were docked starting from a random optimized conformation. The results show that it is possible to reproduce the interactions reported in the literature, hence this protocol can be used for further screening of new compounds.

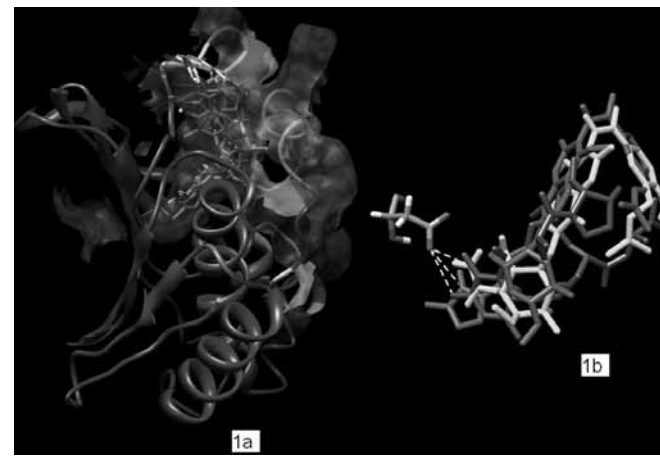


Figure 1. a) Average structure of the enzyme in complex with docked compounds. Temperature factors of the residues are shown in colours, blue for the most stable fragments and red for the most flexible ones. **b)** Hydrogen bonds between docked structures and Asp73 residue. CBN in red, NOV in green and GRX in blue

1. Monaghan, R. L.; Barrett, J. F., Antibacterial drug discovery—then, now and the genomics future. Biochem Pharmacol 2006, 71, (7), 901-9
2. Maxwell, A., DNA gyrase as a drug target. Trends Microbiol 1997, 5, (3), 102-9.

INCORPORATION OF GLUCOSE NUCLEOTIDES INTO A DNA DUPLEX BY A POLYMERASE

Marleen Renders, Mikhail Abramov, Matheus Froeyen, Piet Herdewijn

Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

The enzymatic incorporation in DNA of six-membered ring nucleoside triphosphates – in particular the 6′-triphosphates of (β-D-glucopyranosyl)thymine, (2′,3′-dideoxy-β-Dglucopyranosyl)thymine, (3′,4′-dideoxy-β-D-glucopyranosyl)thymine and (2′,3′-dideoxy-β-D-glucopyranosyl)adenine – was investigated. Elongation of the DNA duplex with all four nucleotide analogues by Vent (exo(-)) polymerase was observed , despite the facts that the pyranose nucleic acids obtained by polymerisation of these monomers do not hybridise in solution with DNA and that the geometry of a DNA strand in a natural duplex differs from that of a pyranose nucleic acid. Molecular modeling showed that hydrogen bonds are formed when 2′,3′-dideoxy-β-homo-T building blocks or β-D-gluco-T building blocks are incorporated opposite adenosine residues in the template but not when they are incorporated opposite thymine residues in the template. The model shows a near perfect alignment of a secondary hydroxy group at the end of the primer and the α-phosphate group of the incoming triphosphate. These experiments provide new information on the role of the active site of the enzyme in the polymerisation reaction and could help in the design of new inhibitors.

POSTER COMMUNICATIONS – PC.06

NEW 4,5-DIHYDRO-*1H*-PYRAZOLE DERIVATIVES AS POTENTIAL NITRIC OXIDE SYNTHASE INHIBITORS

M. Encarnación Camacho (1), M.Dora Carrión (1), Luisa C. López-Cara (1), Esther García (1), Mariem Chayah (1), Antonio Entrena (1), Miguel A. Gallo (1), Antonio Espinosa (1), Ana López (2), Germaine Escamez (2), and Darío Acuña-Castroviejo (2)

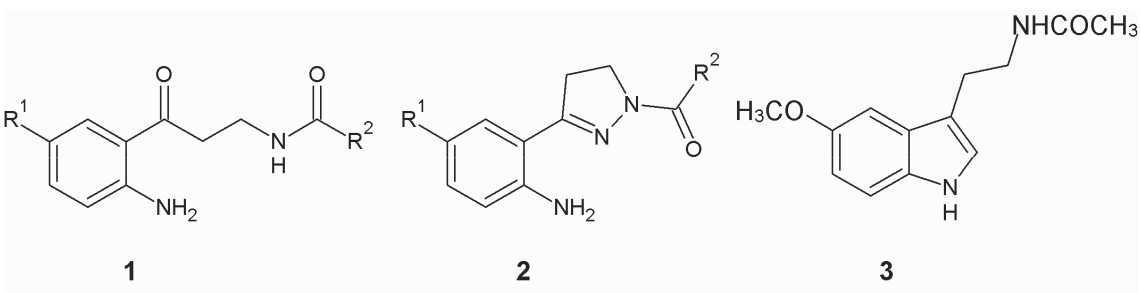
(1) Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, 18071 Granada (Spain). (2) Instituto de Biotecnología, Centro de Investigaciones Biomédicas, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada

Nitric oxide (NO) is produced by oxidation of *L*-arginine[1] in an NADPH- and O2-dependent process catalyzed by isoforms of nitric oxide synthase (NOS), and three isoforms of this enzyme have been identified (nNOS, eNOS, and iNOS)[2]. An NO overproduction by nNOS is implicated in strokes, migraine headaches, Parkinson's disease, and Alzheimer's disease, while NO overproduction by iNOS has been associated with tissue damage, inflammation, rheumatoid arthritis, and the onset of colitis[2,3]. Consequently, selective inhibition of these enzymes by means of synthetic derivatives has became an interesting objective in the potential treating these diseases.

In previous papers, we have described the synthesis of several kinurenamine derivatives **1**[4] and 4,5-dihydro-1*H*-pyrazole derivatives **2**[5], as new types of nNOS inhibitors. The last compound behaves as a rigid analogue of the main brain metabolite of the hormone melatonin **3**, a known NOS inhibitor[6]. Compounds **1** and **2** don't show any inhibitory activity against the Kynurenine 3hydroxylase (KYN3OH), and hence, the potential neuroprotective activity is only due to the nNOS inhibition.

In this communication, the synthesis of a new group of 4,5-dihydro-*1H*-pyrazole derivatives **2** is described. In these molecules**,** two main modifications have been carried out: 1) activation or deactivation of the aromatic ring with different substituents, 2) Modification of the acyl group in position 1 of the ring pyrazole. By means of these modifications we aim to get high NOS inhibition values in 4,5-dihydro-1*H*-pyrazole derivatives.

1. Stuehr, D.J.; Griffith, O.W. *Adv. Enzymol. Relat. Areas Mol. Biol*. **1992**, *65*, 287.
2. (a) Knowles, R. G.; Palacios, M.; Palmer, M: R. J.; Moncada, S. *Proc. Natl. Acad. Sci. USA* **1989**, 86, 5159; (b) McCall,T. B.; Boughton-Smith, N. K.; Palmer, R. M. J.; Whittle, B. J. R.; Moncada, S. *Biochem. J*. **1989**, 261, 293.
3. Ijuin, R.; Umezawa, N.; Higuchi, T. *Bioorganic and Medicinal Chem*. 2006, *14*, 3563.
4. 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.
5. 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.
6. (a) Bettahi, I.; Pozo, D.; Osuna, C.; Reiter, R. J.; Acuña-Casttroviejo, D.; Guerrero, J. *J. Pineal Res*. **1996**, *20*, 205; (b) Leon, J.; Vives, F.; Crespo, E.; Camacho, E.; Espinosa, A; Galllo, M. A.; Escames, G.; Acuña-Castroviejo, D. *J. Neuroendocrinol*. **1998**, *10*, 297.



1 **2** **3**

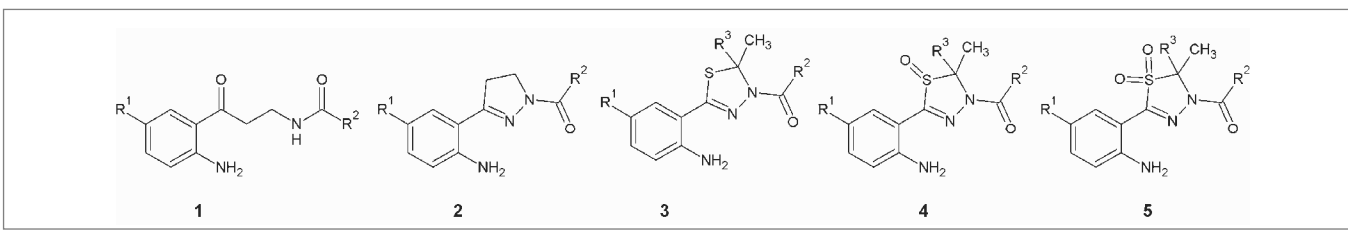
SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-PHENYL-2,3-DIHYDRO-1,3,5- THIAZOLINES AND THEIR OXIDATION DERIVATIVES AS NEURONAL AND INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITORS

Luisa C. López-Cara (1), Antonio Entrena (1), M. Encarnación Camacho (1), M. José Pineda de las Infantas (1), M. Dora Carrión (1), Miguel A. Gallo (1), Antonio Espinosa (1), Germaine Escames (2), Ana López (2), Darío Acuña-Castroviejo (2)

(1) Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, 18071 Granada (Spain).
(2) Instituto de Biotecnología, Centro de Investigaciones Biomédicas, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada

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. An overproduction of NO produces toxicity, and this fact has been associated with several disorders. In mammals, NO is synthesized from *L*-arginine in various cell types by a family of nitric oxide synthase (NOS) isoenzymes[1]. Three NOS isoenzimes are classically known: the constitutive endothelial (eNOS) and neural (nNOS) isoforms, and the inducible isoform (iNOS)[3,4]. More recently, the existence of both constitutive and inducible mitochondrial NOS (cmtNOS and i-mtNOS, respectively) isoforms[3,4], situated in the internal membrane of the mitochondria, has been proven[5]. In previous papers, we have described the synthesis of several kynurenamine derivatives **1**[5] and 4,5-dihydro-1*H*-pyrazole derivatives **2**[6], as a new type of neural nitric oxide synthase (nNOS) inhibitors. Herein we report the synthesis and biological evaluation of a series of new 2-phenyl-2,3-dihydro-1,3,5-thiazolines and their oxidation derivatives **3**, **4** and **5,** respectively**.** The presence of the S atom increases the lipophilicity and simultaneously restricts the conformational freedom in compounds **3**. Sulfone and sulfoxide derivatives are used to improve the SAR information.

1. (a) Sessa, W. C. et al *Circ. Res*. **1994**, *74*, 349; (b) Awolesi, M. A. et al; *Clin. Invest*. **1995**, *96*, 1449. (c) Knowles, R. et al. *Proc. Natl. Acad. Sci.* USA **1989**, *86*, 5159.
2. (a) Schini, V, et al. *Pharmacol. Exp. Ther.* **1992**, *261*, 553; (b) Forstermann, U. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1788.
3. Cho, H. J et al *.J. Exp. Med*. **1992**, *176*, 599.
4. (a) Frandsen, U. et al. *Biochem. Biophys. Res. Commun*. **1996**, *227*, 88. (b) López, L. C.; et al. *Int. J. Biochem. Cell B*. **2006**, *38*, 267.
5. Entrena, A. et al.; *J. Med. Chem.* **2005**, *48*, 8174.
6. Camacho, M. E. et al. *J. Med. Chem*. **2004**, *47*, 5641.



POSTER COMMUNICATIONS – PC.08

HYBRID α-BROMOACRYLOYLAMIDO CHALCONES. DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

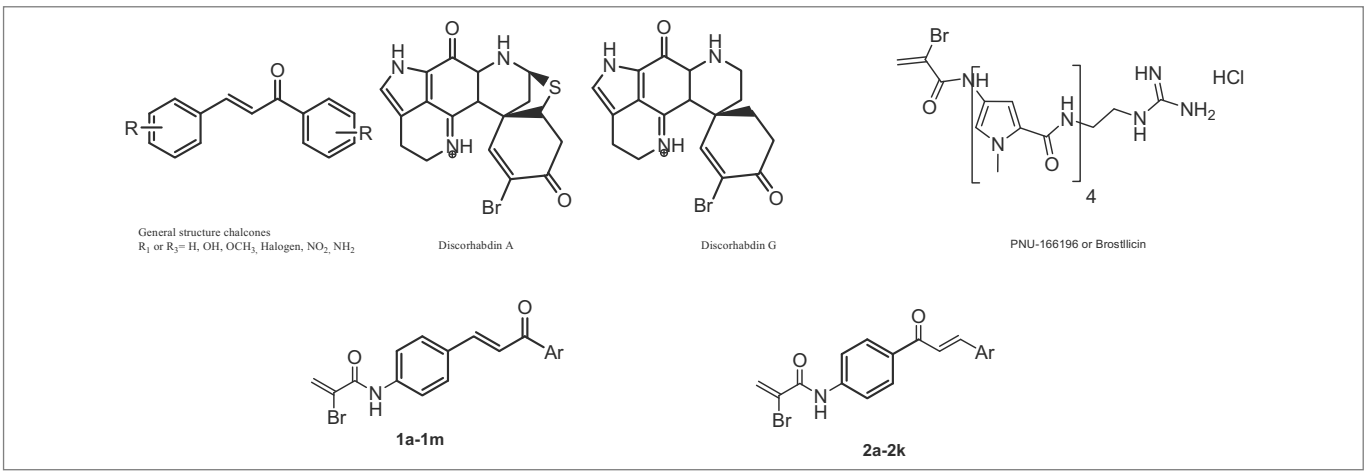
L C López Cara (1), R Romagnoli (1), P. G. Baraldi (1), M. D. Carrión (1), O. Cruz-López (1), Jan Balzarini (2), Ernest Hamel (3), Alessandro Canella (4), Enrica Fabbri (4), Roberto Gambari (4), Giuseppe Basso (5), Giampietro Viola (5)

(1) Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy. (2) Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium. (3) Toxicology and Pharmacology Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, MD 21702, USA. (4) BioPharmaNet, Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, 44100 Ferrara, Italy. (5) Dipartimento di Pediatria, Laboratorio di Oncoematologia, Università di Padova, 35131 Padova, Italy

lcarlotalopez@ugr.es

Research into the anti-tumor properties of chalcones has received significant attention over the last few years. **1 Chalcones** (1,3-diaryl-2-propen-1-ones, are known to exhibit antimitotic properties caused by inhibition of tubulin polymerization by binding to the colchicine-binding site[1]. On the other hand the pyrroloiminoquinone cytotoxic alkaloids **Discorhabdin A6** and **Discorhabdin G7** are characterized by the presence of an α-bromoacryloyl alkylating moiety of low chemical reactivity, an unusual feature for cytotoxic compounds[2]. The same moiety is present in a series of potent anticancer distamycin-like minor groove binders, for example, **PNU166196** (brostallicin), which is currently undergoing Phase II clinical trials[3]. **PNU-166196** is an α-bromoacrylamido derivative of a four-pyrrole distamycin homologue ending with a guanidine moiety. Two novel large series of α-bromoacryloylamido chalcones **1a–m** and **2a–k** containing a pair of Michael acceptors in their structures, corresponding to the α-bromoacryloyl moiety and the a,b-unsaturated ketone system of the chalcone framework, were synthesized and evaluated for antiproliferative activity against five cancer cell lines. Such hybrid derivatives demonstrated significantly increased anti-tumor activity compared with the corresponding amino chalcones[4].

1. Lawrence, N. J.; McGown, A. T. *Curr. Pharm. Des*. **2005**,11, 1679.
2. Cozzi, P.; Beria, I.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. *Bioorg. Med. Chem. Lett*. **2000**, 10, 1269.
3. Geroni, C.; Marchini, S.; Cozzi, P.; Galliera, E.; Ragg, E.; Colombo, T.; Battaglia, R.; Howard, M.; D'Incalci, M.; Broggini, M. *Cancer Res.* **2002**, 62, 2332.
4. Romeo Romagnoli , Pier Giovanni Baraldi, Maria Dora Carrion , Olga López, Carlota López Cara, Jan Balzarini, Ernest Hamel, Alessandro Canella, Enrica Fabbri, Roberto Gambari, Giuseppe Basso, Giampietro Viola. *Bioorg. Med. Chem. Lett*. **2009**, 19, 2022.



DESIGN AND ONE-POT SYNTHESIS OF α-AMINOPHOSPHONATES AND BIS (α-AMINOPHOSPHONATES) BY IRON (III) CHLORIDE AND CYTOTOXIC ACTIVITY

Zahra Rezaei (1), Habib Firouzabadi (2), Nasser Iranpoor (2), Abbas Ghaderi (3), Mohammad Reza Jafari (1), Abbas Ali Jafari (4), Hamid Reza Zare (3)

(1) Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
(2) Department of Chemistry, College of Sciences, Shiraz University, Shiraz, Iran.
(3) Shiraz institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran.
(4) Department of Chemistry, Faculty of Sciences, Yazd University, Yazd, Iran.

α-Aminophosphonic acids and α-aminophosphonates are probably the most important substitutes for the corresponding amino acids in biological systems. Indeed a number of potent antibiotics, enzyme inhibitors, and pharmacological agents are 1-aminophosphonic acids as well as their derivatives, notably peptides. α-Aminophosphonates have been synthesized by various routes. In our study, we used a solution of FeCl3 in THF to facilitate the Manich-type reaction of aldehyde, amine and phosphite compounds to form corresponding α-aminophosphonates in a one-pot, three component reaction. Selected α-Aminophosphonates were entered to a biological assay test and were studied by docking methods, using Autodock 3.0.

The results showed that the reactions were carried out mildly and eco-friendly to form α-aminophosphonates in high yields. An indole derived bis(α-aminophosphonates) showed maximum cytotoxic effect. The docking results are partially in agreement with the experimental results, showing the intercalating properties of the indole derived.

We concluded that an indole derived bis (α-aminophosphonate) is a suitable candidate to be used in further studies.

POSTER COMMUNICATIONS – PC.10

DESIGN, SYNTHESIS, AND ANTIFUNGAL ACTIVITY OF NEW IMIDAZOLE DERIVATIVES

Zahra Rezaei (1), Soghra Khabnadideh (1), Keyvan Pakshir (2), Nargas Sanagoei (1)

(1) Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
(2) Department of parasitology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

This study describes the design, synthesis and evaluation of a novel series of imidazole and bezimidazole derivatives as inhibitors of cytochrome P450 14a-emethylase (14DM). The chemical structures of the new compounds were confirmed by elemental and spectral (1H NMR, 13C NMR, Mass) analyses.

Compounds were designed by a generating virtual library of compounds and docking them into the enzyme active site. Furthermore, they were found to have in vitro activity against Microsporum canis, Trichophyton mentagrophyte, Trichophyton rubrum, Epidermophyton floccosum, and Candida albicans comparable to fluconazole and clotrimazole.

DESIGN AND SYNTHESIS OF SOME TRIAZOLE AND BENZOTRIAZOLE DERIVATIVES AS ANTIFUNGAL AGENTS

Soghra Khabnadideh, Zahra Rezaei, Giti Kashi

Department of Medicinal Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

In recent years the developments of resistance to currently available antifungal azoles in *Candida* spp., as well as clinical failures in the treatment of fungal infections have been reported. Triazole may be considered as a bioisostere of imidazole which is incorporated into the structures of many antifungal compounds.

In this study a series of 1,2,4-triazole, and benzotriazole derivatives were designed as inhibitors of cytochrome P450 14α-demethylase (14DM). The compounds were docked into the active site of MT-CYP51 (PDB code, 1E9X) using Autodock program and then some compounds with the best binding energy were synthesized.

To synthesize the compounds azole or benzotriazole rings, alkyl halide, potassium bicarbonate and tetraethylammonium iodide (TEAI) and NaOH in acetonitrile (30-40ml) were refluxed for 24-90 hr. Then the reaction mixture was filtered and the solid washed with acetonitril for three times and dried over anhydrous Na_2SO_4.

The products were purified by column chromatography using chloroform-ethanol. The yields of reactions were 63%-80%. The structures of the compounds were confirmed by NMR and MS spectroscopy.

Some of the compounds showed good affinity for MT-CytP51 and have been shown to be inhibitors of ergosterol synthesis and thereby fungal growth.

POSTER COMMUNICATIONS – PC.12

STABILITY AND SOLUBILITY OF CYPROTERONE DERIVATIVES IN THE PRESENCE OF HYDROXYPROPYL-β-CYCLODEXTRIN

Soghra Khabnadideh, Zahra Rezaei, Mohammad Mehdi Zarshenas, Andia Khalili, Mohammad Reza Jafari

Department of Medicinal Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Cyproterone acetate is a potent progestagen and a moderate antiandrogen. It is used as a part of contraceptives and also for the treatment of hirsutism and acne. The solubility and stability of cyproterone can be changed by making different esters other than acetate.

Cyclodextrins are cyclic oligosaccharides containing a varying number of glucopyranose rings with ability to increase the solubility and the stability of several steroids such as cyproterone acetate.

In the present study, the influence of hydroxypropyl β-cyclodextrin (HPBCD) on aqueous solubility and stability of some acyl esters of cyproterone were investigated. First a series of cyproterone esters (acetate, propionate, butyrate, hexanoate and benzoate) were synthesized. The phase solubility analysis and the stability of the compounds in the presence of HPBCD in a phosphate buffer solution at pH 7.4 were investigated. For further distinguish of the complexation modes, the compounds were docked inside the HPBCD cavity using the Autodock program.

The results illustrate the interaction between the compounds and HPBCD was AL type and all of the compounds exhibit higher solubility as a result of complexation with HPBCD. The amount of increase in solubility was constantly raised as the chain length was ascending by 4 carbon atoms. The experimental rates for the degradation of the compounds were significantly reduced in the presence of HPBCD and also the docking scores confirm the results.

HOMOCHIRAL DRUGS: THE TALE OF CHIRAL SWITCHES?

M. Eugenia García-Rubiño (1), Ana Conejo-García (1), María C. Núñez (1), Ana M. Gil (2), Carlos Cativiela (2), Alberto Ramírez (3), Juan A. Marchal (3), Houria Boulaiz (3), Miguel A. Gallo (1), Antonio Espinosa (1), Joaquín M. Campos (1)

(1) Departamento de Química Farmacéutica y Orgánica. Facultad de Farmacia, c/ Campus de Cartuja s/n, 18071 Granada (Spain)
(2) Departamento de Química Orgánica y Química Física, Facultad de Ciencias, 50009 Zaragoza (Spain)
(3) Instituto de Biopatología y Medicina Regenerativa (IBIMER). Departamento de Anatomía y Embriología Humana. Facultad de Medicina, Avenida de Madrid s/n, 18071 Granada (Spain)

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 difficult-to-predict (and possibly undesirable side effects).

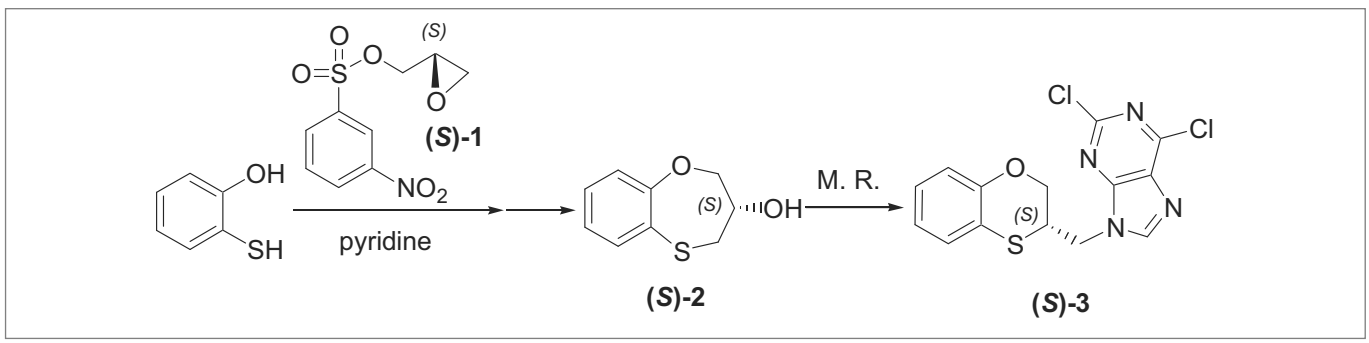
Well aware of this fact and stimulated by the new policy statements issued by the regulatory agencies, the pharmaceutical industry has, over the past decade or so, systematically begun to develop chiral drugs in enantiometrically pure forms.

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

(*S*)-**3** was obtained according to the Scheme. Racemic **3**[1] was resolved in its enantiomers (*S*)-**3** (Retention time = 14.7 min) and (*R*)-**3** (Retention time = 17.5 min), using a CHIRALPAK® IA semi-preparative column and an hexane/^{i}PrOH mixture as eluent. The absolute configurations of the stereocentres were determined by X-ray diffraction. (*R*)- and (*S*)-**3** show different antiproliferative activities against the human breast cancer cell line MDA-MB-231.

Currently, there is a perception in the external environment the the pharmaceutical R&D is no longer innovative, fails to bring out new drugs or, at best, produces a rising number of "me-too" drugs with no advantage over existing treatments. In addition, the cost to discover and develop new medicine has risen dramatically in recent years. Increasing the rate of innovation (including the development of homochiral drugs) is a requirement to secure the future of the pharmaceutical industry.

1. M. C. Núñez, M. Díaz-Gavilán, A. Conejo-García, O. Cruz-López, M. A. Gallo, A. Espinosa, J. M. Campos**.** Design, Synthesis and Anticancer Activity against the MCF-7 Cell Line of Benzo-Fused 1,4-Dihetero Seven- and Six-Membered Tethered Pyrimidines and Purines. Curr. Med. Chem., **2008**, 15, 2614-2631.



POSTER COMMUNICATIONS – PC.14

SYNTHESIS AND ANTICANCER ACTIVITY OF THE (*R,S*)-BENZO-FUSED 1,5-OXATHIEPINE MOIETY TETHERED TO PURINES THROUGH ETHYLENOXY OR PROPYLENOXY LINKERS

María Kimatrai (1), Elena Andreolli (1), Ana Conejo-García (1), Alberto Ramírez (2), Juan A. Marchal (2), Houria Boulaiz (2), Miguel A. Gallo (1), Antonio Espinosa (1), Joaquín M. Campos (1)

(1) Departamento de Química Farmacéutica y Orgánica. Facultad de Farmacia, c/ Campus de Cartuja s/n, 18071 Granada (Spain)
(2) Instituto de Biopatología y Medicina Regenerativa (IBIMER). Departamento de Anatomía y Embriología Humana. Facultad de Medicina, Avenida de Madrid s/n, 18071 Granada (Spain)

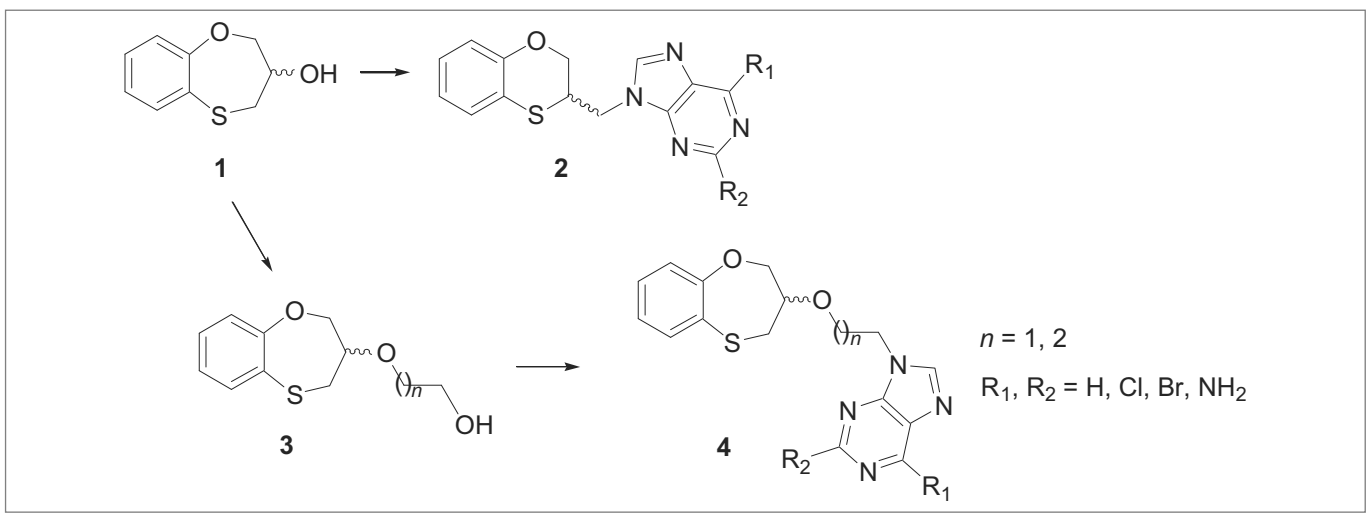
Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. Therefore, the development of new drugs against cancer remains among the priorities of the development of science and fundamental research.

A series of eleven (*R,S*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9H-purines derivatives (**2**) was recently obtained by applying the Mitsunobu protocol that led to a six-membered ring contraction from (*R,S*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-ol (**1**) via an episulfonium intermediate. The results indicated the anticancer activity against the MCF-7 breast cancer cell line for the most active compounds were correlated with their capacity to induce apoptosis[1].

The aim of the research presented in this communication is the preparation and evaluation of substituted purines in which the benzo-fused 1,5-oxathiepine-2-yl fragment is linked to the *N*-9 position of the purine heterocycle through a 2- or 3-carbon linker (**4**). In these cases, the use of a spacer between the sulfur-containing heterocycle and the primary OH group of **3**, prevents the *S* neighbouring participation, maintaining accordingly intact the seven-membered moiety in **4**.

The results provide promising information for further development of potent antiproliferative agents.

1. M. Díaz-Gavilán, A. Conejo-García, O. Cruz-López, M. C. Núñez, D. Choquesillo-Lazarte, J. M. González-Pérez, F. Rodríguez-Serrano, J. A. Marchal, A. Aránega, M. A. Gallo, A. Espinosa, J. M. Campos. Synthesis and Anticancer Activity of (*RS*)-9-(2,3-Dihydro-1,4-Benzoxathiin-3-ylmethyl)-9*H*-Purines. ChemMedChem **2008**, 3, 127-135.



TOWARDS DUAL-ACTION ANTI-MALARIALS - PRIMACENES, FERROCENE DERIVATIVES OF PRIMAQUINE

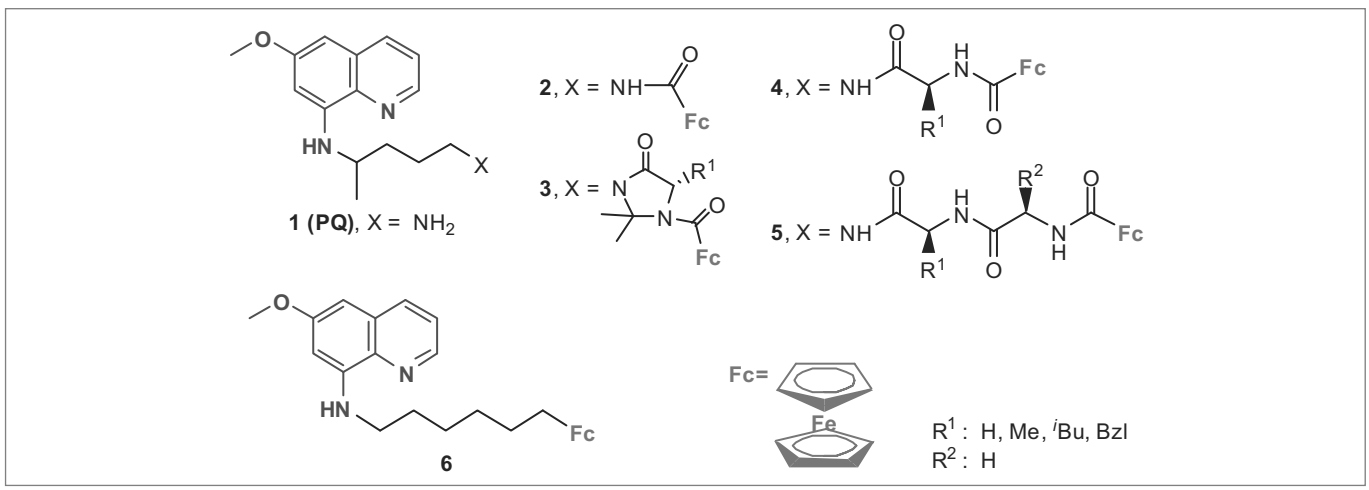
Joana Matos (1), Nuno Vale (1), Rui Moreira (2) and Paula Gomes (1)

(1) CIQUP, Departamento de Química da Faculdade de Ciências da Universidade do Porto, R. Campo Alegre, 687 P-4169-007 Porto, Portugal, (2) iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto P-1649-003 Lisboa, Portugal

Malaria is the world's top-priority tropical disease[1], whose control has been jeopardized since the 1970's by the emergence of *Plasmodium falciparum* strains resistant to the once popular and highly effective chloroquine (CQ). By the end of the XXth century, Biot et al. combined the CQ 4-aminoquinoline core with a ferrocene-based side chain, creating Ferroquine (FQ), an anti-malarial that displayed potent activity against both CQ-sensitive and resistant plasmodia, so apparently establishing a New Frontier in Medicinal Chemistry. Unfortunately, strains soon emerged that were resistant to FQ[2,3]. Nonetheless, Biot's approach was undeniably elegant and initially effective, so we thought that it could be successful if applied to primaquine (PQ, **1**), a 60-year-old anti-malarial against which no clinically relevant resistance has been reported to date[4]. Due to this fact and to PQ's role in controlling the spread of malaria, we had already been working with this anti-malarial by creating *Imidazoquines*, novel anti-malarial derivatives of PQ resistant to premature metabolic inactivation by oxidative deamination[5-13]. So, given that FQ was over 20-fold more potent than CQ as a blood-schizontocide, we believe that insertion of ferrocenic structures on the PQ side chain may contribute not only to improve PQ resistance against metabolic inactivation, but also to enhance its originally modest blood-schizontocidal activity. Thus, we wish to report the synthesis of *Primacenes* (**2-6**), whose activity as dual-action anti-malarials is under study and will be timely reported.

This work is funded by Fundação para a Ciência e Tecnologia (Portugal) through project PTDC/QUI/65142/2006 and post-doc research grant SFRH/BPD/48345/2008 (NV).

1. Hotez, PJ *et al. PLOS Medicine* **2006**, 3, 576.
2. Biot, C *et al. J. Med. Chem*. **1997**, 40, 3715.
3. Dive, D *et al. ChemMedChem* **2008**, 3, 383.
4. Vale, N *et al. Eur. J. Med. Chem*. **2009**, 44, 937.
5. Gomes, P *et al. Tetrahedron* **2004**, 60, 5551.
6. Araújo, MJ *et al. J. Med. Chem*. **2005**, 48, 888.
7. Chambel, P *et al. Tetrahedron* **2006**, 62, 9883.
8. Ferraz, R *et al. J. Org. Chem*. **2007**, 72, 4189.
9. Vale, N *et al. Bioorg. Med. Chem. Lett*., **2008**, 18, 485.
10. Vale, N *et al. Bioorg. Med. Chem. Lett*. **2008**.
11. 4150; [11]Vale, N *et al. Tetrahedron* **2008**, 64, 11144.
12. Vale, N *et al. Eur. J. Med. Chem*. **2009**, 44, 2506.
13. Vale, N *et al.* submitted.



POSTER COMMUNICATIONS – PC.16

SCAFFOLD-HOPPING THROUGH MONOAMINE REUPTAKE INHIBITOR CHEMISTRY SPACE

Ryan C. Schoenfeld

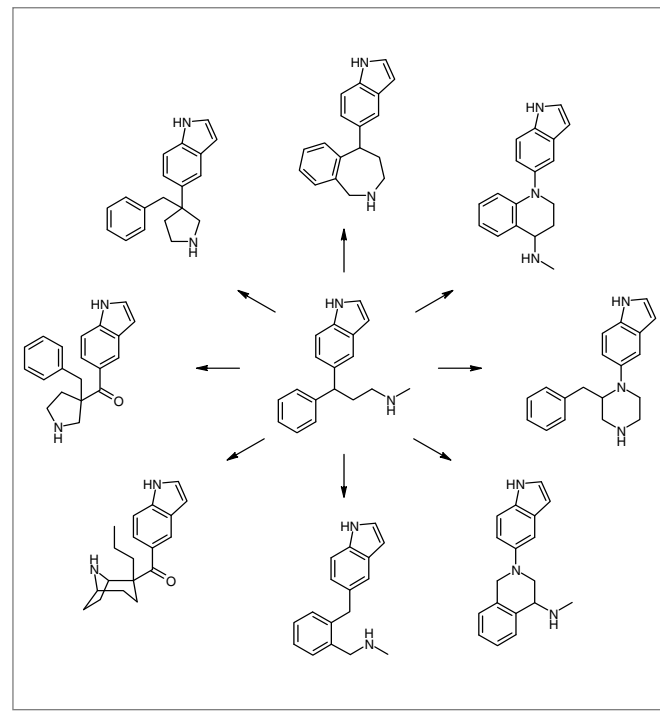
Roche Palo Alto LLC, Medicinal Chemistry
3431 Hillview Avenue, Palo Alto, CA 94304, USA

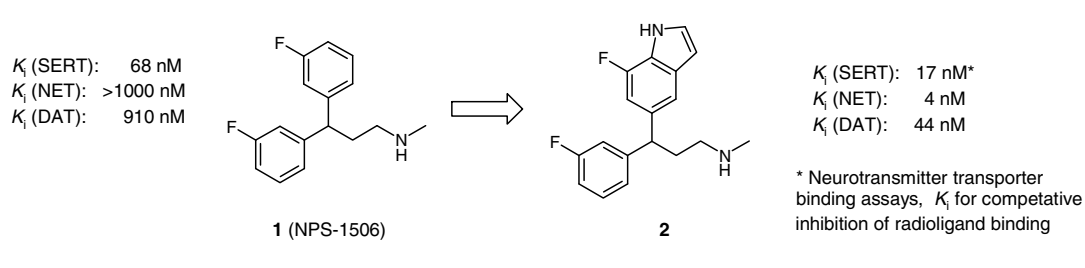
Neurological blockade of monoamine reuptake has long been recognized as a mechanism of treatment for depression[1]. Blockbuster drugs of the past 25 years have focused on selective reuptake inhibition of either serotonin (SSRIs, such as fluoxetine) or of both serotonin and norepinephrine in dual action SNRIs (such as duloxetine)[1]. Recently, the concept of triple reuptake inhibitors (TRIs) that also block uptake of dopamine has attracted much attention in the realm of drug discovery. TRIs are expected to bring increased efficacy to the treatment of major depressive disorder (MDD), as well as to decrease the typical 2-4 weeks lag time seen in the onset of action of current SSRI and SNRI therapies[2].

We found early in our efforts in this field that we could replace one of the phenyl rings of the dual action SSRI / NMDA antagonist NPS-1506 **(1)**[3]. by an indole to achieve a potent triple reuptake inhibitor **(2)**.

With **2** as a starting point, a rich exploration of chemistry space through conformational constraints and scaffold hopping ensued, leading to the discovery of a broad diversity of novel, optimizeable sub-templates. Several of those scaffolds were chosen for further evolution towards a TRI clinical candidate, and the results of some of those efforts will be described.

1. For a recent review: Nierenberg, A. A. *et al. J. Occup. Environ. Med.* **2008**, *50*, 428.
2. Kulkarni, S.K. ; Dhir, A. *Expert Opin. Investig. Drugs* **2009**, *18*, 767.
3. Mueller, A. *et al.* (NPS Pharmaceuticals, Inc.) WO 2000002551.





SPIRO-BENZOPYRAN STRUCTURE: A NEW SCAFFOLD FOR CARDIOPROTECTION

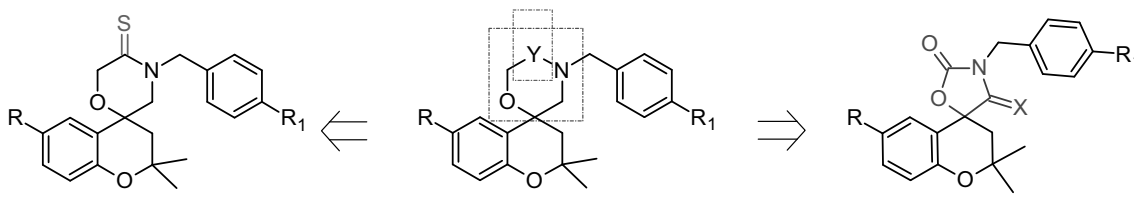
Simona Rapposelli (1), Vincenzo Calderone (2), Maria Digiacomo (1), Alma Martelli (2), Filippo Minutolo (1), Lara Testai (2) and Aldo Balsamo (1)

(1) Dipartimento di Scienze Farmaceutiche, (2) Dipartimento di Psichiatria, Neurobiologia, Farmacologiae Biotecnologie, Università di Pisa

Many evidences underline that the opening of mitochondrial ATP-sensitive potassium channels (mitoK_{ATP}) is cardioprotective in ischemia-reperfusion process. Due to different localization of K_{ATP} channel in various part of the cell (sarcolemmal and inner mitochondrial membrane) and its distribution in many tissues (smooth muscle, pancreas, miocardiocytes) the possibility to manipulate the K_{ATP} channel status has become an important target in the treatment of various diseases, including diabetes, hypertension, heart failure and ischemia. In particular, the selective activation of mitoK_{ATP} channels as protective mechanism against ischemia represents a recent and intriguing field of medicinal and pharmacological investigation.

Recently, we planned the synthesis of a limited number of 4-spiro-morpholone (**A**) and 4-spiro- morpholine (**B**) compounds in order to evaluate their cardioprotective activity. These preliminary works led us to identify new compounds endowed of a good cardioselectivity[1,2] and lacking of some systemic and deleterious effects such as hypotension and vasodilation. With the aim to investigate more deeply this kind of spiro-like structure and the influence of some molecular modifications on their cardioprotective properties, we synthesized new derivatives in which the spiro-nucleus in C4 position is a five-membered heterocycle such as the oxazolidine (**C**) or the 5-imino-oxazolidine (**D**) or a morpholone isoster (i.e.: thiomorpholone (**E**)). The pharmacological evaluation of the cardioprotective effects on cultured cardiomyoblastic cells allow us to select the most interesting compounds to submit to further pharmacological investigations on different experimental models of ischemia/reperfusion.

1. Breschi M.C, Calderone V., Martelli A., Minutolo F., Rapposelli S., Testai L., Tonelli F. and Balsamo A., J. Med. Chem., **2006,** *49*, pp 7600 – 7602.
2. Breschi M.C, Calderone V., Digiacomo M., Manganaro M., Martelli A., Minutolo F., Rapposelli S., Testai L., Tonelli F. and Balsamo A., J. Med. Chem., **2008,** *51*(21), pp 6945 – 6954.



E

A: Y=CO, R=H, Br

B: Y=CH_2, R=H,Br

C: X=H_2, R=H, Br

D: X=NH, R=H,Br

EFFECT OF NOVEL ANTIDIABETIC AGENT CEREBROCRAST ON GLUCOSE AND LIPID METABOLISM, BODY AND ORGAN WEIGHTS, FOOD AND WATER INTAKE OF NORMAL RATS

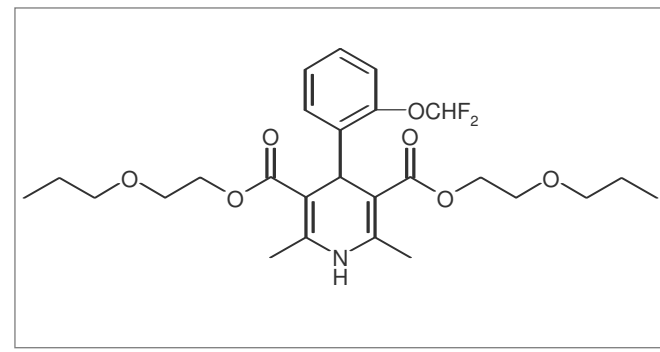
Janina Briede, Mara Stivrina, Dzintra Stoldere, Ilga Dabina, Brigita Vigante and Gunars Duburs

Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., Riga LV-1006, Latvia. E-mail:gduburs@osi.lv

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 of 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 provoked an increase in total cholesterol in the liver and heart, that can be used for plasma membrane formation at cells differentiation and proliferation, as well as for synthesis of steroid hormones in the adrenal gland.



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.

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.

IN VIVO AND IN VITRO ANTI-LEISHMANIAL ACTIVITIES OF BENZENESULFONAMIDES

Mónica Rangel (1), M. Auxiliadora Dea-Ayuela (1,3), Encarna Castillo (2), Pablo Bilbao (3), Francisco Bolás-Fernández (3) and M. Eugenia González-Rosende (1)

(1) Department of Chemistry, Biochemistry and Molecular Biology, (2) department of Physiology, Pharmacology and Toxicology. University CEU Cardenal Herrera, Edificio Seminario s/n, 46113-Moncada, Valencia, Spain. (3) Department of Parasitology. University Complutense of Madrid. Plaza Ramón y Cajal, s/n. 28040-Madrid, Spain.

Leishmaniasis comprises a group of diseases with distinct clinical manifestations caused by different species of the protozoan parasite *Leishmania*. This type of infection is considered within the most relevant group of neglected tropical diseases and targeted by the WHO and other health organizations for prevention, control and eradication. Unfortunately, there is no vaccine against *Leishmania* infection and vector control is difficult. Therefore chemotherapy is the main approach to control this worldwide spread disease. However, the chemotherapeutic treatment is frequently complicated by resistances, cost and toxic side effects. Consequently, the need of novel, efficient and safe agents is imperative[1].

In this context, sulfonamide derivatives with *in vitro* anti-leishmanial activity have been described[2]. Based on our previous results of sulfonamides with *in vitro* leishmanicidal activity[3], we have synthesized a series of sulfonamide analogues and we have evaluated their effect against *L. infantum* promastigotes Compounds exhibiting the highest *in vitro* properties have been also tested *in vivo* in an acute murine model of leishmaniasis.

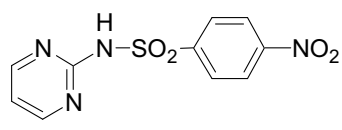
Benzenesulfonamides **1** and **2** were effective against promastigotes of *L. infantum* at concentrations in the micromolar range with no toxicity against mammalian cells and displayed excellent *in vivo* efficacies (parasite burden reduction ca 99%).

These results suggest the potential usefulness of **1** and **2** to be considered as lead compounds for designing new candidates in the therapeutic control of leishmaniasis.

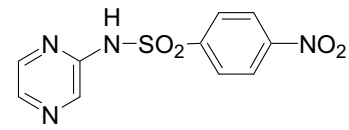
1. Mishra, J.; Saxena, A.; Singh, S. *Curr. Med. Chem.* **2007**, *14*, 1153; Kedzierski, L.; Sakthianandeswaren, A.; Curtis, J. M.; et al. *Curr. Med. Chem.* **2009**, *16,* 599.

2. George, T.G.; Endeshaw, M. M.; Morgan, R. E.; et al. *Bioorg. Med. Chem*. **2007**, *15*, 6071. Andrighetti-Frohner, C. R.; de Oliveira, K. N.; Gaspar-Silva, D.; et al. *Eur. J. Med. Chem.* **2009**, *44,* 755. Everson da Silva, L.; Joussef, A. C.; Kramer Pacheco, L.; et al. *Bio. Med. Chem*. **2007**, *15*, 7553.

3. González-Rosende, M. E.; Dea-Ayuela, M. A.; Castillo, E.; et al. *Drugs Fut.* **2008**, *33* (suppl. A),178.



1



2

POSTER COMMUNICATIONS – PC.20

MICROWAVE SYNTHESIS OF ATORVASTATIN

Juan Hernando, M. Paz Matía, J. Luis Novella, Julio Álvarez-Builla

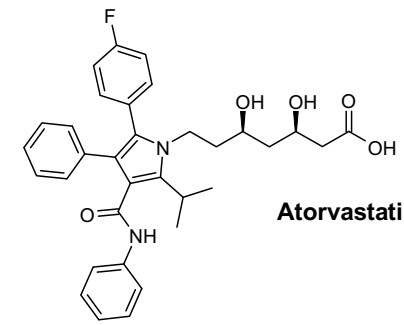
Planta Piloto de Química Fina. Universidad de Alcalá. Ctra. Madrid-Barcelona Km. 33,600. 28871 Alcalá de Henares. Madrid. España.

Atorvastatin, usually marketed under the trade name Lipitor, is a member of the drug class known as statins, used to lower cholesterol biosynthesis[1]. Atorvastatin inhibits the rate-determining step of the enzyme, located in hepatic tissue, that produces mevalonate, a small molecule used in the synthesis of cholesterol and related derivatives. This lowers the amount of cholesterol produced, which in turn lowers the total amount of cholesterol.

Atorvastatin has been prepared following de synthetic method[2,3] indicated in the scheme 1, using microwave irradiation in all steps.

The procedure was optimized in a CEM Explorer apparatus and was scaled up using the CEM Voyager system using the best conditions obtained in the small scale.

Figure 1

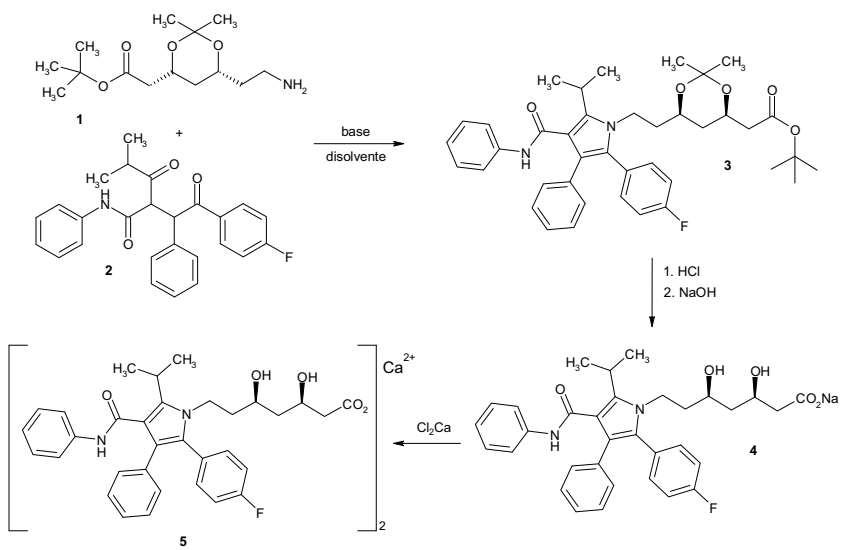


1. Bruce D. Roth, C. J. Blankley, A. W . Chucholowski, E. Ferguson, M. L. Hoefle, D. F. Ortwine, R. S. Newton, C. S. Sekerke, D. R. Sliskovic, M W ilson, *J. Med, Chem.* **1991**, *34*, 357.

2. Kelvin L. Baumann, Donald E. Butler, Carl F. Deering, Kenneth E. Mennen, Alan Millar, Thomas N. Nanninga, Charles W. Palmer, Bruce D. Roth, *Tetrahedron Lett.* **1992**, *17*, 2283.

3. WO 06 097 909.

Scheme 1



SYNTHESIS OF PARACETAMOL OXYDATION PRODUCTS GENERATED IN AQUEOUS SOLUTIONS

Agustín Gutiérrez, Tatiana Cañeque, M. Paz Matía, J. Luis. Novella, Juan J. Vaquero, Julio Álvarez-Builla

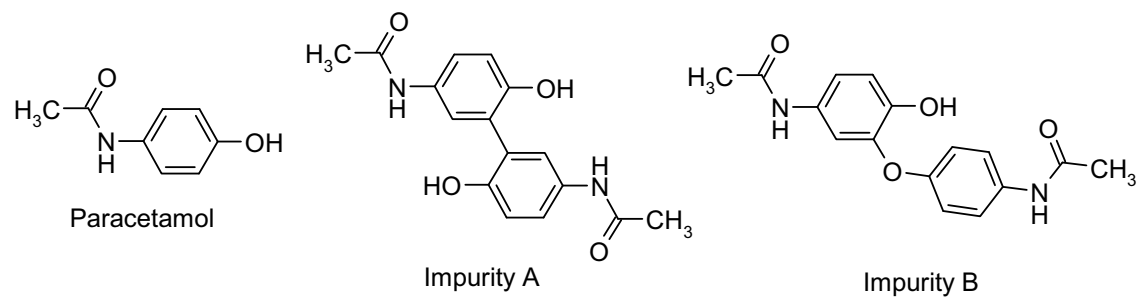
Planta Piloto de Química Fina. Universidad de Alcalá. Ctra. Madrid-Barcelona Km. 33,600. 28871 Alcalá de Henares. Madrid. España.

Paracetamol is a widely available analgesic and antipyretic drug, used in the relief of fever, headaches, and other minor aches and pains. Although considered a safe drug, there are a number of impurities that can be eventually present in its bulk and dosage forms.

In the present work degradation products formed in aqueous solutions of paracetamol have been studied. Two different impurities, A and B (Fig. 1), apparently formed by radical oxidation of the main product[1], were detected by LC-MS[2]. Both have been obtained and identified in comparison with the impurities detected in the paracetamol solutions. Synthesis will be described[3-6].

1. Baumgartner, M. T., Blanco, G. A., Pierini, A. B., *New J. Chem.,* **2008**, *32*, 464.
2. Potter, D., Miller, D., Hinson, J., , *J. Bio. Chem.* **1985**, *260(22)*, 12174.
3. Shiga, M., Yakata, K., Aoyama, M., Saramoto, K., Takagi, M., Veno, K., *Analytical Sciences* **1995**, *11*, 195.
4. PCT/A2193/00326
5. Pinel, E., Bas, C., Neyertz, S., Dominique, N., Petiaud, R., Mercier, R., *Polymer* **2002**, *43*, 1983-1992.
6. Basanogouda, M., Kulkami, M., Kalkhambkar, R., Kulkami, G., *Synthetic Communications* **2008, 38**, 2929-2940.

Figure 1



POSTER COMMUNICATIONS – PC.22

SYNTHESIS OF OMEPRAZOLE, LANSOPRAZOLE AND PANTOPRAZOLE IMPURITIES

M. Paz Matía, J. Luis. Novella, Juan J. Vaquero, Julio Álvarez-Builla

Planta Piloto de Química Fina. Universidad de Alcalá. Ctra. Madrid-Barcelona Km. 33,600. 28871 Alcalá de Henares. Madrid. España.

The final step in the pathway for acid secretion from the parietal cell into the gastric lumen is the so-called 'proton pump'. The proton pump is an active transport system that is powered by the enzyme H^+/K^+-ATPase, which catalyzes the exchange of intra-cellular hydrogen ions for extracellular potassium ions. The inhibition of proton pump will prevent acid secretion from the parietal cell[1].

Omeprazole, pantoprazole and lansoprazole (figure 1) are three irreversible proton pump inhibitors (PPIs). The benzimidazole PPIs are essentially the prodrugs, that in the acidic biophase of the parietal cell form an active metabolite that irreversibly interact with an essential thiol (SH) function on ATPase of the pump.

In the present work we have prepared[2] and analyzed by HPLC-MS[3,4] some of the different impurities of these compounds represented in the figure 2.

1. Karljikovic-Rajic, K.., Novovic, D., Marinkovic, V., Agbaba, D., *J. Pharm. Biomed. Anal.* **2003**, *32*, 1019.
2. Madhusudhan Reddy, G., Mukkanti, K.., Laxmi Kumar, T., Moses Babu, J., Pratap Reddy, P., *Synthetic Commun.* **2008**, *38*,477.
3. Cirilli, R., Ferretti, R., Gallinella, B., Turchetto, L., Zanitti, L., La Torre, F., *J. Pharm. Biomed. Anal.* **2009**, *50*, 9.
4. Espinosa, M., Ruiz, A. J., Sánchez, F., Bosch, C., *J. Pharm. Biomed. Anal.* **2007**, *44*, 831.

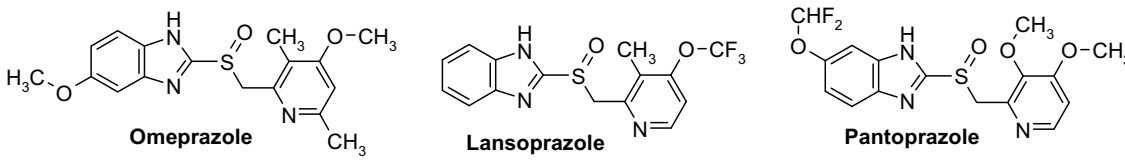


Figure 1

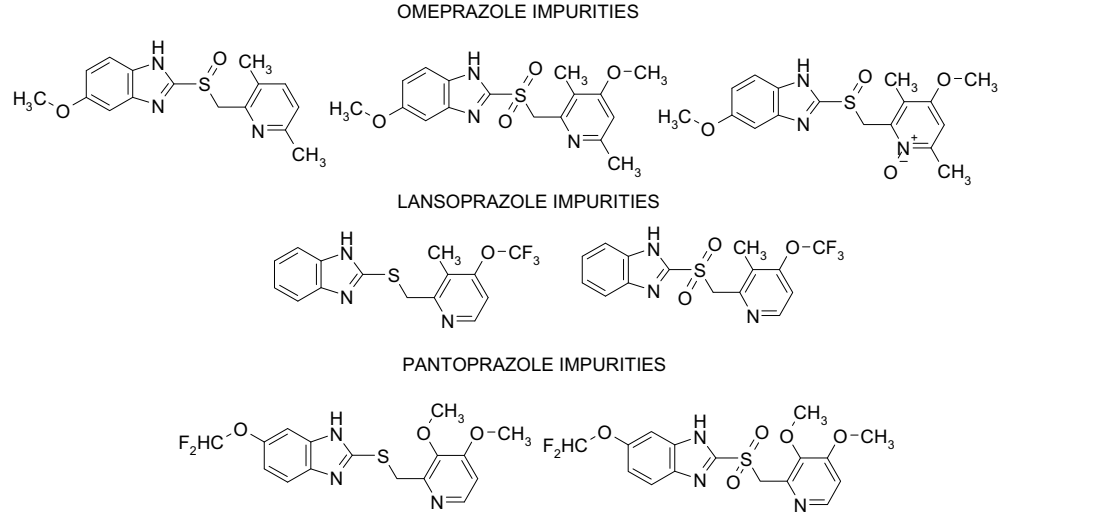


Figure 2

ORAL ANTIDIABETIC DRUGS MODULATE FARNESOID X RECEPTOR ACTIVATION AND TARGET GENE TRANSCRIPTION

Ramona Steri (1), Ewgenij Proschak (2), Gisbert Schneider (2), Manfred Schubert-Zsilavecz (1)

(1) Institute of Pharmaceutical Chemistry/ZAFES/LiFF, Goethe-University Frankfurt, Max-von-Laue- Str. 9, 60438 Frankfurt, Germany
(2) Institute of Organic Chemistry and Chemical Biology/ZAFES/LiFF, Goethe-University Frankfurt, Siesmayerstr. 70, 60323 Frankfurt, Germany

Farnesoid X Receptor (FXR) is a nuclear receptor which acts as a ligand-activated transcription factor. It is highly expressed in liver, intestine and kidney and regulates a large number of target genes which are involved in lipid and glucose homeostasis. Bile acids as well as their metabolites and polyunsaturated fatty acids are known as endogenous FXR ligands, whereas synthetic agonists as GW4064 or Fexaramine lead to deeper insights into the function of FXR[1,2]. In animal models, FXR activation turned out to reduce plasma triglycerides, cholesterol and atherosclerotic lesions accompanied by improved glucose metabolism[3,4].

Recently, antidiabetic thiazolidinediones were reported to modulate activation and target gene expression of FXR[5]. This encouraged us to examine further antidiabetic drugs, which are in clinical use, whether they have an influence on FXR signaling as well.

This work contains the development of a luciferase-based reportergene assay which allows the characterization of FXR ligands. The assay is performed in Cos7 cells, transiently transfected with a Gal4-hybrid protein containing the ligand binding domain of human FXR and a Gal4 DNA binding domain. Beside the data for FXR transactivation, we report the influence of the tested drugs on transcription of FXR target genes in HepG2 cells and support our results with molecular docking experiments, which show key interactions of the molecules within the ligand binding pocket of FXR, comparable to known ligands.

1. Wang et al, Cell Research (2008) 18: 1087-95.
2. Fiorucci et al, Trends in Molecular Medicine (2007) 13 (7): 298-309.
3. Mencarelli et al, Am J Physiol Heart Circ Physiol (2009) 296: 272-81.
4. Kuipers et al, Current Opinion nin Lipidology (2007) 18: 289-297.
5. Kaimal et al, Journal of Pharmacology and Experimental Therapeutics, epub April 15 2009.

POSTER COMMUNICATIONS – PC.24

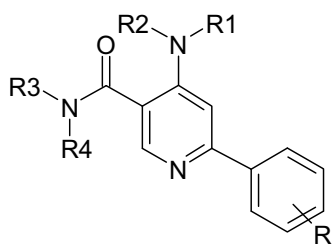
THE IDENTIFICATION OF SELECTIVE, CNS PENETRANT ALPHA2A ANTAGONIST SERIES

Andrew Bell, Paul Erdman, Russell Lewthwaite, Ian Marsh and Robert Smith

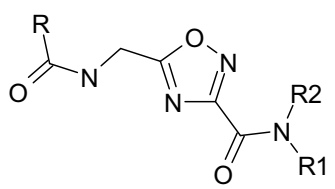
Sandwich Chemistry, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK.

The α_2 receptors have been long known to play a key role in noradrenergic transmission. As a part of our search for novel treatments for urinary incontinence, we have found that non-selective α_2 agonists reduce bladder capacity in rat, and increase urethral tone in dog, while capacity can be increased by the administration of α_2 antagonists. Furthermore, by using sub-type selective α_2 antagonists, we have implicated the α_{2A} receptor as the likely mediator of these effects. Unfortunately, none of the literature α_{2A} antagonists combined the required selectivity and CNS penetration to fully assess the importance of this mechanism, hence alternative chemical matter was required.

We carried out a high-throughput screen of our compound file using a β-lactamase reporter gene functional assay expressing the α_{2A} receptor in CHO cells in order to detect both orthosteric and allosteric antagonists. Hits were triaged based on their potency and selectivity over activity at the α_{2C} receptor. Optimisation of the initial hits provided two structurally distinct series (**1** and **2**) with appropriate potency, selectivity, metabolic stability and CNS penetration to evaluate in our *in vivo* efficacy and safety models. The results of these studies will be disclosed as part of the presentation.



1



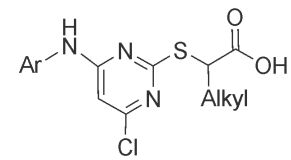
2

IDENTIFICATION OF NEW TARGETS BY MATRIX-ASSOCIATED PIRINIXIC ACID DERIVATIVES

Michaela Dittrich (1), Heiko Zettl (1), Christine Greiner (2), Felix Behnke (2), Oliver Werz (2), Manfred Schubert-Zsilavecz (1)

(1) Goethe-University Frankfurt; Institute of Pharmaceutical Chemistry/ZAFES/LiFF; Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
(2) Eberhard-Karl-University Tübingen; Institute of Pharmaceutical Chemistry; Auf der Morgenstelle 8, 72076 Tübingen, Germany

We have previously shown that pirinixic acid (PA) derivatives address distinct biological targets like peroxisome proliferator-activated receptors (PPARs)[1], which are nuclear receptors, as well as enzymes within the arachidonic acid cascade such as microsomal prostaglandin E2- synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO)[2]. Thereby, selectivity for affecting the appropriate target could be reached by altering the substitution pattern of the PA derivative. Encouraged by our recent results, we are interested to further elucidate the pharmacological profile of PA derivatives, in particular to identify molecular targets. A distinguished method to screen for binding proteins is the so-called "target fishing" approach. In short, the underlying strategy of this method is coupling of a functionalised ligand to a solid matrix. After deprotection of the functional group which was selected for investigation, incubating the matrix-coupled ligand with cell lysates may provide specific ligand-target interaction. After eluting the specific ligand-bound proteins, it is possible to identify these proteins via mass spectrometry or western blot.



In order to identify new promising targets for PA derivatives, this work aims to modify PA itself and selected potent α-alkylated PA derivatives with the purpose to facilitate coupling of these molecules to a solid phase/matrix.

1. Zettl et al, QSAR & Combinatoral Science, 2009, 28, 576-586.
2. Koeberle et al, J. Med. Chem., 2008, 51, 8068-8076.

POSTER COMMUNICATIONS – PC.26

SYNTHESIS OF RADIOLABELLED F15599, A NOVEL 5-HT_{1A} RECEPTOR AGONIST, ITS PHARMACOLOGICAL AND RADIOPHARMACOLOGICAL EVALUATION

Elodie Blanc (1), Laëtitia Lemoine (2), Mathieu Verdurand (2), Luc Zimmer (2), Didier Le Bars (2), Adrian Newman-Tancredi (1) and Bernard Vacher (1)

(1) Institut de Recherche Pierre Fabre, 17 av. Jean Moulin 81100 Castres, France
(2) CERMEP PET Department, University of Lyon & CNRS, Lyon, France

We report the synthesis of radiolabelled F15599 (3-chloro-4-fluorophenyl-(4-fluoro-4{[(5-methyl-pyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methadone), a 5-HT_{1A} receptor agonist. F15599 has nanomolar affinity and high selectivity for 5-HT_{1A} binding sites. After a single oral administration in rats, F15599 totally reverses immobility in the forced swimming test and produces behaviors characteristic of 5-HT_{1A} receptors activation. It is known that 5-HT_{1A} receptors exist in high- and low-affinity states and that agonists bind preferentially to the high-affinity state of the receptor. Since all available PET 5-HT_{1A} radiopharmaceuticals are antagonists, it is of great interest to develop a fluorine-18 labelled agonist that could provide a measure of the functional 5-HT_{1A} receptors. The nitro-precursor of [^{18}F]F15599 is synthesized and then undergoes a fluoronucleophilic substitution. Radiopharmacological evaluations included *in vitro* and *ex vivo* autoradiographies in rat brain and PETscans on rats and cats. The [^{18}F]F15599 *in vitro* binding was consistent with the 5-HT receptors distribution (hippocampus, dorsal raphe nucleus and cortical areas). In *in vivo* studies, the preferential binding of F15599 with 5-HT_{1A} receptors in cortical region was confirmed in both species. Other 5-HT_{1A} agonists from this serie are currently investigated.

THE EFFECT OF C-3 STEREOCHEMISTRY IN THE AROMATASE INHIBITORY ACTIVITY OF 3-HYDROXY-4-ANDROSTENES. A STRUCTURE-ACTIVITY STUDY

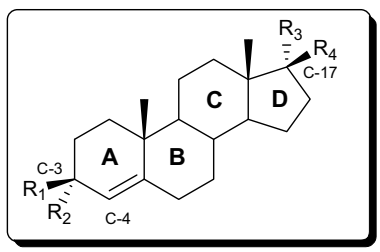
Fernanda Roleira (1), Tânia Sintra (1), Carla Varela (1), Georgina Correia-da-Silva (2), Margarida Cepa (2), Ana Dias (2), Natércia Teixeira (2), Elisiário Tavares-da-Silva (1)

(1) CEF, Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Portugal
(2) IBMC, Institute for Molecular and Cellular Biology and Laboratory of Biochemistry, Faculty of Pharmacy, University of Oporto, Porto, Portugal
froleira@ff.uc.pt

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 some planarity in the A-ring as well as the presence of oxygen functions at C-3 and C-17 positions seems to be important for aromatase inhibition[3]. In this work, we present a structure-activity study, based on the synthesis[4] and aromatase inhibitory activity evaluation, of steroid compounds having double bonds at C-4, which can confer the required planarity in the A/B-ring region, hydroxyl groups at C-3, which assure the oxygen function at C-3 position, and different oxygen functions at C-17. Additionally, we further explore the role of the stereochemistry of the C-3 hydroxyl group, in the aromatase inhibitory activity.

1. (a) Chen, S. *Frontiers of Bioscience* **1998**, *3*(9), *22*. (b) Blickenstaff, R. T. *Antitumor Steroids*, Academic Press (NY);**1992**.
2. Glosh, D.; Griswold, J.; Erman, M; Pangborn, W. *Nature*, **2009**, *457*, 219.
3. (a) Nagaoka, M.; Watari, Y.; Yajima, H.; Tsukioka, K.; Muroi, Y.; Yamada, K.; Numazawa, M. *Steroids*, **2003**, *68*(6), 533; (b) 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; (c) Cepa, M., Correia-da-Silva, G., Tavares da Silva, E.J., Roleira, F.M.F., Teixeira, N. *Steroids*, **2008**, *73,* 1409.
4. Campos Neves, A.S.; Sá e Melo, M.L.; Moreno M.J.M.; Tavares da Silva, E.J.; Salvador. J.A.R.; Costa S.C.P.; Martins R.M. *Tetrahedron,* **1999**, *55* (11), 3255.



1 R_1 (OH); R_2 (H); R_3,R_4 (=O)

2 R_1 (H); R_2 (OH); R_3,R_4 (=O)

3 R_1 (OH); R_2 (H); R_3 (H); R_4 (OH)

4 R_1 (H); R_2 (OH); R_3 (H); R_4 (OH)

5 R_1 (OH); R_2 (H); R_3 (H); R_4 ($OCOCH_3$)

POSTER COMMUNICATIONS – PC.28

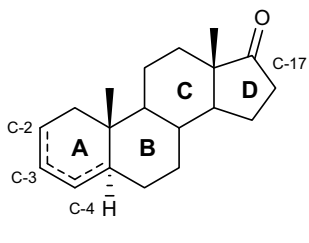
ANDROSTENES *vs* EPOXYANDROSTANES. THE ROLE OF THE PLANARITY OF THE A-RING IN AROMATASE INHIBITION. A STRUCTURE-ACTIVITY STUDY

Carla Varela (1), Fernanda Roleira (1), Georgina Correia-da-Silva (2), Margarida Cepa (2), Ana Dias (2), Natércia Teixeira (2), Elisiário Tavares-da-Silva (1)

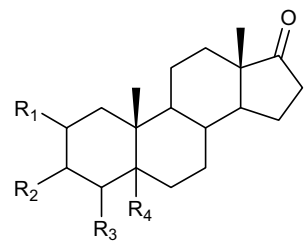
(1) CEF, Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Portugal
(2) IBMC, Institute for Molecular and Cellular Biology and Laboratory of Biochemistry, Faculty of Pharmacy, University of Oporto, Porto, Portugal
etavares@ff.uc.pt

Hormone-dependent breast tumors require estrogens for their growth[1]. One main approach to block estrogen action targets aromatase, a cytochrome P-450 enzyme (CYP19), responsible for estrogen biosynthesis[2]. In breast cancer, intratumoral aromatase is the source for local estrogen production and inhibition of this enzyme 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 has been established[3]. It was found that the volume of the binding pocket is relatively short (no more than 400 Å) allowing to enter into the cleft only molecules with appropriate dimensions such as derivatives of androstenedione with small substituents. 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 some planarity in the A-ring seems to be important for aromatase inhibition[4]. In this work, we present a SAR study, based on the synthesis and aromatase inhibitory activity evaluation of androstenedione derivatives with a simpler structure, containing in the A-ring only C-2, C-3 or C-4 double bonds or, in the other hand, C-2/C-3, C-3/C4 or C-4/C-5 epoxide groups, which can confer the required planarity in the A/B-ring region. Additionally, we further compare which compounds, androstenes or epoxyandrostanes, are the best inhibitors of aromatase.

1. Cuzick, J.; Wang, D.Y.; Bulbrook, R.D..*Lancet*, **1986**, *8472*, 83.
2. Winer, E.P.; Hudis, C.; Burstein, H.J.; Wolff, A.C.; Pritchard, K.I.; Ingle, J.N., *et al*. *J Clin Oncol*, **2005**, *23*, 619
3. Glosh, D.; Griswold, J.; Erman, M; Pangborn, W. *Nature*, **2009**, *457*, 219.
4. (a) Nagaoka, M.; Watari, Y.; Yajima, H.; Tsukioka, K.; Muroi, Y.; Yamada, K.; Numazawa, M. *Steroids*, **2003**, *68*(6), 533. (b) 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. (c) Cepa, M., Correia-da-Silva, G., Tavares da Silva, E.J., Roleira, F.M.F., Teixeira, N. *Steroids*, **2008**, *73,* 1409.



1 Δ^2, **2** Δ^3, **3** Δ^4



4 R_1,R_2 (O) ; R_3,R_4 (H)

5 R_1 (H) ; R_2,R_3 (O) ; R_4 (H)

6 R_1,R_2 (H) ; R_3,R_4 (O)

DISCOVERY OF NOVEL STEAROYL COA DESATURASE ENZYME 1 (SCD1) INHIBITORS

Isabelle Le Roy, Elisabeth Dupont Passelaigue, Christelle Bressanges, Anne-Cécile Roy, Didier Junquero, Yves Rival, Karine Valeille

Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres Cedex, France.

Hyperlipidemia confers increased risk of atherosclerosis. In order to significantly reduce the burden of these cardiovascular diseases, lipid-lowering therapy such as statins is recommended for dyslipidemia. Stearoyl CoA desaturase enzyme 1 (SCD1) is a microsomal enzyme that catalyses the synthesis of monosaturated fatty acids (C16:1; C18:1) by introduction of a *cis* double bound in the delta 9 position of saturated fatty acyl-CoA substrates (C16:0, C18:0). Studies with SCD1 knock out mice have established that these animals are lean and protected from leptin deficiency-induced and diet-induced obesity, with greater whole body insulin sensitivity than wild-type animals. These observations make SCD1 a potential target for the treatment of obesity, dyslipidemia, metabolic syndrome, and diabetes. A series of novel SCD inhibitors were identified by scaffold design based on known SCD inhibitors. Structural changes were made leading to multiple analogs with a comparable or improved potency. Hit compound 1 provided the starting point (HepG2 IC_{50} = 10 μM). We pursued the optimization of potency by changing substituents and scaffolds. For example compound 2 had an HepG2 IC_{50} = 300 nM, but it was also shown to have poor stability upon incubation with mouse or human liver microsomes, making it a poor candidate for *in vivo* studies. In order to overcome this problem, new chemical changes were experimented. Metabolically stable analogs were identified. The discovery, SAR and *in vitro* pharmacology of this new class of SCD inhibitors will be described.

POSTER COMMUNICATIONS – PC.30

THE PREDICTION OF BLOOD–BRAIN BARRIER PENETRATION FOR MOLECULAR IMAGING AGENTS

Ian M. Newington, R. James D. Nairne, and Duncan G. Wynn

GE Healthcare, Medical Diagnostics Research, The Grove Centre, White Lion Road
Amersham, Buckinghamshire HP7 9LL, UK

Brain penetration of positron emission tomography (PET) and single photon emission computed tomography (SPECT) radiotracers is a key requirement for effective imaging. For *in vivo* CNS-targeted molecular imaging agents, brain uptake should rapidly achieve a high level to give a good opportunity to image within the timescale available when using relatively short half-life isotopes. The ability to use *in silico* prediction as a means of pre-screening to avoid the lengthy and costly need to carry out biodistribution studies on many radiolabelled ligands would be an important development. This poster presents progress made in the correlation of *in silico* and *in vitro* parameters with the resultant brain penetration as measured by biodistribution studies of novel tracers that target a number of CNS receptors.

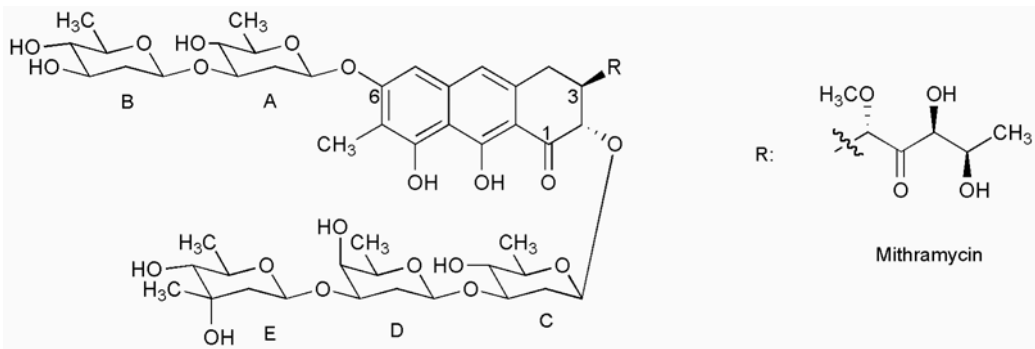
NMR-BASED STRUCTURAL CHARACTERIZATION OF DRUG/TARGET INTERACTION

Pablo Trigo-Mouriño (1), Armando Navarro-Váquez (2) and Víctor M. Sánchez-Pedregal (1)

(1) Dept. Química Orgánica, Facultade de Química, Universidade de Santiago de Compostela. 15782, Santiago de Compostela. Spain.
(2) Dept. Química Orgánica, Facultade de Química, Universidade de Vigo. Spain.

We are interested in some antibiotics of microbial origin that possess antitumour activity due to their binding to DNA. One class of these compounds is the group of derivatives of aureolic acic, that includes chromomycin and mithramycin (MTM) among others[1]. These compounds bind preferentially to the minor groove of CG-rich regions of the DNA. The oligosaccharide and the R side-chains (see figure) are crucial elements in their interaction with DNA. Recently, by using combinatorial biosynthesis strategies[2], novel analogues of mithramycin have been discovered that display better therapeutic index than mithramycin itself. We are especially interested in those derivatives that differ in the side chain R, such as MTM-SK and MTM-SDK[3,4]. Our goal is to understand how those small changes in structure are related to their different affinity for DNA. We are using solution NMR to investigate the structure of some of these ligand/DNA complexes. We expect that this information may help propose new structures with improved binding properties.

1. L.L. Remsing, H.R. Bahadori, G.M. Carbone, E.M. McGuffie, C.V. Catapano, J. Rohr, Biochemistry 2003, 42, 8313.
2. C. Méndez, J.A. Salas, Trends Biotechnol. 2001, 19, 449.
3. L.L. Remsing, A.M. González, M. Nuer-e-Alam, M.J. Fernández-Lozano, A.F. Braña, U. Rix, M.A. Oliveira, C.
Méndez, J.A. Salas, J. Rohr, J. Am. Chem. Soc. 2003, 125, 5745.
4. V. Albertini, A. Jain, S. Vignati, S. Napoli, A. Rinaldi, I. Kwee, M. Nur-e-Alam, J. Bergant, F. Bertoni, G.M. Carbone, J. Rohr, C. V. Catapano, Nucleic Acids Res. 2006, 34, 1721.



POSTER COMMUNICATIONS – PC.32

STRATEGIES FOR IDENTIFICATION OF HTS HITS WITH OPTIMAL PROPERTIES AND GREATEST POTENTIAL FOR SUCCESS

Derek C. Cole, Adam M. Gilbert, John W. Ellingboe, Yongbo Hu

Chemical Sciences, Wyeth Research, 401 N. Middletown Rd. Pearl River, NY, 10956, USA

A typical HTS may identify 100's or 1000's of hits, but it is only possible to optimize several leads. Selection of these leads which lock the Exploratory (Hit-to-Lead) and Discovery teams and significant resources into these series are critical and may have a great impact on the future success or failure of the project. At Wyeth the Exploratory (Hit-to-Lead) phase is broken down into two parts: (i) the HTS confirmed hit to Advanced Hits (4-7 singletons or series) phase and (ii) the Advanced Hits initial optimization and identification of two Lead Series suitable for transition to Discovery Medicinal Chemistry for final optimization. The identification of Advanced Hits with greatest potential requires at least two components: (i) a clear and rational definition of the requirements of the Advanced Hits in terms of physical, chemical, biological and pharmacological properties and (ii) a straightforward strategy and screening tree with validated assays with sufficient throughput to test the HTS hits and allow data driven decisions. This presentation will showcase the strategies, which have been used on various projects following HTS campaigns.

NEW PICOMOLAR AMPA RECEPTOR MODULATORS: DESIGN, SYNTHESIS AND STUDIES

Vladimir Palyulin (1,2), Mstislav Lavrov (1), Vera Lapteva (1), Vladimir Grigoriev (2), Sergey Bachurin (2), Nikolay Zefirov (1,2)

(1) Department of Chemistry, M.V.Lomonosov Moscow State University, (2) Institute of Physiologically Active Compounds, Russian Academy of Sciences

A series of new positive AMPA receptor modulators have been designed using X-ray data for the dimer of the AMPA receptor glutamate binding domain and manual docking techniques (SYBYL8.0 software) with further geometry optimization (Tripos force field). The best fitting to the positive AMPA receptor modulators binding sites was found for N,N'-disubstituted 3,7-diazabicyclo[3.3.1]nonane derivatives, though a number of more simple structures also fitted reasonably to these binding sites. The designed structures were synthesized and the electrophysiological studies were performed for them on isolated Purkinje neurons from rat cerebellum. These studies have revealed that a number of compounds in low concentrations potentiate kainate-induced currents. The results for the best compounds correspond to the potentiation in the range 30-100% for the concentrations 10^{-11}-10^{-8} M while higher concentrations blocked the currents by 30-50%. Thus, the concentration dependence of the effect of the studied compounds on AMPA receptors has a bell shape, *i.e.* the low concentrations potentiate the currents, while the higher concentrations block them. Several compounds have demonstrated the outstanding potency: they caused positive modulation of AMPA receptors at the lowest concentrations among all currently known modulators.

1. Tikhonova I.G., Lavrov M.I., Palyulin V.A., Zefirov N.S., Dokl. Biochem. Biophys., 2004, 399, 351.
2. Patent RU 2333211, 2008.

ENOL CARBAMATES AS A NEW CLASS OF REVERSIBLE INHIBITORS OF FATTY ACID AMIDE HYDROLASE

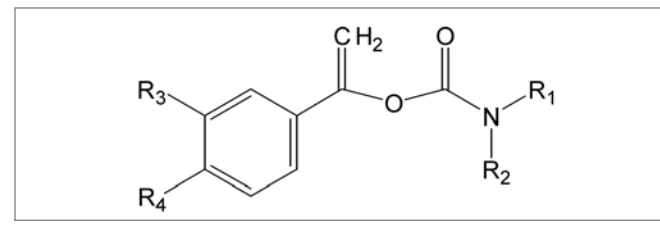
Sonia Gattinoni (1), Chiara De Simone (2,3), Sabrina Dallavalle (1), Filomena Fezza (2,3), Patrizia Minetti (4), Antonio Caprioli (4), Franco Borsini (4), Walter Cabri (4), Sergio Penco (4), Lucio Merlini (1) and Mauro Maccarrone (3,5)

(1) Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Milano, Italy,
(2) Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università degli Studi di Roma "Tor Vergata", Rome, Italy,
(3) Centro Europeo di Ricerca sul Cervello (CERC)/IRCCS Fondazione Santa Lucia, Rome, Italy,
(4) Sigma-tau R&D, Pomezia, Italy,
(5) Dipartimento di Scienze Biomediche, Università degli Studi di Teramo, Teramo, Italy

Inhibition of Fatty Acid Amide Hydrolase (FAAH) increases the endogenous levels of fatty acid amides, with a cascade of effects including analgesic, anxiolytic, antidepressant, sleep-enhancing and anti-inflammatory activities[1]. In the last decade these findings have boosted the search for potent and selective FAAH inhibitors, as potential therapeutics for the treatment of pain, inflammation, anxiety, depression, and other central nervous system disorders[2-4].

We report here the synthesis of new enol carbamates prepared from biphenylethanone derivatives, together with their corresponding inhibitory properties against FAAH enzyme. The best compounds present IC_{50} against the enzyme down to 10 nM.

These compounds behave as reversible, non competitive inhibitors of the enzyme, thus showing their potential for possible development of novel therapeutics.



1. Cravatt, B. F.; Demarest, K.; Patricelli, M. P.; Bracey, M. H.; Giang, D. K.; Martin, B. R.; Lichtman, A. H., Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9371-9376.
2. Seierstad, M.; Breitenbucher, J.G. Discovery and Development of Fatty Acid Amide Hydrolase (FAAH) Inhibitors. *J. Med. Chem.* **2008**, *51*, 7327-7343.
3. Maccarrone, M. Fatty acid amide hydrolase: a potential target for next generation therapeutics. *Curr. Pharm. Des*. **2006**, *12*, 759-772.
4. Fezza F, De Simone C, Amadio D, Maccarrone M. Fatty acid amide hydrolase: a gate-keeper of the endocannabinoid system. *Subcell Biochem*. **2008**, *49*, 101-132.

TUNING THE FOLDING STATES OF β-AMYLOID FRAGMENT Aβ(16-35) WITH DIFFERENT CHARGE OF MICELLE AGGREGATES

Anna Ramunno (1), Manuela Grimaldi (1), Mario Scrima (1), Annamaria Tedeschi (2), Cinzia Esposito (1), Giuseppe Vitiello (2), Gerardino D'Errico (2) and Annamaria D'Ursi (1)

(1) Dipartimento di Scienze Farmaceutiche, Universita' di Salerno, Italy, (2) Dipartimento di Chimica, Università di Napoli Federico II, Italy

Amyloid plaques composed of fibrillar aggregates of amyloid β peptide (Aβ) are considered the characteristic hallmark of Alzheimer's disease. The key molecular event regulating the transition of β-amyloid peptide to the fibril form is the conformational change from random coil-turn to β-strand structures. The knowledge of the structural features corresponding to these different conformational states is strategic to design molecules able to control the conformational transition, and in this respect, acting as anti-Alzheimer agents that prevent or/and reverse the amyloid fibril formation[1]. β-amyloid peptides are enzymatic products of transmembrane protein APP, and have been extensively investigated for their ability to interact with plasma membrane. Although the large amount of data collected until now, the conclusions are controversial and, to some extent, contradictory; nevertheless there is a general agreement on the importance of the charge as key factor driving the interaction of amyloid peptide with plasma membrane[2-5].

Here we report the CD, NMR and EPR study of the β-amyloid fragment Aβ (16-35) in micelle solutions characterized by different charge content. Aβ (16-35) corresponds to the hydrophobic core of the β-amyloid peptide which is proved critical in the aggregation-disaggregation processes.

Our results demonstrate that different contents of negative charges induce different conformational states, potentially corresponding to different folding states of Aβ(16-35). EPR experiments performed on selectively Aβ(16-35) spin labelled samples, show that charges are important to drive the orientation of the peptide on the plasma membrane, promoting or inhibiting membrane catalyzed aggregation processes.

1. Porat, Y. et al, Chem. Biol. Drug Des. 2006, 67, 27–37.
2. Bokvist, M. et al, J. Mol. Biol. 2004, 335, 1039–1049.
3. Chochina, S. V. et al, J. Lipid Res. 2001, 42, 1292–1297.
4. Bowie, J. U. Nature 2005, 438, 581-589.
5. Dobson, C. M. Nature 2003, 426, 884-890.

POSTER COMMUNICATIONS – PC.36

SYNTHESIS AND IN VITRO OPIOID RECEPTOR STUDIES ON BENZOMORPHAN-BASED COMPOUNDS: LP1, A POTENT AND LONG-ACTING ANTINOCICEPTIVE

Lorella Pasquinucci (1), Orazio Prezzavento (1), Emanuele Amata (1), Emanuela Arena (1), Simone Ronsisvalle (1), Carmela Parenti (2), Giuseppina Aricò (2), Giovanna Maria Scoto (2) and Giuseppe Ronsisvalle (1)

(1) Department of Pharmaceutical Sciences-Pharmaceutical Chemistry Unit, University of Catania, Italy
(2) Department of Pharmaceutical Sciences-Pharmacology Unit, University of Catania, Italy

Unrelieved cancer pain significantly decreases the quality of life of patients. Survey data indicate that 5%-10% of patients with non-metastatic solid tumors, 33% with metastatic tumors and at least 70% of dying cancer patients have pain that interferes with quality of life and with function[1]. Cancer pain can be acute or chronic in nature depending on the disease stage. Acute pain comes on quickly, can be severe, but lasts a relatively short time. As opposed chronic pain is greater than 6 months duration pain and is a complex symptom particularly difficult to treat effectively[2]. The most common method proposed by the World Health Organization (WHO) for cancer pain relief consist in a three-step treatment from non-opioid to weak and strong opioids. According to the WHO, opioids alone, or co-administered, are the mainstay of therapy for cancer-related pain[3]. Unfortunately these compounds produce side-effects which reduce the opioids clinical use itself. In particular, the development of tolerance, a gradual loss in opioid efficacy upon repeated administration, represents the therapy limit for patients suffering from chronic cancer pain. In this view, Normolife Project, a "Specific targeted research projects" within the European Sixth Framework Programme, was started to develop new compounds and therapeutic strategy for chronic cancer pain treatment. Compound LP1 (Fig. 1), a benzomorphan-based opioid ligand, has been discovered from a screening of compound libraries.

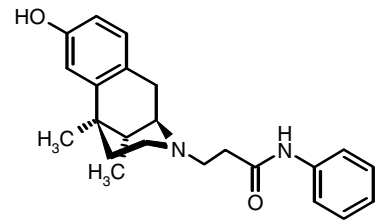


Fig. 1, LP1

LP1 was found to have affinity for μ and δ receptor in nanomolar range (Ki_{μ} = 0.83 nM and Ki_{δ} = 29.1 nM, respectively). Moreover, in tail flick test LP1 showed a potent analgesic effect comparable to morphine. In chronic subcutaneous administration, LP1 maintained its analgesic profile until the fifth day while chronic morphine administration determinated a significative loss of analgesic effect already at the third day of treatment. These results indicate that LP1 could be a new long-acting opioid compound with lower tolerance development. For these reasons we decided to explore SAR of LP1 through the development of a new series of LP1 analogues by replacement of its phenyl ring with different substituted aromatic and heteroaromatic rings. All compounds synthesized were evaluated in vitro with the aim to obtain their affinity and functional profile.

1. D. S. Zhukovsky et al, Journal of Pain and Symptom Management, 1995, 10, 2, 113-119.
2. M. Williams et al, Journal of Medicinal Chemistry, 1999, 42, 9, 1481-1500.
3. B. Lairda et al, European journal of cancer, 2008, 44, 1078-1082.

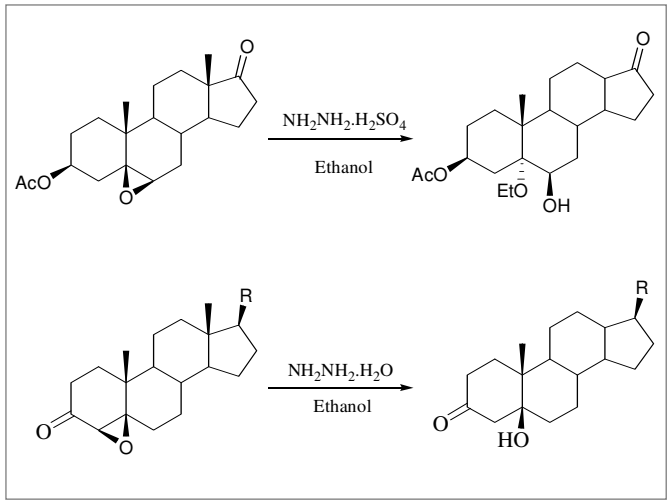
REACTIONS OF EPOXYSTEROIDS WITH METAL-FREE HYDRAZINE REAGENTS

Alcino Leitão (1), Jorge Salvador (2) and Luisa Sá e Melo (1)

(1) Centro de Estudos Farmacêuticos, Lab. Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, 3000-548, Coimbra, Portugal; (2) Lab. Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, 3000-548, Coimbra, Portugal; E-mail: ajleitao@ff.uc.pt

The development of metal-free synthetic methods is an area of great interest to the synthesis of compounds that do not tolerate metal contamination, such as pharmaceutical products. This approach avoids the use of toxic and expensive metals and is particularly important in modern medicinal chemistry[1].

In this communication we show the application of hydrazine and hydrazine salts as non-metallic reagents in the chemistry of steroid compounds. We describe an efficient metal-free process for the ring opening of epoxides, under relatively mild conditions using catalytic amounts of the cheap and commercially available hydrazine sulphate[2] as well as a versatile procedure for the preparation of β-hydroxy ketones, by reductive cleavage of the corresponding α,β-epoxy ketones under mild conditions using $NH_2NH_2.H_20$ in ethanol as solvent[3]. Both β-alkoxy alcohols and β-hydroxy ketones are important intermediates for the synthesis of compounds with pharmaceutical interest.



We thank FCT, through POCI and FEDER, for financial support.

1. *Guideline on the specification limits for residues of metal catalysts*, European Medicines Agency, Pre-authorisation Evaluation of Medicines for Human Use; London, January **2007**; Doc. Ref. CPMP/SW P/QWP/4446/00; http://www.emea.europa.eu
2. Leitão AJL, Salvador JAR, Pinto RMA, Sá e Melo ML *Tetrahedron Lett.* **2008**, *49*, 1694-1697.
3. Salvador JAR, Leitão AJL, Sá e Melo ML, Hanson JR. *Tetrahedron Lett.* **2005**, *46*, 1067-1070.

POSTER COMMUNICATIONS – PC.38

OPTIMIZATION OF NOVEL HETEROARYL DERIVATIVES AS POTENT AND SELECTIVE ADENOSINE A3 RECEPTOR ANTAGONISTS: IN VITRO PHARMACOLOGICAL AND ADME-PK PROFILE

Sylvain Celanire (1), Christelle Bolea (1), Nathalie Lambeng (1), Sonia Poli (1), Nicolas Poirier (1), Fabien Fonteny (1), Sandrine Lasserre (1), Anna Rencurosi (2), Marco Farina (2), Carlo Parini (2), Annalisa Mortoni (2), Stefania Gagliardi (2), Jean-Philippe Rocher (1) and Emmanuel Le Poul (1)

(1) Addex Pharmaceuticals SA, 12 Chemin des Aulx, CH-1228, Plan Les Ouates, Switzerland. (2) NiKem Research s.r.l. Via Zambeletti, 25, 20021, Baranzate (MI) Italy.

Adenosine is a ubiquitous neuromodulator which acts by stimulating four adenosine receptor (AR) subtypes (A_1, A_{2A}, A_{2B} and A_3), all belonging to the G-protein-coupled receptors family. In particular, the adenosine A_3 receptor (A_3R), the most recently discovered adenosine receptor[1], has been shown to mediate adenylate cyclase inhibition, stimulation of phospholipase C and D[2], and calcium mobilization via a Gi/o-dependent pathway[3]. The A_3R has a widespread distribution in the brain and in periphery such as lung, liver, kidney, heart, testis and eyes, which confer potentially numerous therapeutic applications of A_3R agonists as cerebro- and cardioprotective agents, anti-tumor agents, anti-inflammatory agents[4]. A_3R antagonists may bring clinical benefits as renal protective drugs[5], anti-asthmatic[6], neuroprotective[7] and especially as anti-glaucoma agents considering the efficacy of non optimized compounds in preclinical models[8].

Over the last two decades, tremendous efforts have been made to identify potent, subtype-selective human A_3R antagonists leading to a wide variety of structurally diverse heterocycles such as xanthines, dihydropyridines, triazolopyrimidines, naphthyridine, isoquinoline and thiazoles[9]. However, selectivity issues versus other AR subtypes and non optimal physicochemical or pharmacokinetic profile have been important hurdles to the development of novel A_3R antagonists for further clinical investigations.

We report here the synthesis, the pharmacological identification and drug-likeness properties of a new class of A3R antagonists (Figure 1)[10].

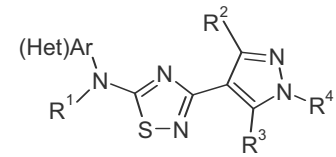


Figure 1

1. Zhou, Q.Y. et al (1992) *Proc. Natl. Acad. Sci. U.S.A.*, 89:7432-7436.
2. Abbracchio, M.P. et al (1995) *Mol. Pharmacol.*, 48:1038-1045.
3. Shneyvays, V. et al (2005) *Am. J. Physiol. Heart Circ. Physiol.*, 288:H2792-2801.
4. (a) Jacobson, K.A. and Gao, Z.G. (2006) *Nature Rev. Drug Disc.*, 5:247-264; (b) Bar-Yehuda, S. et al (2007) *Expert Opin. Investig. Drugs*, 16:1601-1613.
5. Lee, et al (2003) *Am. J. Physiol. Renal. Physiol*. 284: F267-F273.
6. Miwatashi S. et al (2008) *Chem. Pharm. Bull.*, 56:1126-1137.
7. Pugliese, A.M. et al (2007) *Biochem. Pharmacol.*, 74:768-779.
8. (a) Yang H. et al (2005) *Curr. Eye Res.*, 30:747-754; (b) Okamura, T. et al (2004) *Bioorg. Med. Chem Lett.*, 14:3775-3779.
9. (a) Pal, S. et al (2009) *Bioorg. Med. Chem.*, 17:3733-3738; (b) Baraldi, P.G. and Borea, P.A. (2000) *Trends Pharmacol. Sci.*, 21:456-459; (c) Moro, S. et al (2006) *Curr. Med. Chem.*, 13:639-645; (d) Colotta, V. et al (2009) *J. Med. Chem.*, 52: 2407-2419; (e) Ohkawa, S. et al. (2000) PCT Int. Appl., WO2000064894A1.
10. Bolea, C.; Celanire, S.; Le Poul, E.; Gagliardi, S.; Rencurosi, A. and Farina, M. (2009) PCT Int. Appl., WO2009010871A2.

AN IN VIVO MODEL TO ASSESS BRAIN mGLUR2 TARGET ENGAGEMENT: CHARACTERISATION OF ORTHOSTERIC AND NON COMPETITIVE mGLUR2/3 ANTAGONISTS

Françoise Girard, Brice Campo, Sylvain Celanire, Sandrine Lasserre, Olivier Rizzo, Abdelhak Bessif, Isabelle Royer-Urios, Sonia Poli and Emmanuel Le Poul

Addex Pharmaceuticals SA, 12 Chemin des Aulx, CH-1228, Plan Les Ouates, Switzerland.

One of the hurdles in drug discovery is to get an objective measurement of the in vivo target engagement (TE). This is particularly true for CNS drugs that must cross the blood brain barrier and reach specific brain area for modulating target function.

We set up a mGluR2-related in vivo TE assay based on the observation that the mGluR2/3 agonist LY354740 produced a dose-dependent reduction in the locomotor activity (LMA) (3 to 10 mg/kg i.p) in wild-type but not in KO mice[1,2]. We showed that LY341495 (3 or 10 mg/kg i.p), a reference mGluR2/3 orthosteric antagonist, completely reversed the hypolocomotor activity-induced by LY379268 (10 mg/kg i.p)[3], while having no effect when administered alone.

We characterized a non competitive mGluR2/3 antagonist ((4-[3-(2,6-Dimethylpyridin-4-yl)phenyl]-7-methyl-8-trifluoromethyl-1,3-dihydrobenzo[b][1,4]diazepin-2-one, Compound A)[4] in this assay. Cpd A significantly and dose-dependently reversed hypolocomotion induced by 10 mg/kg i.p of LY379268 (ED_{50} = 8.4 mg/kg, p.o.), and did not change LMA when administered alone. This in vivo pharmacological activity of Cpd A is well-predicted with a PK/PD correlation supporting the hypothesis that the in vivo effects are due to activity at mGluR2.

We also showed that a mGluR3 specific antagonist ((3S)-1-(5-bromopyrimidin-2-yl)-N-(2,4-dichlorobenzyl)pyrrolidin-3-amine, Compound B)[5] did not reverse LY379268-induced hypolocomotion, even at doses up to 300 mg/kg p.o, suggesting that hypolocomotion is mGluR2 related to the activation of mGluR2.

This target engagement test is a useful in vivo model to assess the functional blockade of mGluR2 receptors and to characterize mGluR2 antagonists as potential cognitive enhancers and antidepressants[6].

1. Spooren, W.P. et al, (2000) *Eur. J. Pharmacol.*, 397:R1-2.
2. Monn, J.A. et al, (1999) *J. Med. Chem.*, 42:1027-1040.
3. Kingston, A.E. et al, (1998) *Neuropharmacology*, 37:1-12.
4. Ballard, T.M. et al, (2005) PCT Int. Appl. WO2005014002A1.
5. Britton, T.C. et al, (2006) PCT Int. Appl. WO2006044454A1.
6. (a) Chaki, S. et al, (2004) *Neuropharmacology*, 46:457-467; (b) Higgins, G.A. et al, (2004) *Neuropharmacology*, 46:907-917.

POSTER COMMUNICATIONS – PC.40

PHYSICO-CHEMICAL PROPERTIES AND SYNTHESIS OF XANTHONES

Carlos M. G. Azevedo (1), Carlos M. M. Afonso (1), Emília Sousa (1), Sara M. Cravo (1), Salette H. Reis (2), Madalena M. M. Pinto (1)

(1) Laboratório de Química Orgânica, Centro de Estudos Química Medicinal da Universidade do Porto (CEQUIMED-UP), Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal; (2) Laboratório de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal.
cgoncalves.azevedo@gmail.com

Xanthones are oxygen heterocycles with very interesting pharmacological activities[1]. These compounds can be found in Nature (higher plants, lichens and fungi) or can be obtained by synthesis. Xanthones comprise hundreds of members with different pattern of substitutions leading to a great variety of compounds[2].

Our group has been focusing for many years on the synthesis of xanthone derivatives and on the study of some of their biological activities, namely antitumor and antimicrobial[3]. We are also focusing on the physicochemical properties of the more interesting compounds.

Herein we report the study of the lipophilicity of a series of oxygenated xanthones which revealed interesting biological activities. The lipophilicity of the xanthones was determined using the liposome/water system, a model that takes into account not only the hydrophobic interactions between the compound and the membrane, but also the electrostatic interactions[4].

Based on a hit compound (xanthone XP13), which was synthesised by our group and showed very interesting antitumor activity, we also present the synthesis of some XP13 analogues obtained in good yields using classic and MAOS approaches.

FCT (I&D 4040/2007), FEDER, POCI for financial support and for the PhD grant to Carlos Miguel Azevedo (SFRH/BD/41165/2007).

1. M.M.M. Pinto *et al*, Current Medicinal Chemistry, **2005**, 12, 2517-2538;
2. E. Sousa *et al*, Current Medicinal Chemistry, **2005**, 12, 2447-2479;
3. R. Castanheiro *et al*, Tetrahedron, **2009**, 65, 3848-3857;
4. Georgette P. van Balen *et al*, Medicinal Research Reviews, **2004**, 24 (3), 299-324.

CYTOTOXIC EPOXYSTEROLS: CHEMOENZYMATIC SYNTHESIS, BIOLOGICAL EVALUATION AND SAR

João F.S. Carvalho (1), M. Manuel Cruz Silva (1), João N. Moreira (2), Sérgio Simões (2), M. Luisa Sá e Melo (1)

(1) Centro de Estudos Farmacêuticos, Lab. Química Farmacêutica, Faculdade de Farmácia (2) Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia Pólo das Ciências da Saúde, 3000-548, Coimbra, PORTUGAL

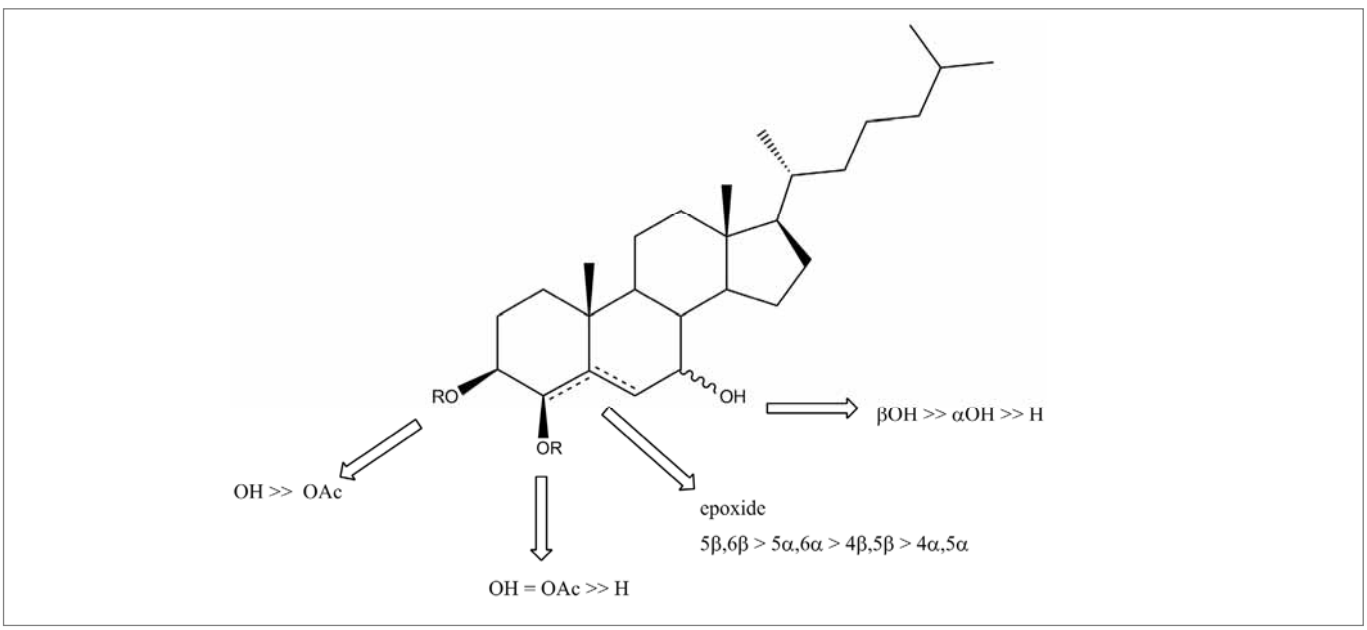
Oxysterols, represent a group of biomolecules gaining much attention in the last years due to their relevant bioactivities. Naturally occurring 5α,6α- and 5β,6β-epoxysteroids have shown important cytotoxic effects in tumor cells.

In this communication, we report the synthesis and evaluation of a library of diastereomerically pure epoxysterols, prepared by combining chemical and enzymatic methodologies. Unsaturated steroids were oxidized by magnesium monoperoxyperhydrophtalate hexahydrate in acetonitrile and the resulting epimeric epoxides were enzymatically discriminated using Novozym 435 or Lipase AY.

The bioactivity of the epoxysterols obtained was studied by evaluation of its cytotoxic potential in two cancer cell lines and in a normal cell line. The results obtained show that these molecules are selectively cytotoxic in a dose-dependent manner at micromolar range for the tumour cells, being the bioactivity strongly affected by the stereochemistry of the 4,5- and 5,6-epoxides and the surrounding hydroxyl groups at 3-, 4- and 7-positions.

Thanks are due to Fundação para a Ciência e Tecnologia (FCT), Portugal, through POCI. JFS Carvalho also thanks FCT for a PhD grant (SFRH/BD/18263/2004).

1. JFS Carvalho, MMC Silva, JN Moreira, S Simões, ML Sá e Melo, *J. Med. Chem.*, 2009, 52, 4007–4019.



LIGAND-BASED DESIGN AND BIOCHEMICAL EVALUATION OF NEW POTENT AROMATASE INHIBITORS

Marco Neves (1), Teresa Dinis (2), Giogio Colombo (3), Maria Luisa Sá e Melo (1)

(1) CEF, Lab. Química Farmacêutica, Fac. Farmácia, Univ. Coimbra, 3000-548, Coimbra, Portugal (2) CNC, Lab. Bioquímica, Fac. Farmácia, Univ. Coimbra, 3000-548, Coimbra, Portugal (3) Istituto di Chimica del Riconoscimento Molecolare - CNR, 20131, Milan, Italy

Aromatase, an enzyme of the cytochrome P450 family, is involved in the conversion of androgens into aromatic estrogenic steroids such as estradiol and estrone. Suppression of estrogen biosynthesis by a mechanism of aromatase inhibition is therefore an effective approach for the endocrine treatment of hormone sensitive breast cancer[1]. Research aiming for the discovery of aromatase inhibitors identified interesting compounds, such as azole non- steroid derivatives[2] and androstenedione analogs[3-5]. In this work we have built and validated new pharmacophore models specific for each of these chemical classes, useful for a fast virtual screening of large compound databases. Small subsets of promising anti-aromatase candidates were extracted from the NCI database using this methodology, and tested on an *in vitro* assay with aromatase extracted from human term placenta. New potent aromatase inhibitors, active in the low nanomolar range, were identified. These results provide the first *in silico* high-throughput screening methodology for potent aromatase inhibitors[6,7].

We thank Fundação para a Ciência e a Tecnologia (FCT), Portugal, through POCTI and FEDER, for financial support. Marco Neves also thanks FCT for a PhD grant (SFRH / BD / 17624 /2004).

1. Smith, I. E. *et al. N. Engl. J. Med.* **2003**, *348*, 2431-2442;
2. Recanatini, M. *et al. Med. Res. Rev.* **2002**, *22*, 282-304;
3. Numazawa, M. *et al. J. Med. Chem.* **1996**, *39*, 1033-1038;
4. Numazawa, M. *et al. J. Steroid Biochem. Mol. Biol.* **1998**, *67*, 41-48;
5. Numazawa, M. *et al. Steroids* **1999**, *64*, 187-196;
6. Neves, M. A. C. *et al. J. Med. Chem.* **2009**, *52*, 143-150;
7. Neves M. A. C. *Eur. J. Med. Chem.* **2009**, doi:10.1016/j.ejmech.2009.05.003.

DESIGN OF ANTICANCER COMPOUNDS: NEW NON SYMMETRICAL INHIBITORS OF CHOLINE KINASE

Belén Rubio-Ruiz (1), Ana Conejo-García (1), Pablo Ríos (2), José M Jiménez-López (2), María Paz Carrasco (2), Miguel A. Gallo (1), Carmen Marco (2), Antonio Espinosa (1), Antonio Entrena (1)

(1) Dpto. Química Farmacéutica y Orgánica, Facultad de Farmacia, Univ. Granada, 18071 Granada (Spain).
(2) Dpto. Bioquímica y Biología Molecular I, Fac. Ciencias, Univ. Granada, 18071 Granada (Spain).

Phospholipid molecules are presumed to participate in the processes of oncogene-induced transformation[1]. Phosphatidylcholine, the major component of the plasma membrane, is hydrolyzed by phospholipase D to yield phosphatidic acid and choline. Choline is phosphorylated by choline kinase (ChoK) to generate phosphorylcholine (PCho). Increased levels of ChoK activity and PCho production in human cancers have been found[2]. We have previously synthesized and characterized more than 250 symmetrical cationic compounds based on structural modifications of hemicholinium-3 (HC-3), a well known ChoK inhibitor (Figure 1). The reported crystal structures of a ChoK isoform from Caenorhabditis elegans[3], and the human one in complex with ADP or PCho[4], provided new opportunities to rationalize the knowledge regarding the inhibition of this enzyme. Docking studies performed on the homology model of human ChoK based on the X-ray crystallographic structure of C. elegans indicate that inhibitors can bind to the binding sites of both substrates (ATP and choline)[5]. Therefore, a consequence for the design of new inhibitors can be obtained. The nature of both choline and ATP binding sites are really different, and hence new non symmetric inhibitors bearing a positively charged group that could be stabilized into the choline binding site, and a noncharged aromatic moiety that mimics the ATP adenine moiety connected by an appropriated spacer can be designed.We describe here the design, synthesis and biological evaluation of a series of 1-{4-[(6-substituted-9H-purin-9-yl)methyl]linker}-4-(substituted)pyridinium bromide (Figure 1).

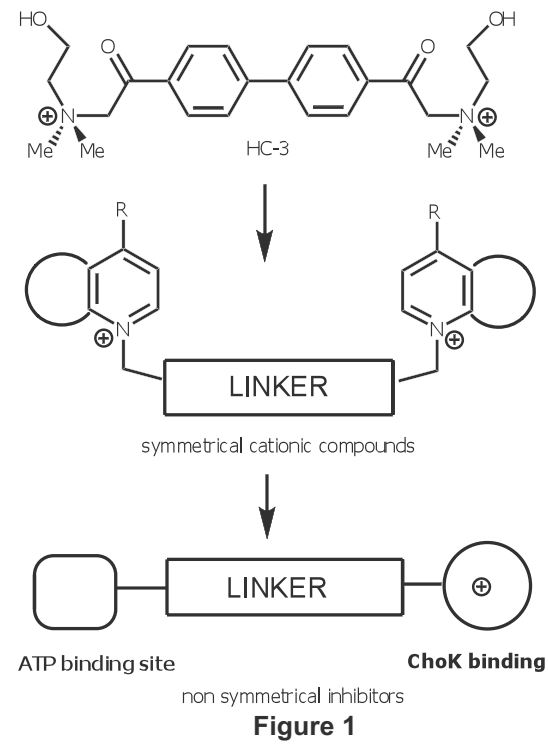


Figure 1

1. J. C. Lacal, A. Carnero, Oncol. Rep. 1994, 1, 677-693.
2. K. Nakagami, T. Uchida, S. Ohwada, et al. Jpn. J. Cancer Res. 1999, 90, 419-424.
3. D. Peisach, P. Gee, C. Kent, Z. Xu, Structure. 2003, 11, 703– 713.4.
4. Malito, N. Sekulic, W.C.S.Too, et al. JMB. 2006, 364, 136-151.
5. L. Milanese, A. Espinosa, J. Campos, et al. ChemMedChem. 2006, 1, 1216-1228.

POSTER COMMUNICATIONS – PC.44

DESIGN AND SYNTHESIS OF NOVEL INDOLOCARBAZOLE DERIVATIVES AS ANTI-CANCER AGENTS

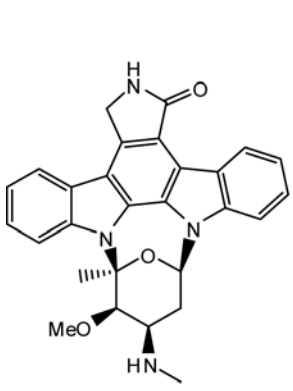
Larry T. Pierce, Michael M. Cahill and Florence O. McCarthy

Department of Chemistry and Analytical and Biological Chemistry Research Facility, University College Cork, Western Road, Cork, Ireland.
e-mail f.mccarthy@ucc.ie

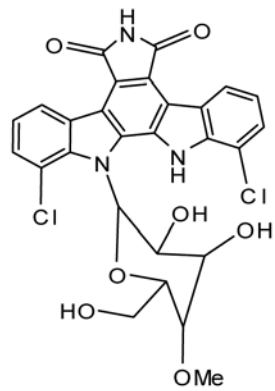
Initial interest in the possible use of indolocarbazole antibiotics as clinical anti-tumour agents beganas a result of the discovery of a natural product, Staurosporine **1**, found to be a nanomolar inhibitor of the enzyme PKC *in vitro*[1]. Rebeccamycin **2**, which also possesses the conserved indolo[2,3-*a*]carbazole framework, exhibits an IC_{50} value of 1.75 μM against Topoisomerase I, yet is virtually inactive towards Topoisomerase II and PKC[2].

Our current research involves modification of the pyrrolidine-2,5-dione heterocyclic component of the polycyclic ring system, engineering its bioisosteric replacement with uracil and other 5- and 6-membered analogues (**3**) in order to investigate the potential synergistic multi-modal activity against protein kinases and supercoiling enzymes (Topo I/ II).

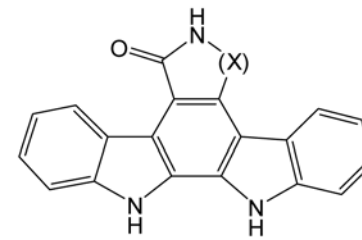
1. M. Prudhomme, Current Pharmaceutical Design, 1997, **3**, 265.
2. M. Prudhomme, Current Medicinal Chemistry, 2000, **7**, 1189.



1



2



3

ENERGY DECOMPOSITION FOR "TUBERCULOSTATIC AGENT – DHFR" COMPLEXES

Elena Afonkina (1), Vladimir Potemkin (2), Maria Grishina (2), Nadezhda Ivshina (2)

(1) Chelyabinsk State University, Russia, Chelyabinsk
(2) Chelyabinsk State Medical Academy, Russia, Chelyabinsk

"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. X-ray data of protein structures have been found in Protein Data Bank. The drug is orientated to the cavity bottom by oxiacilic radical and methyl group, pyrimidine ring is turned to the protein outlet. Aminoacid residus 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.).

Low-active molecules have the following shorter contacts then high-active molecules: 1) of Leu50 with the oxyacylic oxygen, 2) Ile20 with the hydrogen of the aromatic substituent and dihydropyrimidine cycle, 3) Ser49 with the hydrogen of methyl. The following regresiion model was obtained:

$$pMIC = 4.47 - 5.6x_1 - 0.015x_2$$

x_1 – "bond – angle" interaction (MM3 force field), x_2 – electrostatic energy. The cross validation quality is 0.9779 and Fisher's criterion is 43. So, the less steric obstacles for the complexes formation yield in the increase of the activity. Moreover, tuberculostatic activity is depended on full energy of the complex. It has been shown that the more active drug forms the more stable complex.

SKIF–GRID Supercomputer Initiative, RFBR grant (07-03-96041, 07-04-96053).

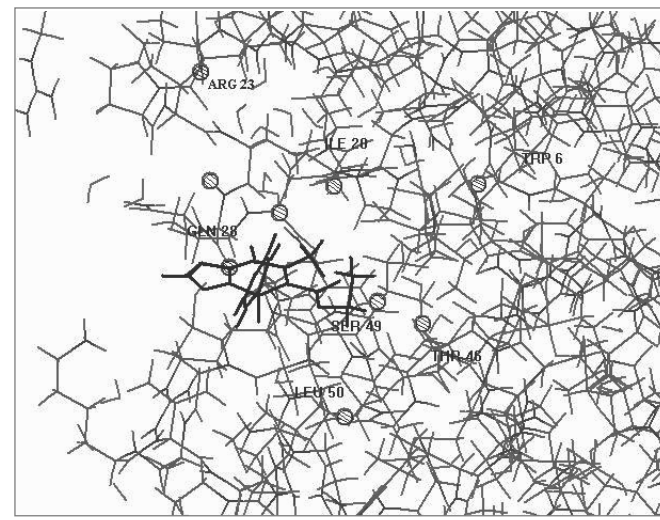


Fig. "Drug – DHFR" complex (atoms of aminoacid residues responsible for binding with the drug are marked).

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., DOI: 10.1021/ci800405n.

POSTER COMMUNICATIONS – PC.46

DESIGN & SYNTHESIS OF NOVEL ELLIPTICINE C-KIT KINASE INHIBITORS

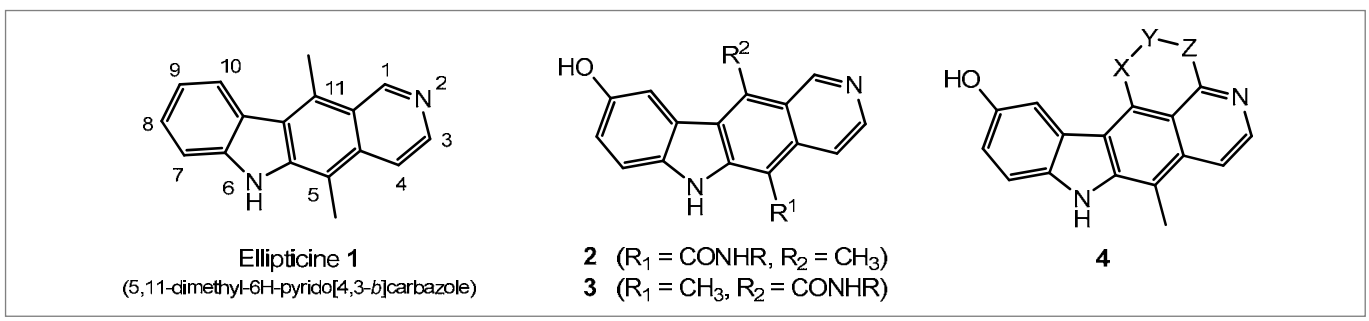
Charlotte Miller (1), Damien Thompson (2) and Florence O. McCarthy (1)

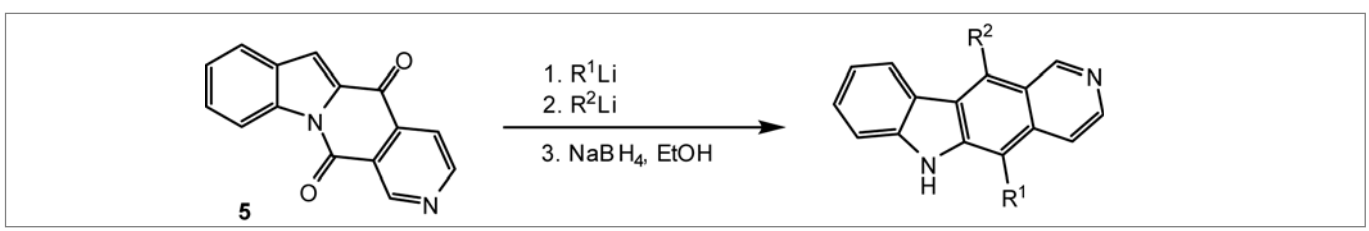
(1) Department of Chemistry, Analytical and Biological Chemistry Research Facility, University College Cork, Western Road, Cork.
(2) Tyndall National Institute, Lee Maltings, Cork, Ireland. email: f.mccarthy@ucc.ie

Receptor tyrosine kinase (RTK) enzymes regulate cell signaling pathways and so are an important target for cancer chemotherapy[1]. Current inhibitors of c-kit kinase, the key RTK stem cell factor receptor, are inactive against the most common mutated variant Asp816Val, associated with highly malignant cancers[2,3]. A recent publication highlighted the utility of the ellipticine pharmacore **1** in inhibiting mutated c-kit[4]. Our recent molecular modeling work[5] applied high-level simulation tools to further probe the binding of ellipticine-based derivatives to c-kit. The resulting binding mode resembles the native complex (ADP) and serves to explain most of the existing experimental data on binding specificities.

On the basis of these results, several key substituted ellipticines **2-4** were selected for synthesis and biological testing. The synthetic route[6,7] employed utilizes regioselective alkylation of the key intermediate, ketolactam **5**, giving access to novel C-5 and C-11 substituted ellipticines.

1. Schlessinger, J., *Cell,* **2000**, *103*, 211 - 225.
2. Ma, Y.; Zeng, S.; Metcalfe, D.; Akin, C.; Dimitrijevic, S.; Butterfield, J.; McMahon, G.; Longley, B. J., *Blood,* **2002**, *99*, 1741 -1744.
3. Liao, A. T.; Chien, M. B.; Shenoy, N.; Mendel, D. B.; McMahon, G.; Cherrington, J. M.; London, C. A., *Blood,* **2002**, *100*, 585 -593.
4. Vendome, J.; Letard, S.; Martin, F.; Svinarchuk, F.; Dubreuil, P.; Auclair, C.; Le Bret, M., *J. Med. Chem,.* **2005**, *48*, 6194-6201.
5. Thompson, D.; Miller, C.; McCarthy, F. O., *Biochemistry,* **2008**, *47*, 10333 - 10344.
6. Gribble, G. W.; Saulnier, M. G.; Obaza-Nutaitis, J. A.; Ketcha, D. M., *J. Org. Chem.,* **1992,** *57*, 5891-9.
7. Modi, S. P.; Michael, M. A.; Archer, S.; Carey, J. J., *Tetrahedron,* **1991**, *47*, 6539-48.





ANTICONVULSANT ACTIVITY OF 2,4(5)-DIARYLIMIDAZOLES IN MICE AND RAT MODELS

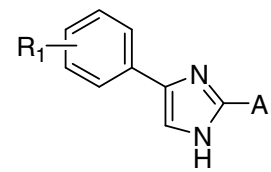
Mirko Rivara (1), Marco Fantini (1), Valentina Zuliani (1), Manoj K. Patel (2)

(1) Dipartimento Farmaceutico, Università degli Studi di Parma, V.le G.P. Usberti, 27/A, I-43124 Parma, Italy, (2) Department of Anesthesiology, University of Virginia, Charlottesville, VA 22908, USA

Epilepsy is a common neurological disorder characterized by recurrent seizures that afflicts approximately 2% of the world's population. The antiepileptic drugs (AEDs) presently used provide adequate seizure control in several patients, but it is estimated that up to 30% of the affected people are still resistant to the available medication and that many AEDs have serious side effects. Voltage-gated sodium (Na_V) channels are considered an important target for the AEDs because of their fundamental role in establishing and regulating the excitability of neurons within the CNS. In particular, the $Na_V1.2$ isoform is important for drug targeting since it is abundantly expressed within the CNS. Furthermore, mutations in *SCN2A*, the gene encoding $Na_V1.2$, have been identified in patients with generalized epilepsy[1]. In fact, a number of the clinical available AEDs, (lamotrigine, phenytoin, carbamazepine and oxcarbazepine) have been shown to inhibit $Na_V1.2$ Na channel currents[2].

Recent researches in our group have analyzed 2,4(5)-diarylimidazoles as new targets of AEDs, founding that many of the compounds synthesized exhibited greater inhibition of $hNa_V1.2$ compared to two clinically used anticonvulsant drugs, lamotrigine and phenytoin, with IC_{50} values in the nanomolar-micromolar range[3-5].

These molecules were then tested in rodent models for anticonvulsant potency (MES and scMet) and AED-induced teratogenicity and the most potent of the synthesized novel compounds emerging from these studies were further investigated for quantification of their anticonvulsant activity (ED_{50}). The encouraging results obtained suggest that the compounds here presented could be novel leads in the search for potent and safe anticonvulsants.



A: phenyl, substituted-phenyl, 3-pyridine, 4-pyridine, cyclohexyl, 2-furan, 3-furan, 2-benzofuran, 3-thiophene

1. Meisler, M.H.; Kearney, J.A. *J. Clin. Invest.* **2005**, *115*, 2010-2017.
2. Liu, G.; Yarov-Yarovoy, V.; Nobbs, M.; Clare, J.J.; Scheuer, T.; Catterall, W.A. *Neuropharmacology* **2003**, *44*, 413-422.
3. Zuliani, V.; Cocconcelli, G.; Fantini, M.; Ghiron, C.; Rivara, M. *J. Org. Chem.* **2007**, *72*, 4551-4553.
4. Rivara, M.; Baheti, A.R.; Fantini, M.; Cocconcelli, G.; Ghiron, C.; Kalmar, C.L.; Singh, N.; Merrick, E.C.; Patel, M.K.; Zuliani V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5456-5458.
5. Fantini, M.; Rivara, M.; Zuliani V.; Kalmar, C.L.; Vacondio, F.; Silva, C.; Baheti, A.R.; Singh, N.; Merrick, E.C, Katari, R.S.; Cocconcelli, G.; Ghiron, C.; Patel, M.K. *Bioorg. Med. Chem.* **2009**, *17*, 3642-3648.

POSTER COMMUNICATIONS – PC.48

MOLECULAR DYNAMICS SIMULATION OF THE mGLUR2/mGLUR3-5HT2A DIMERS IN AN EXPLICIT DMPC-LIPID BILAYER

Gabriele Costantino (1) Agostino Bruno (1), Antonio Entrena Guadix (2)

(1) Dipartimento Farmaceutico, Università degli Studi di Parma Via Usberti 27/A 43100 Parma
(2) Departamento de Química Farmacéutica y Orgánica Facultad de Farmacia Campus de Cartuja s/n. 18071 Granada (Spain)

G-protein coupled receptors (GPCRs) represent one of the major targets of interest in medicinal chemistry, because of their involvement in many physio-pathological processes. Over the last years, experimental evidences have shown that these receptors could form functional homo- and hetero-dimers or oligomers[1,2]. For some subclasses of the GPCR superfamily, and for $GABA_B$ receptors and metabotropic glutamate receptors (mGluRs) in particular, the dimer form is considered to be the constitutive functional unit[1,3,4]. Recently, evidences for the heterodimerization between the mGluR2 subtype of the metabotropic glutamate receptor family and the 5-HT_{2a} subtype of the seretoninergic family, have been reported, thus indicating that dimerization can also occur among unrelated receptor subtypes[5]. Morover it has been supposed that this heterodimer complex could be involved in some physiopathologic disorder of the central nervous system, in particular with important functional consequences related to the mechanism of action of antipsychotic drugs[5], for which both receptor subtypes are considered classical targets. With the aim to investigate the influence that this aspect could have on ligand recognition or receptor activation we have built 3D models of the dimeric assembly between the transmembrane regions of mGluR2, the closely related mGluR3, and $5HT_{2a}$. The dimers were embedded in an explicit phospholipidic bilayer and simulated for 40 ns through molecular dynamics (MD). In separate experiments, the model of the $5HT_{2a}$ monomer and of the $5HT_{2a}$ homodimer were embedded in the same bilayer and simulated, under the same conditions, for 40 ns of MD. For all the systems, a dimyristoylphosphatidylcholine (DMPC)-based bilayer was generated in a rectangular water box in which the ionic strength was kept at 0.15 M by KCl. The simulations were then compared by essential dynamics analysis. From our analysis appear that TM domains and loop regions show different displacements during the simulation, in particular heterodimeric $5HT_{2a}$ accomplishes smaller displacements than monomeric $5HT_{2a}$ compared to reference structure at TM domain level. Furthermore the residues involved in ligand binding show different displacements depending on which receptor form they belong to.

1. Marshall FH; White J; Main M; Green A; Wise A; GABA(B) receptors function as heterodimers. *Biochem Soc Trans. 4:530-535,* **1999**.
2. Liang Y; Fotiadis D; Filipek S; Saperstein DA; Palczewski K; Engel A; Organization of the G protein coupled receptors rhodopsin and opsin in native membranes, *The Journal of BiologicalChemistry, 278:21655-21662,***2003**.
3. Pin JP; Kniazeff J; Binet V; Liu J; Maurel D; Galvez T; Duthey B; Havlickova M; Blahos J; Prezeau L; Rondard P; Activation mechanism of the heterodimeric GABAB receptor. *Biochemical Pharmacology* , 68:1565–1572**,** **2004**.
4. Kniazeff J; Bessis AS; Maurel D; Ansanay H; Prèzeau L; Pin JP; Closed state of both binding domains of homodimeric mGlu receptors is required for full activity, *Nat Struct Mol Biol, 11:706-713,* **2004**.
5. Gonzalez-Maeso J; Ang RL; Yuen T; Chan P; Weiistaub NV; Lopez-Gimènez JF; Zhou M; Okawa Y; Collado LF; Milligan G; Gingrich JA; Filizola M, Meana JJ; Sealton SC; Identification of a serotonin/glutamate receptor complex implicated in psychosis, *Nature, 452:93-97,* **2008**.

3D-QSAR MODELS FOR THE PPARS AGONISTS WITH DUE REGARD TO MEMBRANE TRANSPORT

Shuichi Hirono, Izumi Nakagome, Noriyuki Yamaotsu, Hiroaki Gouda

Department of Pharmaceutical Sciences, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku 108-8641 JAPAN

Peroxisome proliferator-activated receptors (PPARs) as ligand-activated nuclear receptors involve in the transcriptional regulation of lipid metabolism, energy balance, inflammation, and atherosclerosis and are key proteins in the pathogenesis of diabetes and cardiovascular disease[1]. To date, three PPAR subtypes, PPARα, PPARγ and PPARδ have been recognized. Some three-dimensional (3D) structures of the PPARs LBD (ligand-binding domain) complexed with agonists have been determined by X-ray crystallography and their coordinates are available from the protein data bank. Several QSAR studies of PPARs agonists have been published, but most of QSAR models were constructed using either CoMFA fields or 2D-descriptors for IC_{50} which is binding affinity of a PPAR agonist to its receptor measured in vitro or EC_{50} which is transactivation activity of an agonist obtained from a cell-based assay. In this study, we developed 3D-QSAR models of transactivation of PPARα, PPARγ and PPARδ based on the strategy in which the process of ligand binding to the receptor is represented using CoMFA fields and the process of diffusion or transport of the ligand through membranes is described using molecular descriptors.

First, the 3D structures of PPARs LBDs were classified using root mean square deviations of the amino acids within 3Å around ligand binding site and the representative structures were selected as a target protein for the successive computational ligand-docking. Next, the flexible docking of the PPARs agonists whose EC_{50} and IC_{50} are known were carried out for the representative structures of PPARs LBDs using Glide 4.0 (Schrödinger, L.L.C.). The docking poses of each PPARs agonist were evaluated by the scoring function, Glide Score. The top 1 pose of Glide Score was selected as the binding pose of the agonist. This condition was confirmed with PPARs agonists whose binding conformations to PPARs LBD were known. The alignments of PPARs agonists used in CoMFA (SYBYL 7.3, Tripos, Inc.) were generated by superposing the PPARs LBD-agonist complex structures. The binding conformations of PPARs agonists were also used to calculate molecular descriptors by QikProp 2.5 (Schrödinger, L.L.C.). Finally, 3D-QSAR models were constructed by combining the CoMFA fields with QikProp descriptors for transactivation of PPARα, PPARδ and PPARγ. We first constructed two 3D-QSAR models of PPARδ agonists, one of which correlates the binding affinity (IC_{50}) with CoMFA fields and the other of which correlates the transactivation activity (EC_{50}) with CoMFA fields and QikProp descriptors. 29 PPARδ agonists were docked to five PPARδ LBDs and the molecular alignment of them was generated. From CoMFA for the binding affinity (IC_{50}), the good 3D-QSAR model was obtained (r^2 : 0.994, q^2 : 0.424, 6 components). Next we constructed the 3D-QSAR model for transactivation by combination of CoMFA field and 2~4 descriptors among 21 QikProp descriptors. In result, a good 3D-QSAR model for transactivation was obtained using CoMFA field and four descriptors (hydrophilic component of the SASA(FISA), carbon and attached hydrogen component of the SASA (PISA), predicted octanol/water partition coefficient (QPlogPOW), predicted aqueous solubility (QPlogS)) (r^2 : 0.941, q^2 : 0.554, 6 components). Those descriptors are considered to represent the transport of the ligand through membranes. In the same way, we built 3D-QSAR models for PPARα and PPARγ which will be reported at the conference.

1. Ahmed W, Ziouzenkova O, Brown J, Devchand P, Francis S, Kadakia M, Kanda T, Orasanu G, Sharlach M, Zandbergen F, Plutzky J., *J. Intern. Med.*, **262(2)**, 184-98 (2007).

POSTER COMMUNICATIONS – PC.50

GLUCOCEREBROSIDASE INHIBITORS BY EXPLORATION OF CHEMICAL DIVERSITY OF *N*-SUBSTITUTED AMINOCYCLITOLS USING "CLICK CHEMISTRY" AND *IN SITU* SCREENING

Lucía Díaz (1,2), Jordi Bujons (3), Meritxell Egido-Gabás (2), Josefina Casas (2), Amadeu Llebaria (2), and Antonio Delgado (1,2)

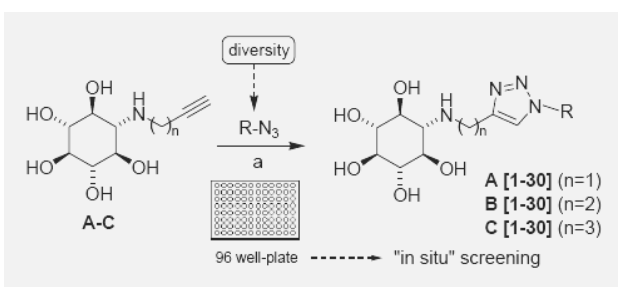
(1) University of Barcelona; Faculty of Pharmacy; Unit of Medicinal Chemistry (Associated to CSIC); Avda. Joan XXIII s/n; 08028 Barcelona, Spain (2, 3) Institute for Advanced Chemistry of Catalonia; (2) Department of Biomedicinal Chemistry and (3) Department of Biological Chemistry and Molecular Modelization; Jordi Girona 18-26; 08034 Barcelona, Spain.

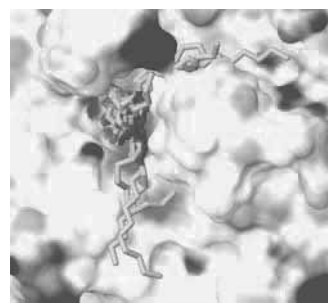
Gaucher disease is one of the most prevalent lysosomal storage disorders, characterized by the accumulation of the sphingolipid glucosylceramide (GlcCer) in the lysosomes. The disease is caused by the deficient activity elicited by several mutated forms of the enzyme glucocerebrosidase (GlcCerase), a β-glucosidase that hydrolyzes GlcCer into glucose and ceramide (Cer)[1]. Among the several therapeutic strategies for this disease[2], the use of selective inhibitors as pharmacological chaperones has become an active field of research[3]. In this work, we describe an approach to explore the chemical diversity on *N*-substituted aminocyclitols by means of the Cu(I)-catalyzed Huisgen cycloaddition reaction (click-chemistry)[4] between a set of 30 azides (R-N_3) and *N*-(ω-alkynylalkyl)aminocyclitols (A-C) with different spacer lengths between the amino group and the terminal alkyne moiety (Scheme 1). Library members were evaluated as potential chaperones based on their ability to induce the recovery of recombinant GlcCerase activity after thermal denaturation[5].

The presence of the triazole unit as well as the nature of the R-substituent turned out to be crucial for enzyme stabilization. Modelization studies have been carried out in order to disclose the structural parameters involved in the interaction of the most potent inhibitors with the enzyme active centre.

The results of this study allowed (1) the identification of a new class of GlcCerase inhibitors with activities that span from moderate (~10^{-4} M) to strong (10^{-8} M), and (2) to propose SAR models for the enzyme-inhibitor interactions that are relevant for the design of new inhibitors.

1. Butters, T. D. *Curr Opin Chem Biol* **2007**, *11*, 412-418.
2. Sawkar, A. R.; D'Haeze, W.; Kelly, J. W. *Cell Mol Life Sci* **2006**, *63*, 1179-1192.
3. Butters, T. D. *Expert Opin Pharmacother* **2007**, *8*, 427-435.
4. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.
5. Egido-Gabas, M.; Canals, D.; Casas, J.; Llebaria, A.; Delgado, A. *ChemMedChem* **2007**, *2*, 992-994.





Scheme 1. Left: Click chemistry approach to *N*-substituted aminocyclitols. (a), Cu_2SO_4, sodium ascorbate, H_2O/THF (1:1). Right: Docked poses of the synthesised inhibitors in the catalytic center of GlcCerase.

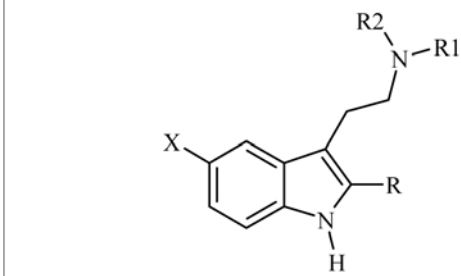
N-ALKYL SUBSTITUTED 1H-INDOLETHYLAMINE DERIVATIVES AS SELECTIVE SEROTONIN 5-HT$_6$ RECEPTOR AGONISTS

Patrizia Minetti (1), Walter Cabri (1), Mauro Marzi (1), Michela Picano (1), Emanuela Tassoni (1), Fabio Bordi (1), Franco Borsini (1), Teresa Riccioni (1), Gilberto Spadoni (2), Giorgio Tarzia (2), and Paolo Carminati (1)

(1) Sigma-Tau Industrie Farmaceutiche Riunite spa, Via Pontina Km 30,400, 00040 Pomezia, Italy
(2) Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino "Carlo Bo", Piazza Rinascimento 6, 61029 Urbino

5-HT$_6$ serotonin receptors represent one of the seven major families of serotonin receptors (5-HT$_1$–5-HT$_7$)[1]. Their unique distribution in the brain and their high affinity for therapeutic antipsychotic and antidepressant agents suggest a possible role of the 5-HT$_6$ receptor in CNS disorders[2]; nevertheless, the exact role of the receptor in these indications has still to be ascertained.

During the past decade, considerable research efforts have been directed towards the identification of selective 5-HT$_6$ receptor modulators as tools for studying the receptor and as potential therapeutic agents, allowing the identification of some interesting 5-HT$_6$ antagonists[3], whereas identification of selective 5-HT$_6$ receptor agonists has proven very challenging. Here we describe our results on novel *N*-alkyl substituted 1H-indolethylamine derivatives showing high affinity and selectivity for 5-HT$_6$ receptor. The ability of *N*-alkyl substituted 1H-indolethylamine derivatives to activate adenylate cyclase was investigated in order to define their functional profile. The compounds were shown to have an agonist behaviour towards 5-HT$_6$ receptors with a potency comparable to that of serotonin.



1. Hoyer, D; Hannon, J P; Martin, G R. Pharmacol. Biochem. Behav. 2002; 71:533.
2. Woolley, M L; Marsden, C A; Fone, K C F. Curr. Drug Top. 2004; 3: 59.
3. Holenz, J; Mercé, R; Diaz, J L. et al. J. Med. Chem. 2005; 48: 1781.

POSTER COMMUNICATIONS – PC.52

NEW IMIDOSELENOCARBAMATES AS CYTOTOXIC AGENTS IN SEVERAL CANCER CELL LINES

Elena Ibáñez (1), Daniel Plano (1), María Font (1), Juan Antonio Palop (1), Ignacio Encío (2), Alfonso Calvo (3) and Carmen Sanmartín (1)

(1) Departamento de Química Orgánica y Farmacéutica. University of Navarra. Irunlarrea, 1. 31008 Pamplona. (2) Departamento de Ciencias de la Salud. Public University of Navarra. Campus Arrosadía s/n. 31006 Pamplona. (3) Oncology Division. Center for Applied Medical Research, CIMA. University of Navarra. Pio XII, 55. 31008 Pamplona. Email: eisopena@alumni.unav.es

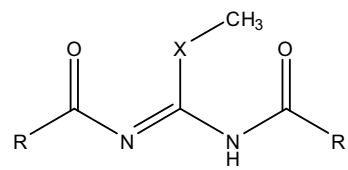
Selenium (Se) is an essential trace element for animals and humans. Its metabolic functions have been attributed to its presence in certain selenoproteins[1]. After numerous studies in animal models and more recently in humans, its chemopreventive and anticancer effects have been demonstrated[2], although mechanisms by which Se acts are not fully understood.

Considering previous investigations carried out by our group[3-8], some structural properties have been concreted for the search of new cytotoxic agents. Due to the encouraging results obtained for the previous imidoselenocarbamates synthesized[7] and after a bibliographical search of aromatic and heteroaromatic structures with cytotoxic properties, we present the synthesis and biological evaluation of 8 new imidotio- and imidoselenocarbamates with general structure:

The cytotoxic activity for all the compounds has been evaluated in five tumoural lines (leukemia, colon, breast, lung and prostate). The following parameters have been established for the first four lines: GI_{50}, TGI and LD_{50}; and the parameter IC_{50} for the last one.

The results show promising cytotoxic activities for compounds with the selenomethyl group, among which the 3-quinolinyl and 2-phenyl-4-quinolinyl derivatives are the most interesting. It can also be noticed that the most sensitive lines for this kind of compounds are leukemia (CCRF-CEM) and breast (MCF-7).

1. Lu, J.; Holmgren, A. *J. Biol. Chem.* **2009**, *284*, 723-727.
2. Zeng, H.; Gerald, F.; Combs Jr. *J. Nutr. Biochem.* **2008**, *19*, 1-7.
3. Sanmartín, C.; Echeverría, M.; Mendivil, B.; Cordeu, L.; Cubedo, E.; García-Foncillas, J.; Font, M; Palop, J.A. *Bioorg. Med. Chem*. **2005**, *13*, 2031-2044.
4. Sanmartín, C.; Ardaiz, E.; Cordeu, L.; Cubedo, E.; García-Foncilla, J.; Font, M.; Palop, J.A. *Lett. Drug Des. Discov*. **2005**, *2*, 341-354.
5. Font, M.; Ardaiz, E.; Cordeu, L.; Cubedo, E.; García-Foncilla, J.; Sanmartín, C.; Palop, J.A. *Bioorg. Med. Chem.* **2006**, *14*, 1942-1948.
6. Sanmartín, C.; Font, M.; Palop, J.A. *Mini-Rev. Med. Chem*. **2006**, *6*, 639-650
7. Plano, D.; Sanmartín, C.; Palop, J.A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6853-6859.
8. Sanmartín, C.; Plano, D.; Palop, J.A. *Mini-Rev. Med. Chem*. **2008**, *8*, 1020-1031.



X= S, Se

R= 3-quinolinyl, 2-phenyl-4-quinolinyl, 2-thienyl, 2-furyl

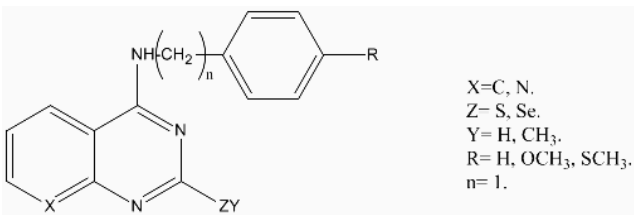
NOVEL PYRIDO[2,3-*d*]PYRIMIDINE AND QUINAZOLINE DERIVATIVES AS CYTOTOXIC AGENTS IN SEVERAL CANCER CELL LINES

Esther Moreno (1), Daniel Plano (1), María Font (1), Juan Antonio Palop (1), Alfonso Calvo (2), Ignacio Encío (3), Carmen Sanmartín (1)

(1) Departamento de Química Orgánica y Farmacéutica, University of Navarra, Irunlarrea, 1, 31008 Pamplona. (2) Departamento de Oncología, Centro de Investigación Médica Aplicada (CIMA), University of Navarra, Pío XII, 53, 31008 Pamplona. (3) Departamento de Ciencias de la Salud. Public University of Navarra, Campus de Arrosadía s/n, 31006 Pamplona. Email: emoreno.1@alumni.unav.es

Cancer is the second cause of death worldwide[1]. Currently, more than 7 million people die each year from cancer[2]. Epidemiological studies have suggested that low blood selenium levels were associated with an increased incidence and mortality from various kinds of cancer[3]. Various potential mechanisms have been proposed for organoselenium compounds in cancer chemoprevention: antiandrogen activity, antioxidant functions, DNA repair[4]...

In order to continue with the investigations of our group[5-7], the synthesis and biological evaluation of new pyrido[2,3-*d*]pyrimidine and quinazoline derivatives have been proposed, according to the general structure:



Cytotoxic activity of all the compounds has been evaluated in five tumoral cell lines: prostate, leukemia, colon, breast and lung; and in a no tumoral line of breast. For all of the cell lines, the following cytotoxic parameters have been determined: IC_{50}, LD_{50}, TGI and GI_{50}.

Several compounds show a promising cytotoxic activity, being up to four times more potent than some of the drugs currently used in clinical trials.

1. Muss, H. B. Cancer in the Elderly: a Societal Perspective from the United States. *Clin. Oncol.*; 21: 92-98, **2009.**

2. www.who.int/es

3. Chen, Y.; Hall, M.; Graziano, J. H.; Slavkovich, V.; Van Geer, A.; Parvez, F.; Ahsan, H.; Selenium and its role in cancer prevention. *Cancer Epidemiol. Biomarkers Prev.*; 16: 207, **2007**.

4. Sanmartín, C.; Plano, D.; Palop, J. A.; Selenium compounds and apoptotic modulation: a new perspective in cancer therapy. Mini Reviews in Medicinal Chemistry; 8: 120-131, **2008**.

5. Sanmartín, C.; Echeverría, M.; Mendivíl, B.; Cordeu, L.; Cubedo, E.; García-Foncillas, J.; Font, M.; Palop, J. A.; Synthesis and bilogical evaluation of new symmetrical derivatives as cytotoxic agents and aopotosis inducers. *Bioorg. Med. Chem.*; 13: 2031-2044, **2005**.

6. Cubedo, E.; Cordeu, L.; Bandrés, E.; Rebollo, A.; Malumbres, R.; Sanmartín, C.; Font, M.; Palop, J. A.; García-Foncillas, J.; New symmetrical quinazoline derivatives selectively induce apoptosis in human cancer cells. *Cancer Biol. Ther.*; 5:7, 850-859, **2006**.

7. Cordeu, L.; Cubedo, E.; Bandrés, E.; Rebollo, A.; Sáenz, X.; Chozas, H.; Domínguez, V.; Echeverría, M.; Mendivil, B.; Sanmartín, C.; Palop, J.A.; Font, M.; García-Foncillas, J.; Biological profile of new apoptotic agents based on 2,4- pyrido[2,3-*d*]pyrimidine derivatives. *Bioorg. Med. Chem.*; 15: 1659-1669, **2007**.

POSTER COMMUNICATIONS – PC.54

LYSINE TRIMETHYLATION, A TOOL FOR IMPROVING THE THERAPEUTIC INDEX OF ANTIMICROBIAL PEPTIDES

Beatriz G. de la Torre (1), María Fernández-Reyes (2), Dolores Díaz (2), Ania Cabrales (1), Mariona Vallès (1), Jesús Jiménez-Barbero (2), Luis Rivas (2) and David Andreu (1)

(1) Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain;
(2) Center for Biological Research-CSIC, Madrid, Spain.

Despite their interest as alternatives to conventional antibiotics in the face of an alarming worldwide antibiotic resistance crisis, antimicrobial peptides (AMPs) are still to gain a foothold in therapeutics, largely due to the limitations intrinsic to their peptide nature. A number of structural modifications tending to enhance AMP biological lifetimes have been proposed with various results. Lys trimethylation, despite its predicament in epigenetic studies, has surprisingly received little attention from peptide medicinal chemists. Among other effects, Lys trimethylation (i) preserves global charge but (ii) abrogates the hydrogen bond-forming ability of the amino group, and (iii) increases side chain bulkiness as well as hydrophobicity. We have examined the effect of Lys trimethylation using a well-known AMP platform, the cecropin A-melittin hybrid CA(1-7)M(2-9), KWKLFKKIGAVLKVL-amide. Permethylation of all five Lys residues caused a drastic loss of antimicrobial potency but, interestingly, an even more pronounced reduction in cytotoxiciy (measured as hemolysis). Singly and doubly Lys-trimethylated analogues were next evaluated, with encouraging results: most analogues were non-cytotoxic up to 60 μM but retained antimicrobial activities close to 1 μM that amounted to considerable enhancements in therapeutic index. The solution structures of the analogues by 2D NMR revealed significant differences with the parent non-trimethylated CA(1-7)M(2-9), and thus provide some basis for explaining the improved therapeutic profile of the Lys-trimethylated analogues.

AMINOPYRIDINE *N*-OXIDES AS POTENT, ORALLY BIOAVAILABLE INHIBITORS OF p38 MAP KINASE: DESIGN, SYNTHESIS, SAR AND *IN VIVO* ANTI-INFLAMMATORY ACTIVITY

Wenceslao Lumeras, Francisco Caturla, Laura Vidal, Cristina Esteve, Cristina Balagué, Adelina Orellana, María Domínguez, Ramón Roca, Josep M. Huerta, Núria Godessart and Bernat Vidal

Almirall Research Center, Laboratorios Almirall S.A., Ctra. Laureà Miró 408, E-08980 Sant Feliu de Llobregat, Barcelona, Spain

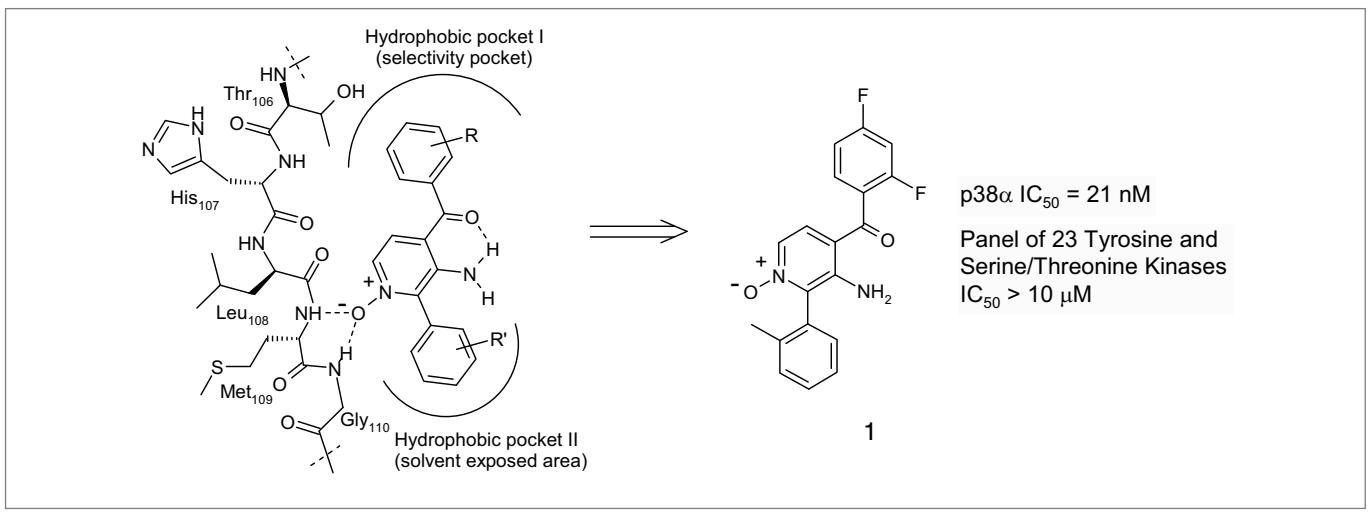
The p38α mitogen-activated protein (MAP) kinase is an intracellular Ser/Thr kinase that is activated by a range of environmental stimuli such as TNFα, IL-1β and stress[1]. In its activated state, p38α phosphorylates a range of intracellular protein substrates that post-transcriptionally regulate the biosynthesis of TNFα and IL-1β. The pathophysiological consequence of excessive production of TNFα and IL-1β is thought to be significant mediation of the progression of many inflammatory diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease[2]. The proven ability of p38α MAP kinase to efficiently regulate those pro-inflamatory cytokines has attracted numerous pharmaceutical companies and independent researchers into pursuing p38α inhibitors primarily as novel anti-inflammatory drugs[3].

In this poster, a novel series of aminopyridine *N*-oxides were designed, synthesized and tested for their ability to inhibit p38α MAP kinase. Some of these compounds showed a significant reduction in the LPS-induced TNFα production in human whole blood. SAR studies revealed that *N*-oxide oxygen was essential for activity and was probably a determinant factor for a marked selectivity against other related kinases. Compound **1** was identified as a potent and selective p38α inhibitor with an appropriate balance between potency and pharmacokinetics. *In vivo* efficacy of **1** was demonstrated in reducing TNFα levels in an acute murine model of inflammation (ED_{50} =1 mg/kg in LPS-induced TNFα production when dosed orally 1.5 h prior to LPS administration). The oral efficacy of **1** was further demonstrated in a chronic model of adjuvant arthritis in rats when administered orally (ED_{50} = 4.5 mg/kg).

1. Raingeaud, J.; Gupta, S.; Rogers, J. S.; Dickens, M.; Han, J.; Ulevitch, R. J.; Davis, R. J. Characterization of the structure and function of a novel MAP kinase kinase (MKK6). J. Biol. Chem. 1996, 271, 2886-2891.

2. Foster, M. L.; Halley, F.; Souness, J. E. Potential of p38 inhibitors in the treatment of rheumatoid arthritis. Drug News Perspect. 2000, 13, 488-497.

3. Kumar, S.; Boehm, J.; Lee, J.C. p38 MAP kinases: key signaling molecules as therapeutic targets for inflammatory diseases. Nature Rev. Drug Disc. 2003, *2*, 717-726.



POSTER COMMUNICATIONS – PC.56

GRAMICIDIN S ANALOGUES WITH PHENYLALANINE DERIVATIVES AT THE *i*+2 POSITION OF THE β-TURN

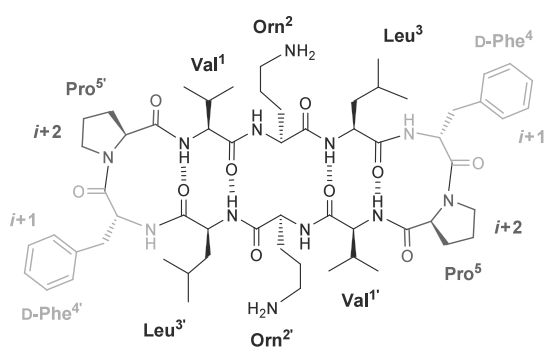
Concepción Solanas (1), Beatriz G. de la Torre (2), María Fernández-Reyes (3), Clara M. Santiveri (4), M. Ángeles Jiménez (4), Luis Rivas (3), Ana I. Jiménez (1), David Andreu (2), and Carlos Cativiela (1)

(1) Departamento de Química Orgánica, ICMA, Universidad de Zaragoza–CSIC, Zaragoza, Spain. (2) Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain. (3) Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. (4) Instituto de Química-Física Rocasolano, CSIC, Madrid, Spain

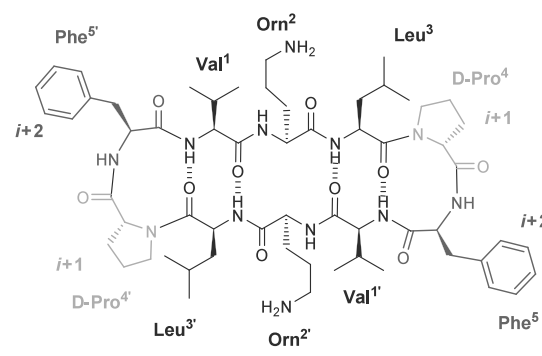
Increasing resistance of bacteria to conventional antibiotics requires the development of new drugs to combat microbial infection. Naturally occurring antimicrobial peptides represent a viable alternative, since these molecules target the cell membrane and therefore development of resistance is unlikely. The cationic antimicrobial peptide gramicidin S, *cyclo*(Val-Orn-Leu-D-Phe-Pro)[2], is active against a wide range of bacteria and fungi, but also exhibits a strong hemolytic effect. This peptide adopts a pleated β-sheet structure where the Val, Orn and Leu residues align to form the antiparallel β-strands, while D-Phe and Pro occupy positions *i*+1 and *i*+2, respectively, of the βII′-turns.

In a recent study[1], we have shown that replacement of D-Phe in gramicidin S by different non-coded aromatic residues can induce important changes in the biological profile. We present now a new series of analogues generated by changing the D-Phe-Pro sequence at the β-turns by D-Pro-Phe, and further replacement of Phe by non-coded residues bearing aromatic side chains with diverse size, orientation and flexibility. NMR analysis has shown that this double modification preserves the β-sheet conformation, while the microbicidal and hemolytic potencies are much dependent on the particular structure of the aromatic amino acid incorporated. Some peptides with significantly improved therapeutic index have been found.

1. Solanas, C.; de la Torre, B. G.; Fernández-Reyes, M.; Santiveri, C. M.; Jiménez, M. A.; Rivas, L.; Jiménez, A. I.; Andreu, D.; Cativiela, C. J. Med. Chem. **2009**, 52, 664–674.



cyclo(Val-Orn-Leu-**D-Phe-Pro**)$_2$ (GS)



cyclo(Val-Orn-Leu-**D-Pro-Phe**)$_2$

SYNTHESIS, BIOLOGICAL EVALUATION AND QSAR OF NEW QUINOXALINE DERIVATIVES AS ANTIOXIDANT AND ANTI- INFLAMMATORY AGENTS

Asunción Burguete (1, 2), Eleni Pontiki (2), Dimitra Hadjipavlou-Litina (2), Elsa Moreno (1), Enrique Torres (1), Carlos Barea (1), Roberto Díez (1), Javier Gurbindo (1), Silvia Pérez-Silanes (1), Ignacio Aldana (1) and Antonio Monge (1)

(1) Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain, (2) Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece. Email: mburguete@alumni.unav.es

Quinoxaline 1,4-di-*N*-oxide derivatives are a class of compounds having a great interest in medicinal chemistry as they display a broad range of biological properties such as antibacterial, anticancer or antiparasitic[1].

In a recent study[2] we have demonstrated that quinoxaline 1,4-di-*N*-oxide derivatives show also very interesting antioxidant and anti-inflammatory properties, some of them displaying an *in vivo* anti-inflammatory effect higher than the reference drug, indomethacin IMA, and promising *in vitro* inhibition values of LOX (< 1 μM).

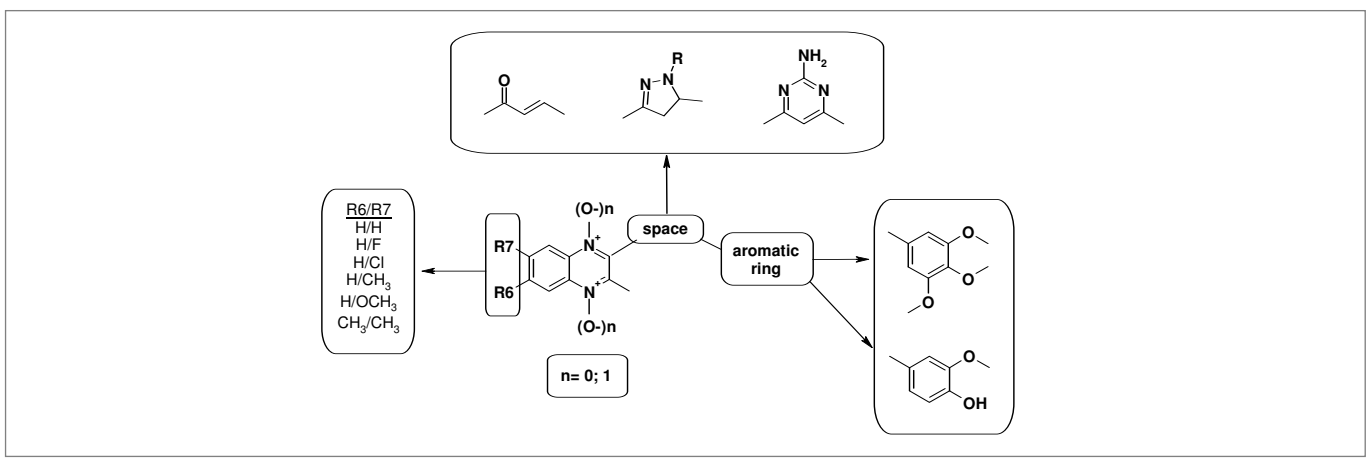
Based on these results and with the aim of obtaining new compounds with improved activities we now describe the synthesis, anti-inflammatory, antioxidant activities and QSAR studies of novel quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives. Microwave assisted methods have been used to synthesize some of the compounds in order to optimize reaction times and to improve the yields.

The tested compounds presented important scavenging activities and promising *in vitro* inhibition of soybean lipoxygenase. Two of the best lipoxygenase inhibitors were evaluated as *in vivo* anti-inflammatory agents using the carrageenin-induced edema model. One of them showed important *in vivo* anti-inflammatory effect (41%) similar to that of indomethacin (47%) used as the reference drug. A QSAR analysis is presented.

Asunción Burguete was awarded a PhD scholarship supported by the "Gobierno de Navarra". Eleni Pontiki is grateful to the "Foundation for Education and European Culture" for financial support during the PostDoc.

1. Burguete, A.; Estévez, Y.; Castillo, D.; González, G.; Villar, R.; Solano, B.; Vicente, E.; Pérez, S.; Aldana, I.; Monge, A.; Sauvain, M.; Deharo, E. *Mem. Inst. Oswaldo Cruz.* **2008**, *103*, 778-780.

2. Burguete, A.; Pontiki, E.; Hadjipavlou-Litina, D.; Villar, R.; Vicente, E.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6439-6443.



POSTER COMMUNICATIONS – PC.58

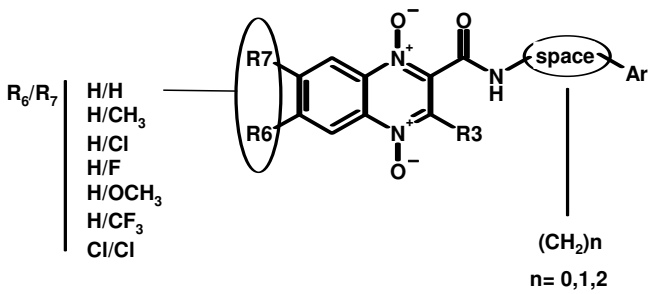
NEW QUINOXALINE-2-CARBOXAMIDE 1,4-DI-*N*-OXIDE DERIVATIVES AS ANTI-MYCOBACTERIUM TUBERCULOSIS AGENTS

Elsa Moreno (1), Enrique Torres (1), Asunción Burguete (1), Saioa Ancizu (1), Carlos Barea (1), Roberto Díez (1), Silvia Pérez-Silanes (1), Ignacio Aldana (1) and Antonio Monge (1)

(1) Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain. Email: emoreno4@alumni.unav.es

Tuberculosis (TB) is a respiratory transmitted disease affecting nearly 32% of the world's population, more than any other infectious disease. It is estimated that 1.7 million deaths resulted from TB in 2006, and there are an estimated 8 million new cases each year. Moreover, up to 50 million people are infected with drug-resistant forms of TB. The magnitude and extent of drug-resistant strains have increased concern that TB may once again become an incurable disease. In this sense, the discovery of new active and promising compounds with antimycobacterial activity remains essential for control and prevention of TB.

As a continuation of our research in quinoxaline 1,4-di-*N*-oxide and with the aim of identifying new antitubercular drug candidates, new series of compounds had been designed. Taking into account the data showed by specific analogues, we propose new quinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives according to the following structure:



In vitro assays are conducted 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).

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. Elsa Moreno was awarded a PhD scholarship supported by the "Gobierno de La Rioja".

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.

MICROWAVE ASSISTED SYNTHESIS OF NEW ISONICOTINIC HYDRAZIDE QUINOXALINE 1,4-DIOXIDE DERIVATIVES

Enrique Torres, Beatriz Solano, Elsa Moreno, Asunción Burguete, Saioa Ancizu, Carlos Barea, Roberto Díez, Silvia Pérez-Silanes, Ignacio Aldana and Antonio Monge

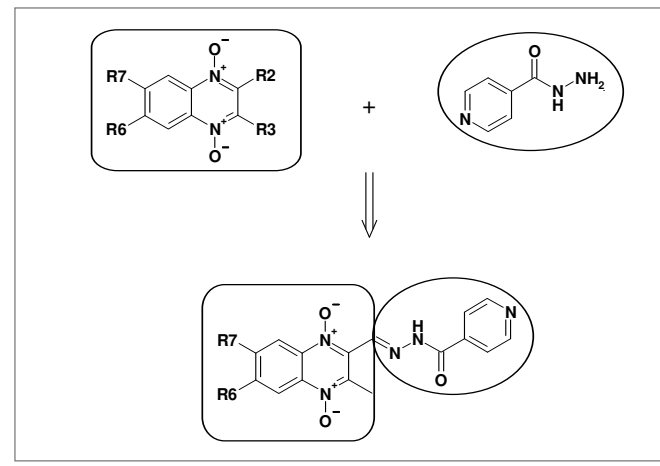
Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain. Email: etpastor@alumni.unav.es

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.

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. The design of those compounds considered the incorporation of isoniazid in a quinoxaline-1,4-di-*N*-oxide derivative. Isoniazid is one of the most effective first-line anti-TB drugs.

Nine new quinoxalines 1,4-di-*N*-oxide derivatives containing isoniazid pharmacophore were initially prepared by the traditional method and a microwave assisted method has been 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.

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).



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.

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.60

HELIOS, A LIGAND-BASED VIRTUAL SCREENING SOLUTION

Óscar Villacañas, Joan Díaz, Xavier Maresma, Raimon Grau, Óscar Huertas and Ignasi Belda

Intelligent Pharma, S.L.

Intelligent Pharma has developed a new technology called HELIOS, which is able to identify novel compounds that mimic the behavior of a given reference compound, but with completely different structural properties (non-structural analogues). This type of scaffold hopping has several applications in modern drug discovery. For instance, if the reference compound is an already known hit (for example, a hit coming from natural product screening or even from a third-party patent), HELIOS can find structurally unrelated compounds that reproduce some three-dimensional physico-chemical properties. Therefore, these new compounds will feature the same biological activities but with a different molecular structure. In addition, scaffold hopping can provide novel hits that may have different ADME/tox profiles or better synthesability, compared to the reference ligand.

In a typical HELIOS run, the user selects the reference compound and the data base where HELIOS is going to look for. Therefore, depending on the configuration of those two selections, HELIOS can be used to:

1. To design "mee-too's" or to identify new active compounds to substitute "old" drugs.
2. To extend patent protection or to circumvent patentability issues.
3. To "rescue" failed molecules or to identify "back-ups" in early drug discovery stages.
4. To identify more synthesisable or scalable compounds.
5. To perform drug reprofiling.
6. To perform ADME/Tox prediction.
7. To identify mechanisms of action.

MS-BINDING ASSAYS – WITH MALDI TOWARDS HIGH THROUGHPUT

Georg Höfner (1), Klaus T. Wanner (1) and Dietrich Merkel (2)

(1) Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, D-81377 München, (2) Applied Biosystems, Frankfurter Str. 129b, D- 64293 Darmstadt

MS-binding assays have the potential to be a highly versatile technique in the drug discovery process. They offer all advantages of conventional and widely accepted radioligand binding assays without drawbacks such as the need for labelling or the handling of hazardous radioactive material[1]. Recently, we were able to demonstrate the feasibility of this concept for GAT1 - the most abundant GABA transporter subtype in the CNS representing a validated drug target for the therapy of epilepsy. The developed MS-binding assay uses NO711 as nonlabelled marker that can be quantified by LC-ESI-MS/MS[2]. Although the LC-MS procedure achieves a considerable capacity, its throughput is decisively limited by the chromatographic separation step.

Only recently a new MALDI-MS/MS system combining sensitivity, precision, and dynamic range with the high throughput capacity of MALDI sources was introduced. As this MALDI-MS/MS system - branded as FlashQuant™ - could be assumed to provide an ideal tool to analyse MS binding assays, we attempted to demonstrate its applicability for this purpose taking NO 711 binding to mGAT1 as anexample.

First a method to quantify the marker NO 711 employing [$^2H_{10}$]NO 711 as internal standard was established. A linear calibration function in the range of 208 pM to 16.7 nM NO 711 was obtained that enabled reliable MALDI-MS/MS quantification of NO 711 in MS binding assays. Based on this NO 711 binding to mGAT1 was characterized in saturation experiments and additionally in competition experiments for a series of 13 known test compounds. The resulting affinity constants of both saturation and competition experiments were in excellent agreement with those obtained in MS binding assays based on LC-ESI-MS/MS quantification. As the MALDI-MS system takes only a few seconds for quantification per sample and the whole assay procedure is executed in the 96 well format, the presented technique offers the opportunity for high throughput screening[3].

1. G. Höfner, C. Zepperitz, K,T. Wanner in *Mass Spectrometry in Medicinal Chemistry* (Eds.: K.T. Wanner, G. Höfner), Wiley VCH, Weinheim **2007**, 247-283.
2. Zepperitz C, Höfner G, Wanner KT, *ChemMedChem*, **2006**, 1, 208 – 217.
3. Höfner G, Merkel D, Wanner KT, *ChemMedChem*, **2009**, in press.

POSTER COMMUNICATIONS – PC.62

NEW LOW CALCEMIC 2-METHYLENE-22-ENE-19,26-DINOR-1α,25-DIHYDROXY VITAMIN D_3 ANALOGS: SYNTHESIS AND BIOLOGICAL PROPERTIES

Grazia Chiellini (1,2), Pawel K. Grzywacz (1), Lori A. Plum (1), Margaret Clagett-Dame (1), Hector F. DeLuca (1)

(1) Department of Biochemistry, University of Madison-Wisconsin, Madison, WI, USA, (2) Dipartimento di Scienze Scienze dell'Uomo e dell'Ambiente, Università di Pisa, Pisa, Italia

The natural hormone, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] regulates various biological events including bone and calcium metabolism and cell differentiation[1]. The response to the hormone is triggered by its nuclear receptor, the vitamin D receptor (VDR), a member of the nuclear hormone receptor superfamily. 1α,25$(OH)_2D_3$ has significant therapeutic potential in the treatment of osteoporosis rickets, secondary hyperparathyroidism, psoriasis, renal osteodystrophy and certain malignancies[1]. However, the therapeutic use of 1α,25$(OH)_2D_3$ is limited because it induces significant hypercalcemia. A number of vitamin D analogs have therefore been synthesized, and some of them have been shown to have desirably low calcemic activity[2]. In a continuing effort to explore the 19-nor class of pharmacologically important vitamin D compounds, we report here the synthesis and the biological profile of four new 2-methylene- 22-ene- 19,26- dinor-1α,25$(OH)_2D_3$ compounds: RR-22, SOR-1, REN and SS-22. Our synthetic strategy to obtain these new side chain modified 2-methylene-19-nor-1α,25$(OH)_2D_3$ compounds is based on a convergent approach, using the Wittig-Horner reaction of the A-ring phosphine oxide with the 25-hydroxy-Grundmann's ketones. A similar strategy was previously used in our laboratory for the preparation of other 2-substituted 19-norvitamins[3]. While the in vitro activity of these new compounds is comparable to the natural hormone, in vivo they all have limited calcemic activity when measured either by intestinal calcium transport or bone calcium mobilization. Accordingly, some of these novel analogs may be useful as new and safer therapeutic agents.

1. Vitamin D; Feldman D., Glorrieux F.H., Pike J.W., Eds.; Academic: New York, 1997.

2. Yamada S. et al., Med. Res. Rev. 23(1): 89-115 (2003).

3. Sicinski R. et al., J. Med. Chem. 37(22): 3730-8 (1994).

ANALYSIS OF CARBOHYDRATE-BINDING PROTEINS USING SPR AND MASS SPECTROMETRY

Carmen Jiménez-Castells (1), Adrián Moise (2), Beatriz G. de la Torre (1), Michael Przybylski (2), Ricardo Gutiérrez-Gallego (1,3) and David Andreu (1)

(1) Department of Experimental and Health Sciences, Pompeu Fabra University
(2) Department of Chemistry, University of Konstanz, 78457 Konstanz, Germany
(3) Bio-analysis group, Neuropsychopharmacology program, IMIM-Hospital del Mar
(1,3) Barcelona Biomedical Research Park, Dr. Aiguader 88, 08003 Barcelona, Spain

Interest in lectins (carbohydrate-binding proteins of non-immunological origin) has steadily grown over the last decades because of their decisive role in numerous pathological processes. Examples of lectin-sugar interactions include the recognition occurring during bacterium-host adhesion, viral entry, or metastasis. Concomitantly, the development of new, powerful, nanosized analytical tools to study these interactions has accelerated.

Here, two complementary analytical techniques are described that provide both quantitative and qualitative data on carbohydrate-protein interactions with high sensitivity, low sample consumption, and without sample labelling. With the first technique, based on surface plasmon resonance (SPR), both kinetic and thermodynamic parameters are determined in real time. In this approach, the sugar immobilisation through a peptide module[1,2] allows to capture the carbohydrate-binding protein, to characterise the interaction and, subsequently, to identify the interacted lectin by mass spectrometry.

The second technique employs proteolytic excision of protein-carbohydrate complexes and mass spectrometric analysis (CREDEX-MS)[3] and enables the identification of the peptide motifs at the carbohydrate binding site. In this approach, the sugar is immobilised to a functionalised Sepharose support and the lectin passed through. After on-column digestion of the complex, sugar-bound peptides are eluted and identified[3]. The identification of the amino acid residues directly involved in the specific interaction may help delineate the minimal structural determinants for the development of pharmaceuticals capable of modulating or inhibiting carbohydrate-targeting pathogens.

Here, the combination of these two methodologies for sugar-protein interaction studies and their applicability will be described with legume lectins as test models.

1. Vila-Perelló, M., Gutiérrez-Gallego, R., Andreu, D. Chembiochem 6, 1831-1838 (2005).
2. Jiménez-Castells, C., de la Torre, B.G., Gutiérrez-Gallego, R., Andreu, D. Bioorg. Med. Chem. Lett. 17, 5155-5158 (2007).
3. Przybylski M., Moise A., Svobodova E., Siebert H., Gabius H. J Pept. Sci. Supplement to Vol. 14, 40 (2008).

POSTER COMMUNICATIONS – PC.64

MICROWAVE ASSISTED SYNTHESIS OF NEW 3-(1,4-DI-*N*-OXIDE-QUINOXALIN-2-YL)-1-PHENYL-PROPENONE DERIVATIVES AS POTENTIAL ANTICANCEROUS AGENTS

Saioa Ancizu, Enrique Torres, Raquel Villar, Elsa Moreno, Asunción Burguete, Nerea Castrillo, Silvia Galiano, Silvia Pérez-Silanes, Ignacio Aldana, Antonio Monge.

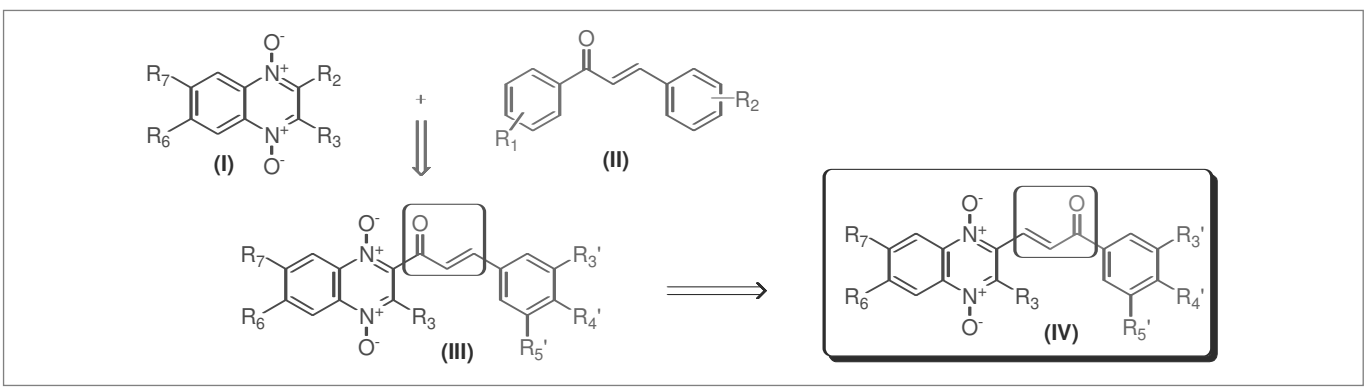
Unidad de I+D de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, C/ Irunlarrea s/n, 31008 Pamplona, Spain.

The interest of quinoxalines in medicinal chemistry is still in progress due to the important pharmacological properties they present. In fact, recent studies have put in evidence that quinoxalines and their fused ring derivatives are endowed with antimycobacterial, antiprotozoal, anticandida and anticancer activities[1]. Furthermore, the quinoxaline 1,4-di-*N*-oxide derivatives **(I)** have shown selective cytotoxicity against hypoxic cells present in solid tumors and some action mechanism have been proposed for them[2]. In the same way, chalcones **(II)** are a kind of chemical structures displaying a wide range of biological activities[3] with an outstanding activity as anticancerous agents.

As a result of our anticancerous research project, a new series of quinoxaline 1,4-di-*N*-oxide derivatives containing α,β-unsaturated ketone system were synthesized by our group **(III)** and tested for their anticancerous activity[4]. With the aim of improving the previous results, new quinoxaline 1,4-di-*N*-oxide derivatives containing inverted α,β-unsaturated ketone system **(IV)** were designed, synthesized and tested as potential anticancerous agents.

This work has been carried out thanks to the financial support of the FIS project (PE 1080817, October 2008). S. Ancizu indebted to the Navarra Government for a grant.

1. Solano, B. et al. *Journal of Medicinal Chemistry* **2007**, *50*, 5485-5492.
2. Ganley, B. et al. *Bioorganic & Medicinal Chemistry* **2001**, *9*, 2395–2401.
3. Lin, Y. M. et al. *Bioorganic & Medicinal Chemistry* **2002,** *10,* 2795-2802.
4. Burguete, A. et al. *Bioorganic & Medicinal Chemistry Letters* **2007,** *17*, 6439-6443.



SYNTHESIS AND PHARMACOLOGICAL SCREENING OF SELENYLACETIC ACID DERIVATIVES AS CYTOTOXIC AND ANTIPROLIFERATIVE AGENTS

Enrique Domínguez (1), Esther Moreno (1), Iosu Jiménez (1), Ylenia Baquedano (1), Daniel Plano (1), María Font (1), Alfonso Calvo (2), Celia Prior (2), Ignacio Encío (3), Juan Antonio Palop (1), Carmen Sanmartín (1)

(1) Departamento de Química Orgánica y Farmacéutica. University of Navarra. Irunlarrea. 1. 31008-Pamplona, Spain
(2) División de Oncología del Centro de Investigación Médica Aplicada (CIMA). University of Navarra. Pío XII, 53. 31008-Pamplona, Spain
(3) Departamento de Ciencias de la Salud. Public University of Navarra. Campus Arrosadía s/n. 31006-Pamplona, Spain

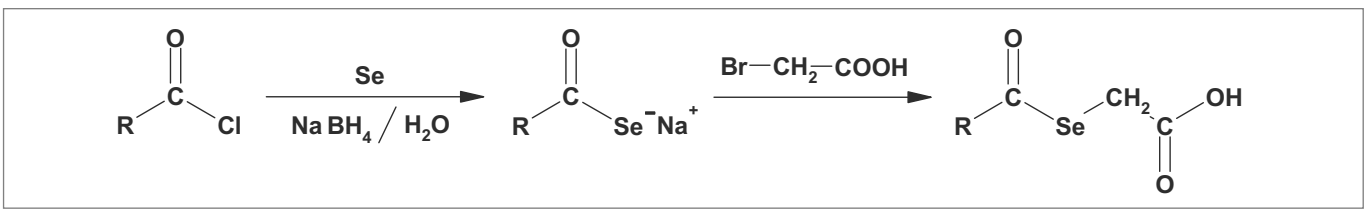
Recent studies have demonstrated the chemopreventive and antiproliferative properties of certain selenocompounds[1-5]. According to these findings, our research group planned the synthesis and biological evaluation of twentysix aroyl and heteroaroyl selenylacetic acid derivatives with the general formula Hetar-CO-Se-CH_2-COOH or Ar-CO-Se-CH_2-COOH.

The synthesis was carried out following the procedure shown below6. In a first step the appropriate aroyl or heteroaroyl chloride reacts with sodium hydrogen selenide prepared *in situ* leading to the formation of the sodium aroylselenyde, which reacts in a second step with α-bromoacetic acid to produce the corresponding selenylacetic acid derivative.

All the compounds were tested against prostate cancer cell line (PC-3) and some of the most active against a panel of four cancer human cell lines (CCRF-CEM, HTB-54, HT-29, MCF-7) and one mammary gland-derived non-malignant cell line (184B5).

Some of them exhibited remarkably cytotoxic and antiproliferative activities against MCF-7 and PC-3 higher than the reference compounds doxorubicin and etoposide respectively. So, in MCF-7 when Ar = phenyl, 3,5-dimethoxyphenyl or benzyl the TGI values were 3.69, 4.18 and 6.19 μM. On the other hand, in PC-3 these compounds have shown values such as 6.8, 4 and 2.9 μM. Furthermore, benzoylselenylacetic acid didn't provoke apoptosis and didn't perturb cell cycle in MCF-7.

1. Jackson, M.I.; Combs Jr, G.F. *Curr. Opin. Clin. Nutr. Metab. Care*. 2008, 11(6): 718-726.
2. Naithani R. Mini. Rev. *Med. Chem*. 2008 8(7): 657-668
3. Lee, J.T.; Lee, T.J.; Park, J.W.; Kwon, T.K. *Int. J. Oncol*. 2009, 34(5): 1455-1460.
4. Huang, F.; Nie, C.; Yang, Y.; Yue, W.; Ren, Y.; Shang, Y.; Wang, X.; Jin, H.; Xu, C; Chen, Q. Free. Radic. Biol. Med.
2009, 46(8): 1186-1196.
5. Sanmartin, C.; Plano, D.; Palop, *J.A. Mini. Rev. Med. Chem*. 2008 8(10): 1020-1031
6. Filgueiras de Athayde-Filho, P.; Gouveia de Souza, A.; Alves de Morais, S.; Botelho, J.R.; Barbosa-Filho, J.M.; Miller, J.; Lira, B.F. *Arkivoc*, 2004; 2004(VI): 22-26.



POSTER COMMUNICATIONS – PC.66

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DERIVATIVES AS CYTOTOXIC AND PROAPOPTOTIC COMPOUNDS

Ylenia Baquedano (1), Esther Moreno (1), Daniel Plano (1), Elena Ibáñez (1), Iosu Jiménez (1), Enrique Domínguez (1), Juan Antonio Palop (1), María Font (1), Ignacio Encío (2), Carmen Sanmartín (1)

(1) Departamento de Química Orgánica y Farmacéutica. University of Navarra. Irunlarrea, 1. 31008- Pamplona
(2) Departamento de Ciencias de la Salud. Public University of Navarra. Campus Arrosadía s/n. 31006- Pamplona

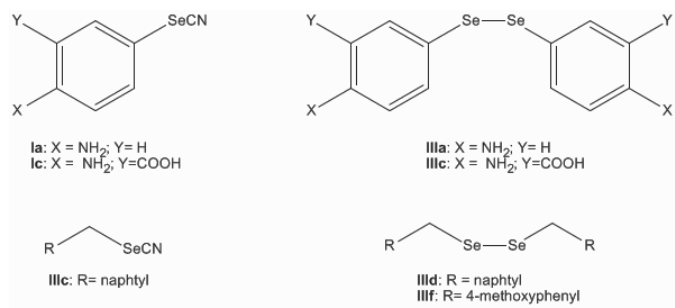
It is well known that the trace elemental selenium (Se) appears to have cancer preventive properties based on a converging body of evidence from epidemiologic, clinical and experimental studies[1,2]. Organoselenocyanates are an important class of chemopreventive agents which possess antioxidative, antimutagenic and anticarcinogenic properties. 1,4-phenylenebis(methylene)selenocyanate inhibits tumorgenesis of lung tumors in A/J mice[3] and carcinogenesis in rat mammary tumor cells[4]. Diphenylmethyl selenocyanate is an effective cancer chemopreventive agent against lung carcinogenesis when applied at the post-initiation phase[5]. In addition, it inhibits mouse skin carcinogenesis by inducing apoptosis[6].

As a result of these studies and previous work of our research group in organoselenium compounds[7], we carried out the synthesis and biological evaluation of seven organoselenium compounds as cytotoxic and proapoptotic compounds.

We evaluated cytotoxic activity of these compounds in four cancer cell lines, leukaemia (CCRF- CEM), lung (HTB-54), colon (HT-29) and breast (MCF-7). Results are expressed as GI_{50} (concentration that reduces by 50% the growth of treated cells with respect to untreated controls), TGI (dose that inhibits 100% of cell growth) and LD_{50} (dose that kills 50% of cells). Furthermore, we determined the cell cycle distribution of compound **IIIa.**

Compounds **IIIa** and **IIIc** showed a remarkable cytostatic effect (GI_{50} and TGI < 10μM) in HTB-54 and MCF-7 cell lines respectively. Moreover compound **IIIa** induces apoptosis in CCRF-CEM and MCF-7 cell lines, and a significant decrease of G_0/G_1 phase cell population and increase of cell population was observed in $SubG_1$, S and G_2/M phases.

1. Gurusamy, K. *Biol. Trace Elem. Res.*, 2007, *118*, 191-206.
2. Cheung, E.; Wadhera, P.; Dorff, T.; Pinski, J. *Expert Rev. Anticancer Ther.* 2008, *8*, 43-50.
3. El-Bayoumy, K.; Das, A.; Boyiri, T.; Desai, D. H.; Sinha, R.; Pittman, B.; Amin, S.G. *Chemico-Biological Interactions*, 2003, *146*, 179-190.
4. Richie, J.P.; Kleinman, W.; Desai, D.H.; Das, A.; Amin, S.G.; Pinto, J.T.; El-Bayoumy, K. *Chemico-Biological Interactions*, 2006, *161*, 93-103.
5. Das, R.K.; Banerjee, S.; Bhattacharya, S. *Exp. Toxicol. Pathol.* 2007, *58*, 351-360.
6. Das, R.K.; Hossain, S.K.U.; Bhattacharya, S. *Cancer Lett.* 2005, *230*, 90-101.
7. Plano, D.; Sanmartín, C.; Moreno, E.; Prior, C.; Calvo, A.; Palop, J.A. *Bioorg. Med. Chem. Lett.*, 2007, *17*, 6853-6859.



General structure of diselenide and selenocyanate compounds.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL SYMMETRICAL AND ASYMMETRICAL BENZYLAMINOPHTALAZIN DERIVATIVES

Iosu Jiménez (1), Elena Ibáñez (1), Daniel Plano (1), Ylenia Baquedano (1), Enrique Domínguez (1), Juan Antonio Palop (1), Ignacio Encío (2), Carmen Sanmartín (1).

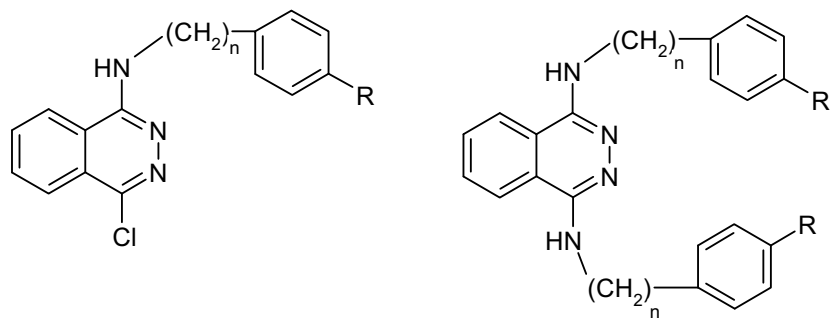
(1) Departamento de Química Orgánica y Farmacéutica. University of Navarra. Irunlarrea,1. 31008- Pamplona.
(2) Departamento de Ciencias de la Salud. Public University of Navarra. Campus de Arrosadía s/n. 31006-Pamplona.

Is well known that phthalazine derivatives, like others members of the isomeric benzodiazine series, have therapeutical activity such as DNA bisintercalator, phosphodiesterase 5 inhibitors, vasorelaxant, VEGFR-2 inhibitors and cytotoxic agents[1-3].

In our effort to find news cytotoxic heterocyclic derivatives inductors of apoptosis and study if it exists a relationship between molecular symmetry and compound's biological activity[4] we have synthesized several pairs of 1-benzylamino-4-chlorophthalazines and 1,4-bis-benzylaminophthalazines.

These compounds were evaluated as cytotoxic agents by a MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide]) assay against five types of 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 nontumoral cell line 184B5 (breast ephithelium).

1. Rodríguez-Ciria, M.; Sanz, A.M.; Yunta, M.J.R. Bioorganic & Medicinal Chemistry 11 2143-2148.
2. Watanabe, N.; Kabasawa, Y.; Takase, Y.; Matsukura, M.; Miyazaki, K.; Ishihara, H.; Kodama, K.; Adachi, H. J.Med.Chem
1998, 41. 3367-3372.
3. Duncton, M.A.J. et al. Bioorganic & Medicinal Chemistry 17 (2009) 731–740.
4. Sanmartín, C.; Font, M.; Palop, J.A. Mini-Reviews in Medicinal Chemistry, 2006, 6, 639-650.



n = 0,1.
R = H, Cl, OCH_3, SCH_3, CF_3, SeH, SeCN, $SeCH_3$.

SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF $\{Co^{3+}–M^{2+}\}$ (M = Zn, Cd, Hg) HETEROBIMETALLIC COMPLEXES

Nagendra K. Kaushik (1), Akhilesh K. Verma (2), Anurag Mishra (2), J. S. Adhikari (2), and Rajeev Gupta (2)

(1) Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, Delhi – 110 007, India.
(2) Dept. of Chemistry University of Delhi, Delhi – 110 007, India.

A novel series of heterobimetallic complexes, $\{Co^{3+}–M^{2+}\}$ where M = Zn, Cd, and Hg, have been synthesized using cobalt(III) complexes $Na[Co(L^1)_2]$ and $[Co(L^2)_3]$ as the building blocks. These heterobimetallic complexes have been characterized by various spectroscopic methods (^{1}H NMR, ^{13}C NMR, UV-vis and IR), microanalytical results, and conductivity measurements. Screening results for anticancer and antibacterial studies against the human brain tumor U87 cell line and a range of resistant and standard bacterial strains indicate promising activity that varies with changes in the ligand and the coordinated secondary/peripheral metal ions. Treatment induced cell death (MTT and macro-colony assay), growth inhibition, cytogenetic damage (micronuclei formation), cell cycle delay and apoptosis were studied as parameters for the cellular response. Treatment with complexes enhanced growth inhibition and cell death in a concentration dependent manner in both U87 and HEK cell lines. At higher concentrations (>15.6 µg/ml) the cytotoxic effects of the complexes were highly synergistic and mainly mediated through apoptosis implying the possible interactions of lesions caused by the agents. The enhanced cell death due to complexes was accompanied by a significant increase (2–3 folds @ 62.5 µg/ml) in micronuclei formation in U87 cells. Under these conditions, complexes also enhanced the cell cycle delay mainly due to S and G2 blocks. The increase in micronuclei formation observed after treatment indicates that these complexes may interfere with the rejoining of DNA strand breaks. Amongst all compounds; **1**, **2**, **4**, **6** and **7** exhibited potent activity against both bacteria and U87 cells. Despite potent in-vitro activity, all complex exhibited diminished cytotoxicity against the normal human HEK cells at all effective concentrations.

1. A. Mishra , **N. K. Kaushik** , A. K. Verma , R. Gupta. Eur. J. Med. Chem., 43 (2008) 2189 -2196.
2. A. P. Singh, **N. K. Kaushik**, A. K. Verma, G. Hundal, R. Gupta. Eur. J. Med. Chem, 44 (2009) 1607-1614.

ANTIMALARIAL 4(1H)PYRIDONES

Marga Puente, José M. Bueno, Pilar Manzano, Jesús Chicharro, Jorge Fernández, M. Cruz García, Beatriz Díaz, Juan Carlos de la Rosa, Esperanza Herreros, José M. Fiandor

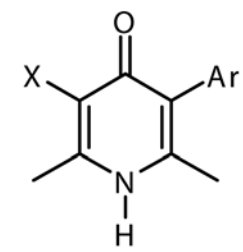
ID CEDD/CEEDD. DDW DPU Tres Cantos, Drug discovery Chemistry Department

4(1H)-pyridones 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 play 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. In summary, the presence of 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 b.

The general structure of the antimalarial 4(1H)pyridones is depicted in Fig 1.



X= Cl, Br
Ar= Aromatic moiety

Fig 1. General formula of Antimalarial 4(1H)Pyridones

POSTER COMMUNICATIONS – PC.70

IDENTIFICATION OF NOVEL INDANYLAMIDINOHYDRAZONES AS 5-HT$_6$ ANTAGONISTS

Neus Mesquida (1), Sara López-Pérez (1), Immaculada Dinarès (1), Jordi Frigola (2), Ramon Mercè (2), Jörg Holenz (2), Raquel Pérez (2), Javier Burgueño (2) and Ermitas Alcalde (1)

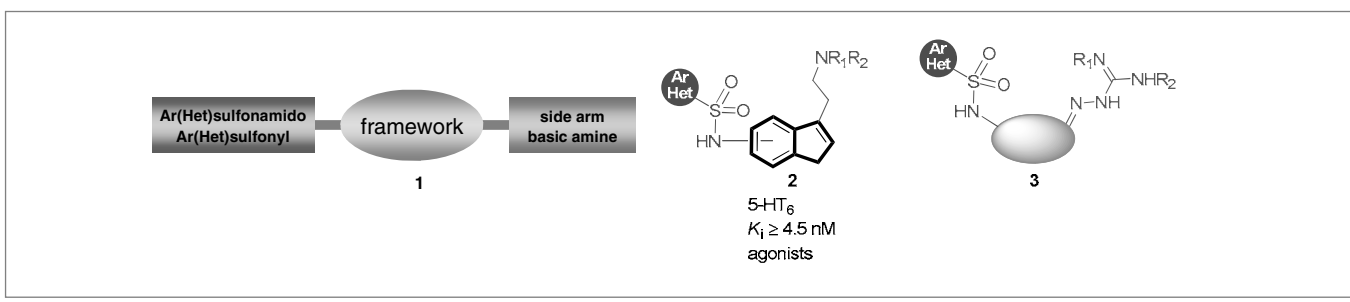
(1) Laboratori de Química Orgànica, Departament de Farmacologia i Química Terapèutica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, 08028 Barcelona, Spain. (2) ESTEVE, Av. Mare de Déu de Montserrat, 221, 08041 Barcelona, Spain.

The 5-HT$_6$ serotonin receptor has become an attractive and promising therapeutic target for the development of new drug-like selective CNS agents, and recent research has developed a broad array of small-molecules as potent and selective 5-HT$_6$ antagonists. However, agonists have proved elusive because of their generally modest selectivity. Many 5-HT$_6$ ligands **1** are comprised of an indole or other heterocyclic system with a basic amine side arm and an arylsulfonamido or arylsulfonyl moiety[1]. By developing an indole-to-indene switch in quest of 5-HT$_6$ ligands, we have been able to examine several sets of indene-based sulfonamides **2**[2,3].

Following our current search for 5-HT$_6$ serotonin receptor ligands related to disubstituted 3-(aminoethyl)indenylsulfonamides **2**, the present study was designed to examine a structural change of the basic amine moiety at the indene 3-position. The replacement of the conformationally flexible *N*,*N*-(dimethylamino)ethyl side chain by a rigid carboximidamide moiety led to the identification of new indanylsulfonamides **3**, which exhibited excellent binding affinity and antagonistic response at the 5-HT$_6$ receptor, with K_i and IC_{50} values in the nanomolar range.

This research was supported by ESTEVE, Barcelona, Spain, through projects FBG2004/05-302663 and FBG2006/07-303647 Fundació Bosch i Gimpera-Universitat de Barcelona, Spain. S.L.-P thanks ESTEVE for a graduate fellowship (2004-07) and the AGAUR for a predoctoral 2008-F.I. fellowship. Thanks are also due to AGAUR, Grup de Recerca Consolidat 2005SGR00158.

1. (a) Liu, K. G.; Robichaud, A. J. *Drug Dev. Res.* **2009**, *70*, 145–168. (b) Johnson, C. N.; Ahmed, M.; Miller, Neil D. *Curr. Opin. Drug Discov. Dev.* **2008**, *11*, 642–654. (c) Glennon, R. A. *J. Med. Chem.* **2003**, *46*, 2795–2812.
2. Alcalde, E.; Mesquida, N.; Frigola, J.; López-Pérez, S.; Mercè, R. *Org. Biomol. Chem.* **2008**, *6*, 3795–3810.
3. Alcalde, E.; Mesquida, N.; López-Pérez, S.; Frigola, J.; Mercè, R. *J. Med. Chem.* **2009**, *52*, 675–687.



AN APPLICATION OF THE INDOLE-TO-INDENE SWITCH TO 5-HT$_6$ SEROTONIN RECEPTOR LIGANDS: RING CONSTRAINT IN INDENYLSULFONAMIDES USING CYCLIC AMINES AND STRUCTURALLY ABBREVIATED COUNTERPARTS

Sara López-Pérez (1), Neus Mesquida (1), Jordi Frigola (2), Ramon Mercè (2), Jörg Holenz (2) Marta Pujol (2), Enrique Hernández (2) and Ermitas Alcalde (1)

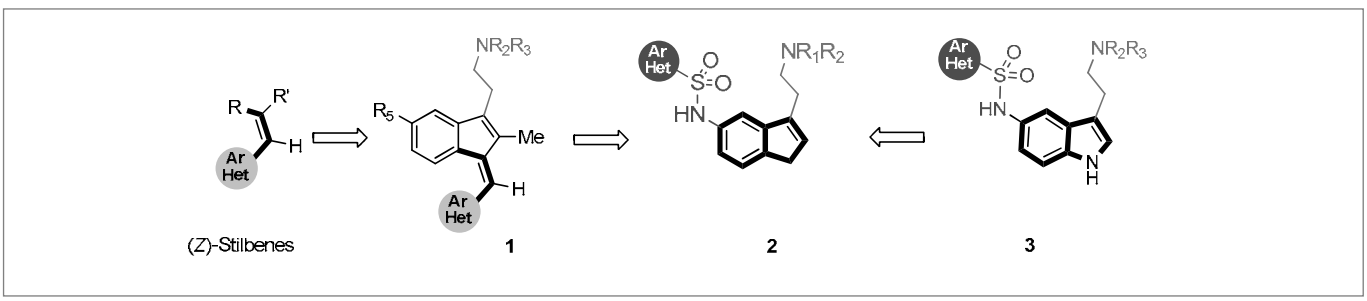
(1) Laboratori de Química Orgànica, Departament de Farmacologia i Química Terapèutica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, 08028 Barcelona, Spain. (2) ESTEVE, Av. Mare de Déu de Montserrat, 221, 08041 Barcelona, Spain.

In the context of a research project whose aim was to find (*Z*)-stilbenes with potential biological effects on the central nervous system, we began by applying a scaffold selection approach to indene systems such as **1**. The next selection step was the incorporation of a sulfonamide functionality at the 5-position of both *cis*-indenes **1** and their reduced counterparts **2**, which exhibited 5-HT$_6$ serotonin receptor affinity with $K_i \geq 20$ nM[1]. The design and synthesis of structural analogs based on an indole-to-indene switch from the potent indolylsulfonamides **3** to the indene counterparts has led to the discovery of potent agonists **2**[2].

As part of our continuing efforts in identifying new 5-HT$_6$ ligands, we focused our attention on the *N*-(inden-5-yl)sulfonamides featuring a ring-constrained aminoethyl side chain at the indene 3-position, some of which exhibited high binding affinity. Selected compounds were tested in a cyclic AMP functional assay and found to be 5-HT$_6$ full antagonists.

This research was supported by ESTEVE, Barcelona, Spain, through projects FBG2004/05-302663 and FBG2006/07-303647, Fundació Bosch i Gimpera-Universitat de Barcelona, Spain. S.L.-P thanks ESTEVE for a graduate fellowship (2004-07) and the AGAUR for a predoctoral 2008-F.I. fellowship. Thanks are also due to AGAUR, Grup de Recerca Consolidat 2005SGR00158.

1. Alcalde, E.; Mesquida, N.; Frigola, J.; López-Pérez, S.; Mercè, R. *Org. Biomol. Chem.* **2008**, *6*, 3795–3810.
2. Alcalde, E.; Mesquida, N.; López-Pérez, S.; Frigola, J.; Mercè, R. *J. Med. Chem.* **2009**, *52*, 675–687.



POSTER COMMUNICATIONS – PC.72

SYNTHESES AND CDK/GSK-3 SELECTIVITY OF 11-METHOXY-SUBSTITUTED PAULLONE DERIVATIVES

Nadine Tolle (1), Lutz Preu (1), Yoan Ferandin (2), Laurent Meijer (2), and Conrad Kunick (1)

(1) Technische Universität Braunschweig, Institut für Pharmazeutische Chemie, Beethovenstraße 55, 38106 Braunschweig, Germany
(2) C.N.R.S., Station Biologique de Roscoff, Place G. Teissier, B.P. 74, 29682 Roscoff Cedex, France

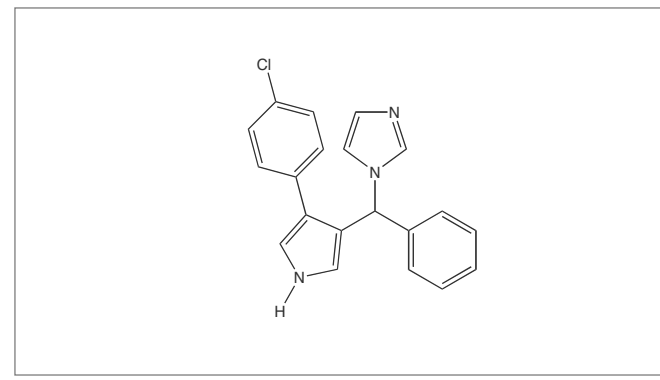
Glycogen synthase kinase-3 (GSK-3) is a widely distributed serine/threonine kinase playing a significant role in manifold cellular signalling pathways by phosphorylating metabolic, signalling and structural proteins and thereby regulating their functions. Dysfunction of GSK-3 is involved in a variety of diseases (e.g. diabetes mellitus, Alzheimer's disease and cancer). Therefore, selective GSK-3 inhibitors are not only of particular importance as putative drugs, but also as biochemical tools useful in the research of the pathobiochemistry underlying these major ailments. 1-Azakenpaullone is a commercially available GSK-3 inhibitor, acting competitively with respect to ATP and exhibiting up to a hundredfold selectivity against the closely related cyclin-dependent kinase 1/cyclin B complex. In an approach to optimize selectivity and potency of 1-azapaullone derivatives, we have probed different attachment positions around the parent paullone scaffold. In the present poster, the synthesis of 11-methoxy-1-azakenpaullone will be reported, which turned out to be a promising starting molecule for a further development campaign in the 1-azapaullone series. Furthermore, the kinase inhibitory selectivity and potency of this new 1-azapaullone will be compared to its 1-carba congener.

1-[(ARYL)(4-ARYL-1*H*-PYRROL-3-YL)METHYL]-1*H*-IMIDAZOLES ARE NOVEL ANTI-*TRYPANOSOMA CRUZI* AGENTS

Roberto Di Santo (1), Alberto Iacovo (1), Gaetano Miele (1), Giuliana Cuzzucoli Crucitti (1), Federica Rosi (1), Valentina Pepe (1), Christophe Verlinde (2), Michael Gelb (3), Roberta Costi (1)

(1) Dipartimento di Chimica e Tecnologie del Farmaco, "Sapienza" Università di Roma, Roma, Italia
(2) Departmets of Biochemistry & Biological Structure Biomolecular Structure Center 1959 NE Pacific Ave, University of Washington, Seattle WA 98195, USA
(3) Departments of Chemistry and Biochemistry Campus Box 351700, 36 Bagley Hall, University of Washington, Seattle, WA 98195 alberto.iacovo@uniroma1.it

Trypanosoma cruzi (TC) is the causative agent of Chagas disease threatening lives of more than 100 million people. This parasite has a life cycle that involves obligatory passages through four stages: i) proliferative epimastigote, ii) infective trypamastigote in triatomine insect as vectors, iii) proliferative intacellular amastigote, and iv) infective bloodstream trypamastigote in mammalian host. The infection is transmitted among humans predominantly by insect vectors or through blood transfusion, and primarily affects the hearth (chagastic cardiopathy), gastrointestinal tract (megasyndromes) and nervous system (dementia)[1]. The current treatment is based on benznidazole or nifurtimox that cause a number of side effects and show poor clinical efficacy. These two drugs are effective against the circulating form of the parasite (trypomastigotes) during the acute phase of this disease, but not during the chronic stage. Thus there is an urgent need for new anti-TC drugs[2]. Growing knowledge on the basic biology of TC opens new opportunities for rationally developed approaches to treatment of Chagas disease. One of the approaches is to block a key metabolic pathway in the parasite[3]. Conserved in eukaryota, sterol biosynthesis is one such target pathway. Within this pathway, one of the most important enzymes is the sterol 14α-demethylase (CYP51) that is a member of the cytochrome P450 superfamily. CYP51 catalyzes the oxidative removal of the 14-methyl group from post-squalene sterol precursors. Since our group was recently engaged in the research of novel antimycotic agents targeted to the lanosterol demethylase of fungi, we decided to screen our library of antifungal



agents on a panel of parasites. RDS 416 was identified as an hit active against TC at 14 ng/ml concentration (EC_{50}). We also tested RDS 416 for binding to TC lanosterol 14-demethylase in vitro and found that it binds well this target, consistently with the EC_{50} data on parasites. Subsequently, a series of derivatives of this hit were designed, synthesized and tested against TC, The results of the biological assays will be shown.

1. Lepesheva GI et al. *J. Biol. Chem.* **2006**, *281*, 3577-3585.
2. Aguirre G et al. *Eur. J. Med. Chem.* **2004**, 39, 421–431.
3. Urbina, J.A *Curr. Opin. Infect. Dis.* **2001**, *6*, 733–741.

POSTER COMMUNICATIONS – PC.74

FESCDIPINE II IS A NEW ANTIHYPERTENSIVE DRUG. COMPUTATIONAL STUDIES OF ITS INTERACTION WITH ANGIOTENSIN CONVERTER ENZYME

Ana Velázquez, Víctor Vázquez, Víctor Abrego, Luisa Martínez, Miguel Hernández, Mónica Saucedo, Nalleli Solís, Pablo Martínez, Miguel Flores, Denise Ramírez and Enrique Ángeles

Medicinal Chemistry Laboratory, Departamento de C. Químicas FES Cuautitlán UNAM, Campo1 Cuautitlán Izcalli Estado de México, México. Email: angeles@unam.mx

The heart disease has become a very important problem in the whole world. In 2002, the WHO reported as one the main mortality facts the arterial hypertension (1.7%). In USA, the FDA reported that in 2006 there were 65 millions of people with arterial hypertension. In Mexico (2005), 2.7% of the people died by hypertension, for 2030 year the mortality percentage will be 32.5. Stout and his group had studied changroline as an effective antiarrhythmic agent; they concluded that without the region II of the molecule the activity doesn't exist. And if in the region III they include carbonyl groups the antiarrhythmic activity increases and the toxicity decreases. In UNAM-FESC we re-take this research. We are proposing antihypertensive activity for changrolina and an *in silico* comparative study of its analogues (LQM-300), FESCDIPINE II with captopril.

We made a conformational analysis, validated by X-Ray diffraction. With the global minimum the molecular dockings were made using SYBYL 6.9 and MOE 2008, Ver.10 programs. The binding energy for SYBYL was computed by: ΔEbin = Eest + Eelectros + ΔE[Ecomplex-(Eenz + Elig)]. The enzyme active domain was determined from the complex structure of ACE- captopril of the PDB with a 1.82 Å of resolution.

The molecular docking is a predictive model and its results with both programs show us that there is a good inhibition activity between the LQM-300 compounds and the ACE for the hypertension treatment. When we compared this activities with a reported molecule like captopril this results were alike between them. These results confirm our biological activity studies. We will discuss in the poster why FESCDIPINE II was the best compound with antihypertensive activity.

The authors wish to acknowledge to PAPIIT/UNAM Projects No IN203609, IN211108 and CONACYT Project No 82473 by partially support this work. We would like to thank to C. Barajas, F. Sotres, M. Hernández, P. García and D. Jiménez for their skillful technical assistance and DGSCA-UNAM for their support. As a part of Project Cátedra Química Medicinal of FESC-UNAM

1. Alvadalejo, P., Bouaziz, H., Duriez, M., Gohlke, P., Levy, B. I., Safar, M. E., Benetos, A. **1994.** Angiotensin converting enzime inhibition prevents the increase in aortic collagen in rats. *Hypertension.* 23, 74-82.
2. Velázquez, A. Ma., Torres, L. A., Díaz, G., Ramírez, A., Hernández, R., Santillán, H., Martínez, L., Martínez, I., Díaz-Barriga, S., Abrego, V., Balboa, M. A., Camacho, B., López Castañares, R., Dueñas-González, A., Cabrera, G and Ángeles, E. *ARKIVOC*. (ii), 2006, 150 – 161.
3. Synthesis and antihypertensive effects of new methylthiomorpholinphenol derivatives. A. Ma. Velázquez[1], L. Martínez[1], V. Abrego[1], M. A. Balboa[2], L. A. Torre[1], B. Camacho[1], S. Díaz-Barriga[1], A. Romero[1], R. López- Castañares[3], E. Angeles, *European Journal of Medicinal Chemistry*, *43*, 486-500 (2007).

CAPE AND CAFFEIC ACID ANALOGUES AS ANTINEOPLASIC DRUGS. DOCKING STUDIES

Enrique Ángeles (1), Víctor Vázquez (1), Roberto Macías (2), Olga Beltrán (2), Ana Velázquez (1), Víctor Abrego (1), Miguel Hernández (1), Mónica Saucedo (1), Nayeli Solís (1), Pablo Martínez (1), Miguel Flores (1), Denise Ramírez (1), Saúl Villa (2)

(1) Medicinal Chemistry Laboratory, Departamento de C. Químicas FES Cuautitlán UNAM, Campo 1 Cuautitlán Izcalli, Estado de México, México, (2) Departamento de Biología Celular, CINVESTAV, IPN, México DF, México.
Email: angeles@unam.mx

One of the current handicaps of cancer treatments is the difficulty of aiming these treatments at destroying malignant cells without killing healthy cells in the process. There are many reports that propolis, is a very safe healthcare product from bee hives, and it contains anticancer ingredients, one of them is called CAPE (caffeic acid phenethyl ester), its potential therapeutic effect on NF tumors was active *in vivo* and is found to have the following properties: antimitogenic, anti-carcinogenic, anti-inflammatory, immunomodulatory, and antioxidant. Recent reports suggest that CAPE also has a neuronal protective property against ischemic injury and in inducing apoptosis in human pancreatic cancer cells.

In this work we present the results of docking studies of new caffeic acid analogues as antineoplasic drugs. The studies were performed in a Silicon Graphics Octane 2 Worksation, using MOE software. We are using derivatives of caffeic acid as targets on subunits of Cytochrome P450 as a receptor. We found that evidence that CAPE and new derivatives have a excellent molecular interaction when we calculated the Interaction Binding Energy. The all results will be discussed in the meeting.

1. Chemoprotective effect of caffeic acid phenethyl ester on promotion in a medium-term rat hepatocarcinogenesis assay. Claudia E. Carrasco-Legleu, Lucrecia Márquez-Rosado, Samia Fattel-Fazenda, Evelia Arce-Popoca, Julio I. Pérez-Carreón and Saul Villa-Treviño, *Int. J. Cancer:* **108,** 488–492 (2004).
2. Caffeic acid phenethyl ester preferentially sensitizes CT26 colorectal adenocarcinoma to ionizing radiation without affecting bone marrow radioresponse**.** Yu-jen Chen, Hui-Fen Liao, Tung-hu Tsai, Sheng-yuan Wang, and Ming-shi Shiao. Int. J. Radiation Oncology Biol. Phys., Vol. 63, No. 4, pp. 1252–1261 (2005).
3. Evidence that the Anticarcinogenic Effect of Caffeic Acid Phenethyl Ester in the Resistant Hepatocyte Model Involves Modifications of Cytochrome P450. Olga Beltrán-Ramírez,* Leticia Alemán-Lazarini,* Martha Salcido-Neyoy,* Sergio Hernández-García,* Samia Fattel-Fazenda,* Evelia Arce-Popoca,* Jaime Arellanes-Robledo,* Rebeca García-Román,* Patricia Vázquez-Vázquez, Adolfo Sierra-Santoyo, and Saúl Villa-Treviño, TOXICOLOGICAL SCIENCES 104(1), 100–106 (2008).

POSTER COMMUNICATIONS – PC.76

SYNTHESIS OF NEW 1-ARYL-3-ARYLAMIN-PROPAN-1-OL DERIVATIVES AS ANTIMALARIAL AGENTS

Adela Mendoza (1), Silvia Pérez-Silanes (1), Luis Berrade (1), Adriana Pabon (2), Eric Deharo (3), Ignacio Aldana (1), Antonio Monge (1)

(1) Unidad en Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), Universidad de Navarra, C/Irunlarrea s/n, 31080 Pamplona, Spain. (2) Grupo Malaria, Universidad de Antioquía, Calle 62; 52-59 Medellín, Colombia 3Mission IRD, Casilla 18-12-09, Lima 18, Perú

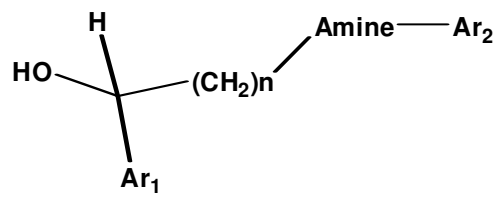
Malaria is one of the most dangerous diseases affecting primarily poor people of tropical and subtropical regions. The last report last report from WHO informed that 109 countries showed endemic malaria in 2008. The search for novel drugs with cheap and easy synthetic methods against specific parasites is an important goal for antimalarial drug discovery.

The increasing resistance of the malaria parasite *Plasmodium falciparum* to currently available drugs and especially to chloroquine demands a continuous effort to develop new effective therapeutic options. Identification of new molecular scaffolds structurally unrelated to existing antimalarial agents represents a valuable strategy to bypass resistance phenomena.

Accordingly, a series of new 1-aryl-3-arylamin-propan-1-ol derivatives, was evaluated *in vitro* against a chloroquine-sensitive strain (FCR-3) of *Plasmodium Falciparum*[1]. The antimalarial activity of these structures is determinated using 3H- Hypoxantine incorporation inhibition by parasite method[2].

We wish to express our gratitude to the PIUNA project from the University of Navarra. Adela Mendoza was awarded a PhD scholarship supported by the "Gobierno de Navarra".

1. Bertami S., Bourdy G., Landau I., Robinson J.C., Esterre Ph., Deharo E.(2005). Evaluation of French Guiana traditional remedies. Journal of Ethnopharmacology 98(1-2),45.
2. Desjardins, R.E., Canfield, C.J., Haynes, J.D .1979. Quantitative assessment of antimalarial activity invitro by a semiautomated microdilution technique. Antimicrobial agents and Chemotherapy16,710.



Ar_1 = 3-benzo [b]thiophenyl and 2-naphthyl
n = 1 or 2
Amine = Piperazine and *S*-Aminopyrrolidine
Ar_2 = (4-nitro-2-trifluoromethyl)phenyl and (2-nitro-4-trifluoromethyl)phenyl

PYRIDAZIN-3(*2H*)-ONE DERIVATIVES AS NOVEL PDE IV INHIBITORS

Núria Aguilar (1), Jordi Gràcia (1), Marta Carrascal (1), Vittorio dal Piaz (2), Maria Paola Giovannoni (2), Claudia Vergelli (2), Maria Antonia Buil (1), Carme Masdeu (1), Yolanda Garrido (1), Wenceslao Lumeras (1), Begoña Hernández (1), Mónica Córdoba (1), Elena Calaf (1), Miriam Andrés (1), Montse Miralpeix (1) and Amadeu Gavaldà (1)

(1) Almirall Research Center, Laboratorios Almirall S.A., Ctra. Laureà Miró 408, E-08980 Sant Feliu de Llobregat, Barcelona, Spain
(2) Department of Pharmaceutical Sciences, University of Florence, Italy. Via Ugo Schiff 6, 50019 Sesto Florentino, Italy.

Phosphodiesterases (PDE's) comprise a superfamily of enzymes responsible for the hydrolysis and inactivation of the second messengers cAMP and cGMP. Eleven different PDE families have been identified to date. Among them the PDE4 isoenzyme family exhibits a high affinity for cyclic AMP. Increased levels of cAMP caused by PDE4 inhibition are associated with the suppression of cell activation in a wide range of inflammatory and immune cells. Moreover, PDE4 inhibitors decrease the release of the cytokine TNFα.

Therefore, diseases such as asthma, chronic obstructive pulmonary disease (COPC) and other inflammatory conditions could potentially be treated with a selective PDE4 inhibitor. Thus, several PDE4 inhibitors such as **roflumilast** or **oglemilast** are in active development for the oral treatment of such diseases.

Herein we described the discovery of pyridazin-3(2H)-one as novel structural class of potent and efficacious PDE4 inhibitors. The structure activity relationship is described along with the pharmacokinetic and in vivo profile of key compounds.

POSTER COMMUNICATIONS – PC.78

NEW BENZO[b]THIOPHENE DERIVATIVES AS HITS FOR THE DEVELOPMENT OF CHAGAS DISEASE DRUGS

Silvia Pérez-Silanes (1), Luis Berrade (1), Adela Mendoza (1), Diego Benítez (2), Hugo Cerecetto (2), Mercedes González (1), Ignacio Aldana (1) and Antonio Monge (1)

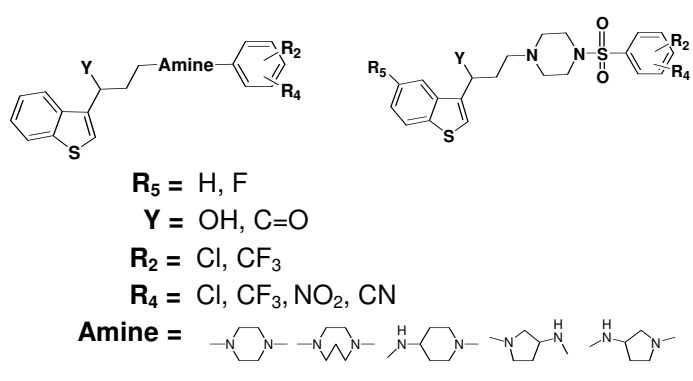
(1) Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, C/Irunlarrea s/n, 31008 Pamplona, Spain; E-mail:sperez@unav.es
(2) Laboratorio de Química Orgánica, Facultad de Ciencias/Facultad de Química, Universidad de la República, Iguá 4225, 11400 Montevideo, Uruguay.

Neglected diseases represent a major health problem. This year marks the 100th anniversary of the Discovery of Chagas disease. The illness mainly affects the poor in remote areas and is endemic in Latin America, where an estimated 18 million people are infected with the causative parasite *Trypanosma cruzi*[1]. Currently, there are only two clinically used drugs, Nifurtimox and Benznidazole[2]. Both possess important toxic effects and relative clinical efficacy; therefore, the pharmacotherapy of Chagas disease is very deficient and there is an urgent need for the development of safe and effective drugs.

We have focused our recent efforts on the development of new hits with activities against the agents responsible for some neglected diseases. We have synthesized and evaluated the capacity of seventeen benzo [b] thiophene derivatives at 25 μM doses to inhibit the growth *T. cruzi* Tulahuen 2 strain. We have found the new compounds to have very good *in vitro* activity, even better than the reference compound, Nifurtimox and Benznidazole.

Adela Mendoza was awarded a PhD scholarship supported by the "Gobierno de Navarra".

1. WHO: http://www.who.int/neglected_diseases/diseases/chagas/en/index.html. Accessed June 14, 2009.
2. Cerecetto, H.; González, M. Chemotherapy of Chagas' disease: Status and new developments. Curr. Top. Med. Chem. 2002. 2, 1187-1213.



REQUIREMENTS AT THE PURINE MOIETY FOR THE INHIBITORY ACTIVITY OF 9-ARYLPURINES AGAINST COXSACKIE VIRUS B3 (CVB3) REPLICATION

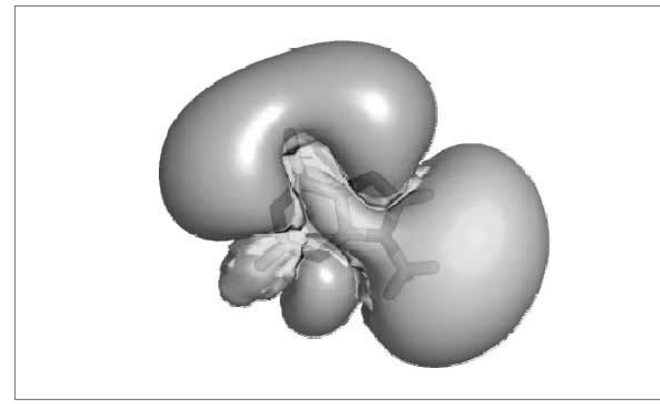
Eva-María Priego (1), Leire Aguado (1), Hendrik Jan Thibaut (2), María Luisa Jimeno (3), Antonio Morreale (4), Federico Gago (5), María-José Camarasa (1), Johan Neyts (2) and María-Jesús Pérez-Pérez (1)

(1) Instituto de Química Médica (CSIC), 28006 Madrid, Spain; (2) Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium; (3) Centro de Química Orgánica Lora-Tamayo (CSIC), 28006 Madrid, Spain; (4) Unidad de Bioinformática, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), 28049 Madrid, Spain; (5) Departamento de Farmacología, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain

Enteroviruses are responsible for a broad spectrum of conditions in man including respiratory infections, meningitis, encephalitis, pancreatitis, myocarditis and neonatal sepsis. In particular Coxsackie virus type B3 (CVB3) is an important human pathogen that may result in acute and chronic viral myocarditis (which may progress to cardiomyopathy requiring transplantation). So far there is no drug approved for the treatment of CVB3 or any enteroviral infection.

We recently reported on the first compounds among 9-arylpurines able to selectively inhibit CVB3 replication with EC_{50} values in the range 4-8 μM; cytotoxicity was not observed at 250 μM for the most selective compounds[1,2]. This family of compounds is characterized by their simplicity in structure, synthetic accessibility and their structural novelty in comparison to previously reported anti-CVB3 compounds.

Different substitutions have been efficiently introduced at positions 2, 6 and/or 8 of the purine moiety. The results obtained indicate that the nature of these substituents has a significant impact on the antiviral activity. In order to rationalize these results, different computational tools have been used to find a common pattern among antivirally effective compounds. The synthesis, antiviral evaluation and theoretical calculations of these 9-arylpurines will be presented in detail.



E-M. P. has a CSIC contract from the I3P programme financed by F.S.E. L.A. thanks the Spanish Ministerio de Educación y Ciencia for a FPU predoctoral fellowship. This work has been supported by a grant of the Spanish CICYT (SAF2006-12713-C02-01) and BIPEDD (S2006/BIO-0214).

1. Aguado, L; Thibaut, HJ; Camarasa, MJ, et al. *Antivir. Res*. **82**, A55, 2009.
2. Thibaut, HJ; Aguado, L; van der Linden, L, et al. *Antivir. Res*. **82**, A73, 2009.

POSTER COMMUNICATIONS – PC.80

STRUCTURE-BASED DISCOVERY OF NEW SMALL-MOLECULE INHIBITORS OF THE ANTIAPOPTOTIC PROTEIN Bcl-x_L

Marta Pinto (4), María del Mar Orzáez (3), Enrique Pérez-Payá (3), Juan J. Pérez (2) and Jaime Rubio-Martínez (1)

(1) Dept. de Química Física; Universitat de Barcelona (UB); Martí i Franqués 1; E-08028 Barcelona, Spain and the Institut de Química Teòrica i Computacional (IQTCUB) (2) Dept. d'Enginyeria Química; Universitat Politècnica de Catalunya (UPC); ETS d'Enginyeria Industrial; Av. Diagonal 647; E-08028 Barcelona, Spain. (3) Dept. of Medicinal Chemistry. Centro de Investigación Príncipe Felipe, Av. Autopista del Saler, 16. E-46012 Valencia, Spain. (4) Unitat de Recerca en Informàtica Biomèdica (GRIB), UPF, Dr. Aiguader 80, E-08003 Barcelona, Spain

Overexpression of different Bcl-2 anti-apoptotic proteins has been described in many cancers and is connected to an elevated resistance to chemotherapeutic treatments. Accordingly, inhibition of their protective function has emerged in recent years as an interesting strategy to develop new anti-cancer drugs. In the present work, a pharmacophore-directed virtual screening approach has been used to discover new Bcl-x_L inhibitors. The pharmacophore model was derived from molecular dynamics studies of different BH3 peptides bound to the Bcl-xL protein. Screening of different small molecule databases using Catalyst (Catalyst, Accelrys Inc.) permitted to identify a small set of small-molecule binders. After *in vitro* testing, five of these compounds were found to disrupt the Bcl-xL/Bak(BH3) complex . Furthermore, one of them (UBQF19), exhibited cell-permeability inducing apoptosis in HBL2 and Jurkat cells with concentrations in the low micromolar range. The strategy followed and the results obtained in this work are presented. These new leads identified open a new opportunity to develop a new structural class of anti-cancer agents by the optimization of these compounds.

PRODRUGS OF AMINE-CONTAINING THERAPEUTIC AGENTS BASED ON THE DIPEPTIDYL-PEPTIDASE-IV (DPPIV/CD26) ENZYME FOR IMPROVING WATER SOLUBILITY

Silvia Cabrera (1), Alberto Díez-Torrubia (1), Jan Balzarini (2), María-José Camarasa (1) and Sonsoles Velázquez (1)

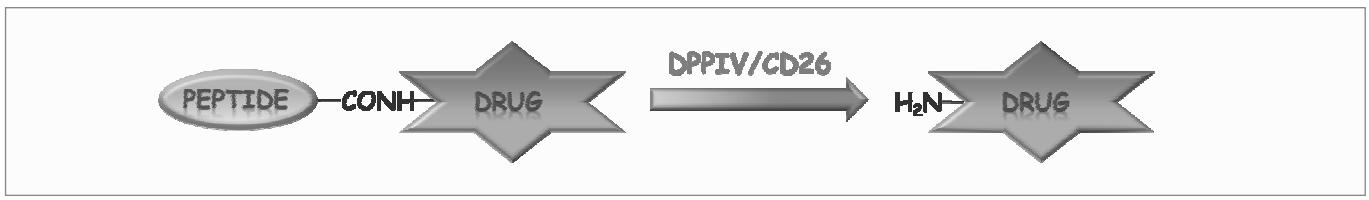
(1) Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain. (2) Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium.

We have described recently[1] a novel enzyme-based prodrug approach that provides conjugates of therapeutic agents with a peptide moiety as a carrier where the conjugate [peptide]-[drug] is specifically cleavable by the endogenous dipeptidyl-peptidase IV enzyme (DPPIV), present on the surface of certain cells or in plasma. This enzyme, also known as CD26, belongs to a group of atypical serine proteases and preferentially cleaves X-Pro (or X- Ala) dipeptides from the N-terminus of a variety of natural peptides. For proof-of-concept of this novel prodrug technology, we focused on the anti-HIV-1 lipophilic TSAO compounds. [$(Xaa\text{-}Pro)_n$]-[TSAO-T] conjugates bearing di- and tetrapeptides sequences of different nature were prepared and studied[2,3]. In all cases, DPPIV/CD26 was able to efficiently hydrolyze those "artificial substrates" different from natural peptides.

Herein, we will describe the extension of this study to other amine-containing therapeutic agents of different nature such as doxorubicine, 6-aminoquinoline and purine or pyrimidine nucleoside drugs[4]. Efficient procedures for the synthesis of dipeptidyl and tetrapeptidyl amide prodrugs including *N*-acylation protocols of the exocyclic amino function of cytidine and adenosine nucleosides were performed. Furthermore, the ability of these prodrugs to act as efficient substrates of DPPIV/CD26 enzyme, their human or bovine serum hydrolysis profiles and the improvement of the water solubility compared with that of the parent drugs will be reported.

We thank the Spanish MEC/MICINN. (SAF2006-12713-C02) and the K. U. Leuven (GOA no. 05/19) for financial support. A. D.-T. and S. C. thank the CSIC and MICINN for their "I3P" and "Juan de la Cierva" contracts, respectively.

1. Balzarini, J.; Camarasa, M. J.; Velázquez, S. "Prodrugs cleavable by CD26" Patent PCT WO **2004**/098644.
2. García-Aparicio, C.; Bonache, M. C.; De Meester, I.; San-Félix, A.; Balzarini, J.; Camarasa, M.-J.; Velázquez, S. *J. Med. Chem.* **2006**, *49*, 5339-5351.
3. García-Aparicio, C.; Díaz-Torrubia, A.; Balzarini, J.; Lambeir, A.-M.; Velázquez, S.; Camarasa, M.-J. *Antivir. Res.* **2007**, *76*, 130-139.
4. Díaz-Torrubia, A.; García-Aparicio, C.; Cabrera, S.; Balzarini, J.; De Meester, I.; Camarasa, M.-J.; Velázquez, S. *J. Med. Chem.* **2009**, *submitted*.



POSTER COMMUNICATIONS – PC.82

FURTHER INSIGHTS IN THE INHIBITORY ACTIVITY OF ACYCLIC THYMINE NUCLEOSIDES AGAINST THYMIDINE MONOPHOSPHATE KINASE OF *M. TUBERCULOSIS*

Olga Familiar (1), Hélène Munier-Lehmann (2), José-Antonio Ainsa (3), María-José Camarasa (1) and María-Jesús Pérez-Pérez (1)

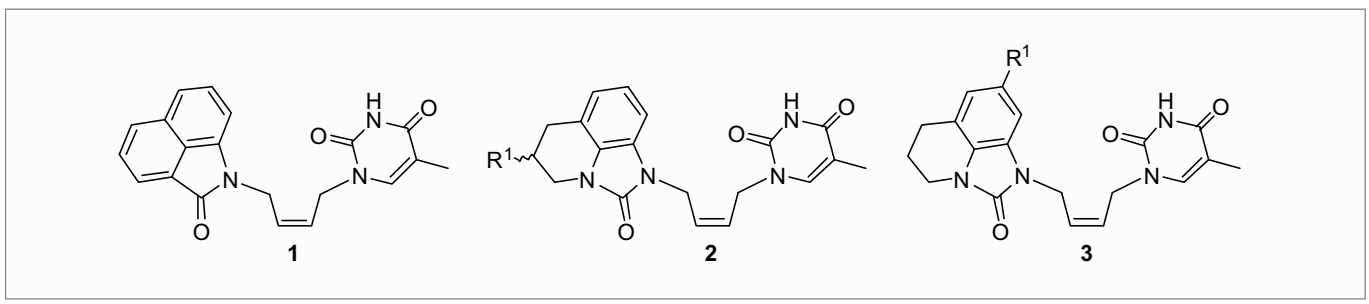
(1) Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain. (2) Institut Pasteur, Unité de Chimie Organique, CNRS URA 2128, Rue du Dr. Roux, 75724 Paris Cedex, France. (3) Departamento de Microbiología, Medicina Preventiva y Salud Publica, Universidad de Zaragoza, Domingo Miral s/n, 50009 Zaragoza and CIBER Enfermedades Respiratorias, Spain

Thymidine monophosphate kinase (TMPK) catalyzes the phosphorylation of thymidine monophosphate to the diphosphate, using ATP as the phosphate donor in the presence of magnesium ion. TMPK is crucial for maintaining the thymidine triphosphate (dTTP) pools that are required for DNA synthesis in replicating organisms. The enzyme TMPK from *Mycobacterium tuberculosis* has been proposed as a suitable target in the search of novel compounds able to halter the replication of this worldwide distributed pathogen. The differences between the mycobacterial enzyme and its human analogue are significant enough to support the required degree of selectivity[1].

We have reported on a novel series of acyclic thymine nucleosides represented by compound **1** that inhibit TMPKmt with *K*i values in the submicromolar range[2]. Our data, supported by molecular modeling simulations, revealed that both the thymine base and the *cis*-butenyl spacer were crucial for the right location of the inhibitors at the dTMP binding site in TMPKmt, while the carbonyl group at the distal site establishes an hydrogen bond interaction with the side chain of Arg95. Thus these demands have been kept unaltered in the new series of compounds where the modifications have been performed at the distal site by replacing the naphtolactam ring of the lead compound (**1**) by different imidazoquinolinones (**2** and **3**) with the aim of improving the solubility of our first series of inhibitors without compromising the inhibitory activity against TMPKmt.

OF thanks the Spanish Ministerio de Educación y Ciencia (MEC) for a FPI predoctoral fellowship. This work has been supported by grants of the Spanish MEC (SAF programme), the Pasteur Institute (GPH tuberculose), the CNRS and INSERM.

1. Pochet, S.; Dugue, L.; Douguet, D.; Labesse, G.; Munier-Lehmann, H. *ChemBioChem* **2002**, *3*, 108-110.
2. Familiar, O.; Munier-Lehmann, H.; Negri, A.; Gago, F.; D. Douguet, D.; Rigoud, L.; Hernández, A. I.; Camarasa, M. J.; Pérez-Pérez, M. J. *ChemMedChem* **2008,** *3*, 1083-1093.



NEW 1,3-DIARYLUREA DERIVATIVES AS INHIBITORS OF CYCLIN CDK4: SYNTHESIS, BIOLOGICAL EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIPS

Núria Mur, José M. Rodríguez, Vanessa Prieur, Arturo Vinuesa, M. Dolors Pujol

Laboratori de Química Farmacèutica. Facultat de Farmàcia. Universitat de Barcelona. Av. Diagonal 643. 08028 Barcelona (Spain). Fax: (0034)93403594. E-mail: mdpujol@ub.edu

Cyclin-dependent Kinases (CDKs) phosphorylate several substrates directly involved in the cell growth control, such as retinoblastoma protein (pRB). In normal cells, CDK4 and CDK6 activities are negatively regulated by the tumour suppressor 16[1].

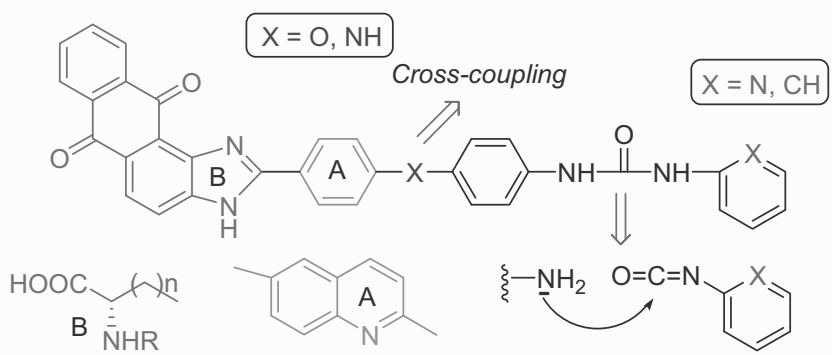
Recent biological studies suggest that selective inhibition of CDK4 may restore normal cell activity and could be a valuable approach to cancer therapy[2].

As part of our ongoing programme to design new types of selective CDK4 inhibitors, we report in this work the preparation of new compounds possessing the 1,3-diarylurea moiety. The target 1,3-diarylurea derivatives were synthesized via the route outlined in **Scheme 1**.

The intermediate diphenylether or diphenylamine was obtained from the phenol or aniline and bromo- or chloroaryl by *cross-coupling* reaction or by nucleophilic aromatic substitution (nitro-compounds). The corresponding aniline was treated with the appropriate phenylisocyanate to give the diarylurea[3].

Other compounds containing quinoline instead of phenyl (**A**) were synthesized to investigate the effect of these substituents on CDK selectivity and potency. Also effects of different **B** substituents at C-4 of phenyl (or at C-6 of quinoline) will be reported.

1. Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem*. **2000**, *43*, 1-12.
2. Stevens, K. L. et al. *Bioorg. Med. Chem. lett*. **2008**, *18*, 5758-5762.
3. Franz, R. A.; Zebardast, T.; Hakimion, F.; Shirazi, H. F.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem*. **2006**, *14*, 7044-7050.



Scheme 1

POSTER COMMUNICATIONS – PC.84

OPTIMIZATION OF A POTENT HIV-1 INTEGRASE STRAND TRANSFER INHIBITOR (INSTI): MOLECULAR MODELING, SYNTHESIS AND ANTI-HIV ACTIVITY

Sara De Grazia (1), Stefania Ferro (1), Laura De Luca (1), Maria Letizia Barreca (2), Zeger Debyser (3) and Alba Chimirri (1)

(1) Dip. Farmaco-Chimico, Università di Messina, Viale Annunziata, I-98168, Italy; (2) Dip. Chimica e Tecn. del Farmaco, Università di Perugia, Via del Liceo 1, I-06123, Italy; (3) Div. of Molecular Medicine, Katholieke Univ. and IRC KULAK, Kapucijnenvoer 33, B-3000, Leuven, Belgium

Since many years our research group is engaged in the development of anti-HIV agents targeting essential steps in the viral life cycle. In this context the design of new HIV-1 Integrase Strand Transfer Inhibitors (INSTIs) plays a key role.

To date only one active drug against this enzyme, raltegravir (Isentress), has received FDA approval. Raltegravir belongs to the family of β-diketo acids analogues which display potent antiviral activity and their IN-binding mechanism is connected to the presence of DKA pharmacophoric motif which could be involved in a functional sequestration of one or both divalent metal ions in the enzyme catalytic site.

In our previous studies we reported three-dimensional pharmacophore models for HIV-1 integrase inhibitors which led to the discovery of a new series of benzylindole diketo acids derivatives able to inhibit the ST step of integration at nanomolar concentration. Particularly, a potent antiviral agent named CHI-1043 was identified as "lead compound"[1-3].

Taking in account these findings and in order to obtain further useful information for its optimization, different structural modifications were planned and a small library of novel CHI-1043 analogues was prepared.

All the synthesized compounds were tested for their inhibitory effect on IN enzymatic activity and against HIV replication.

Innovative eco-friendly methods and technologies have been used for the synthesis of the new compounds and in particular the microwave irradiation was applied in several steps of the synthetic pathway leading a significant improvement of reaction conditions and yields.

Research supported by THINC project (HEALTH-F3-2008-201032)

1. Barreca ML, Ferro S, De Luca L, Monforte AM, Debyser Z, Witvrouw M, Chimirri A. *J. Med. Chem.* **2005**, 48, 7084-7088.
2. De Luca L, Barreca ML, Ferro S, Iraci N, Michiels M, Christ F, Debyser Z, Witvrouw M, Chimirri A. *Bioorg. Med. Chem. Lett.* **2008**, 18, 2891-2895.
3. Ferro S, De Luca L, Barreca ML, Iraci N, De Grazia S, Christ F, Witvrouw M, Debyser Z, Chimirri A. *J. Med. Chem.***2009**, 52, 569-573.

NOVEL CB1 CANNABINOID LIGANDS: CHROMENOPYRAZOLES

José Cumella (1), Laura Hernández-Folgado (1,2), Dow P. Hurst (2), Patricia Rodríguez (2), María Gómez (3), Diana C. G. A. Pinto (4), Pilar Goya (1), Patricia H. Reggio (2), Javier Fernández-Ruiz (3), Artur M. S. Silva (4), and Nadine Jagerovic (1)

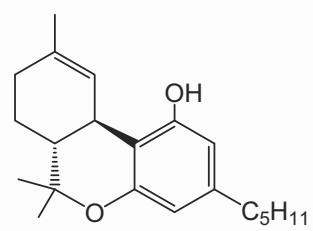
(1) Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain, (2) Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro NC27402,USA, (3) Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain, (4) Departamento de Química, Universidade de Aveiro, Aveiro, Portugal.

Two G-protein-coupled cannabinoid receptors, CB_1 and CB_2, have been identified so far. The cannabinoid receptors CB_1 are mainly expressed on central and peripheral neurons whereas CB_2 cannabinoid receptors are located primarily in the immune system. These receptors have emerged as promising therapeutic targets. With the discovery of CB_1 and CB_2 endogeneous, natural and synthetic ligands, considerable effort has been directed toward the structure-activity relationships and molecular modeling of the different cannabinoid families. Among these families, the classical cannabinoids are characterized by a dibenzopyran structure exemplified by phytocannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC). Even though the first generation of classical cannabinoids showed potent activity in vivo, they lacked CB_1/CB_2 selectivity. SAR studies of classical cannabinoids revealed the important role played by the substitutions in the C-1, C-3, and C-9 positions in the recognition of CB_1 or CB_2 receptors. In this study, chromenopyrazoles with different pyrazole substitution have been explored as cannabinoid ligands[1].

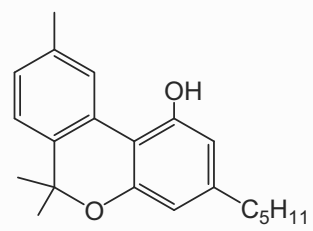
We will present an optimised synthetic route to a series of chromenopyrazole derivatives and their binding evaluation towards both receptors, CB_1 and CB_2. Newly cannabinoid designed ligands show high affinity and selectivity for CB_1 receptor. Docking studies has been performed on receptor homology model.

These studies were supported by SAF2006-13391-C03-02, CANNAB-CM (S-SAL-0261-2006), and FCT-CSIC bilateral convention (2007PT0038).

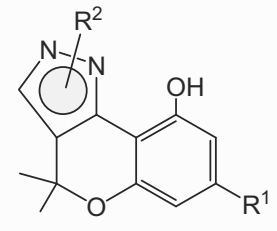
1. Patent application number ES P200900802.



Δ^9-Tetrahydrocannabinol (Δ^9THC)
CB_1/CB_2 cannabinoid ligand



Cannabinol (CNB)
CB_1/CB_2cannabinoid ligand



Selective CB_1 cannabinoid ligand

POSTER COMMUNICATIONS – PC.86

NEURONAL NETWORKS FOR THE DISCOVERY OF PDE7 INHIBITORS ACTIVE ON CNS DISEASES

Miriam Redondo (1), Valle Palomo (1), Daniel Pérez (1), Nuria Campillo (1), José Brea (2), Isabel Cadavid (2), Isabel Loza (2), Concepción Pérez (1), Ana Pérez-Castillo (3), Carmen Gil (1) and Ana Martínez (1)

(1) Instituto de Quimica Medica-CSIC, Juan de la Cierva 3, 28006 Madrid (Spain), (2) Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela (Spain), (3) Instituto de Investigaciones Biomédicas-CSIC, Arturo Duperier 4, Madrid (Spain)

Phosphodiesterases (PDEs) have been emerged, in the last years, as an important family of druggable targets with a central role in signal transduction by regulating intracellular levels of cyclic AMP and GMP[1]. Specifically, PDE7 is one of the eleven isoforms expressed abundantly in different brain areas, which suggests its potential involvement in different CNS diseases interfering with both the inflammatory and neurotransmitter cascades[2]. Therefore, the identification of selective inhibitors targeted against PDE7 enzyme has become an attractive area of research[3].

Using neuronal networks and descriptors generated by CODES, here we present an *in silico* model to predict the PDE7 inhibitory activity of entirely new molecules. CODES codifies molecules from a topological point of view and the generated descriptors are related to the chemical nature of the atoms, the atomic bonds and the connectivity with the rest of the molecule[4]. The predictive neuronal network tool here designed has provided excellent results both in the PDE7 inhibitor classification of different novel quinazolines[5] and, more importantly, in the discovery of a completely new chemical family as PDE7 inhibitors. Experimental enzymatic inhibition using a specific HTS methodology developed for this project confirmed this last result. Moreover, we have evidence showing that these compounds reduce the inflammatory activation of primary cultures of astrocytes, microglia, and neurons in response to lipopolysaccharide.

1. Conti, M,.; Beavo, J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem*. **2007**, *76*, 481-511.
2. Menniti, F. S.; Faraci, W. S.; Schmidt, C. J. Phosphodiesterases in the CNS: targets for drug development. *Nat. Rev. Drug Discov*. **2006**, *5*, 660-670.
3. Gil, C.; Campillo, N. E.; Pérez, D. I.; Martínez, A. "Phosphodiesterase 7 (PDE7) inhibitors as new drugs for neurological and anti-inflammatory disorders". *Exp. Opin. Ther. Patents* **2008**, *18*, 1127-1139.
4. Stud, M. CODES_ v1.0 (revisión 3).
5. Castro, A.; Jerez, M. J.; Gil, C.; Calderón, F.; Doménech, T.; Nueda, A.; Martínez, A. CODES, a novel procedure for ligand-based virtual screening: PDE7 inhibitors as an application example. *Eur. J. Med. Chem*. **2008**, *43*, 1349-1359; Castaño, T.; Wang, H.; Campillo, N. E.; Ballester, S.; González-García, C.; Hernández, J.; Pérez, C.; Cuenca, J.; Pérez- Castillo, A.; Martínez, A.; Huertas, O.; Gelpí, J. L.; Luque, F. J.; Ke, H.; Gil, C. "Synthesis and biological evaluation of thioxoquinazoline derivatives as phosphodiesterase 7 inhibitors". *Chem. Med. Chem.* **2009**, *4*, 866-876.

TARGETING CINNAMIC ANTIOXIDANTS TO MITOCHONDRIA: A NEW THERAPEUTIC DIRECTION ON NEURODEGENERATIVE DISEASES

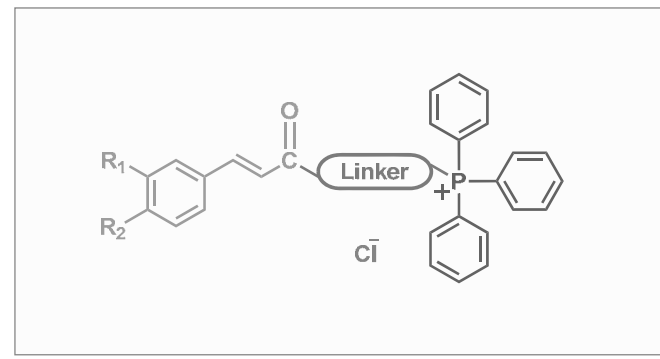
Jorge Garrido (1), Alexandra Gaspar (2), Elsa Reis (2), Tiago Silva (2), Nuno Milhazes (3), E. Manuela Garrido (1), Fernanda Borges (2)

(1) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal; (2) Departamento de Química, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; (3) Instituto Superior de Ciências de Saúde-Norte, Gandra, PRD, Portugal.

Neurodegenerative diseases confront mankind in a variety of guises and induce chronic suffering and debilitation for a significant percentage of the worldwide population. The quest for new targets within cell machinery is one of the major issues in this field of study. Apart from the major classic targets in neuronal system a battery of new putative targets has been proposed nowadays.

In fact, targeting mitochondria could be an effective way to slow the neurodegenerative disease progression and consequently the ageing processes of the brain. Although the molecular mechanisms responsible for mitochondria-mediated disease processes are not fully elucidated yet, the oxidative stress appears to be critical. The matrix space in mitochondria is the intracellular compartment which is potentially the most active in production of ROS and is the most vulnerable to ROS damaging effects. In this context, the control of mitochondrial redox processes is an attractive perspective and the development of mitochondriotropic compounds is of great importance in such an effort.

The aim of our project is the design and synthesis of several hydroxycinnamic antioxidant derivatives harbouring positive charges at physiological pH (hence capable of mitochondrial accumulation)



that could be used as potent and selective agent(s) throughout specific the target mitochondria. Some of the results obtained so far will be presented in this communication.

1. P. Toogood (2008) *Mitochondrial drugs*, Curr. Opinion Chem. Biol., 12: 1-7.
2. F. Borges, A. Gaspar, N. Milhazes, E. M. Garrido, J. Garrido, E. Reis, T. Silva (2009) *Targeting cinnamic antioxidants to mitochondria: a new therapeutic approach on neurodegenerative diseases*, National Patent 104560 E.

POSTER COMMUNICATIONS – PC.88

CHROMONE AS A PRIVILEGED SCAFFOLD FOR THE DEVELOPMENT OF MONOAMINE OXIDASE INHIBITORS

Alexandra Gaspar (1), Nuno Milhazes (2), Jorge Garrido (3), Fernanda Borges (1), Eugénio Uriarte (4), Matilde Yáñez (5), Francisco Orallo (5), Stefano Alcaro (6) and Francesco Ortuso (6)

(1) Departamento de Química, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; (2) Instituto Superior de Ciências da Saúde-Norte, Gandra, PRD, Portugal; (3) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal; (4) Departamento de Química Orgânica and (5) Farmacologia, Instituto de Farmacia Industrial, Facultad de Farmacia, Universidad de Santiago de Compostela, Spain; (6) Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università "Magna Græcia" di Catanzaro, Italy.

Chromone scaffold ((4H)-1-benzopyran-4-one) has been nowadays recognized as a pharmacophore of a great number of bioactive molecules, either of natural or synthetic origin. At present, numerous biological effects, especially in the popular medicine, have been ascribed to this benzo-γ-pyrone nucleus such as anti-inflammatory, antitumoral and antimicrobial activities. Enzymatic inhibition properties towards different systems, such as oxidoreductases, kinases, cyclooxygenases, have been also recognized.

Accordingly, our project has been focused on the discovery of new chemical entities (NCEs) for MAO inhibition, based on the development of versatile chemistries suitable for the preparation of libraries incorporating privileged structures with benzo-γ-pyrone substructure. In this work functionalized chromone scaffolds suitable to establish structure-activity relationships were obtained by expedite synthetic strategies and screened toward human MAOs isoforms (hMAOs) to evaluate their potency/selectivity. Additionally, molecular modelling studies were performed using available hMAO-A and hMAO-B structures deposited into the Protein Data Bank (PDB) as receptor models to understand the enzyme-inhibitor interactions and to explain the selectivity of the most active compounds.

The SAR study allow to conclude that chromones that have substituents in position-3 of γ-pyrone nucleus act preferably as MAO-B inhibitors with IC50 values in the micromolar to nanomolar range. The hMAOs molecular modeling studies performed for two isosteric chromones allow to get insight in the enzyme-inhibitor interactions and to explain the displayed selectivity.

Our findings pointed out a crucial, undisclosed role of the presence of a carboxylate group in position 3 of the pyrone ring able to establish hydrogen bond interactions with active site residue to obtain highly potent and selective MAO-B inhibitors. Additional studies are warranted for a systematic lead optimization, modulated by appropriate modifications of length, size, and chemical nature of the substituents, process that can lead to a drug candidate.

The authors thank financial support from FCT (Portugal)-Project PTDC/QUI/70359/2006

1. Borges, M.F.M.; Gaspar, A.M.N.; Garrido, J.M.P.J.; Milhazes, N.J.S.P.; Batoreu, M.C.C.(2008) Chromone derivatives for use as antioxidants/preservatives. WO2008104925A1 and PT103665.
2. Borges, M.F.M.; Gaspar, A.M.N.; Garrido, J.M.P.J.; Milhazes, N.J.S.P, Uriarte, E.; Yáñez, M.; Orallo, F. (2009) Utilização de cromonas, seus derivados, seus sais farmaceuticamente aceitáveis e seus pró-fármacos com actividade inibidora da monoamina oxidase e aplicações terapêuticas relacionadas. PT104487.

NEW Trp(NPS)-CONTAINING DIPEPTIDE DERIVATIVES AS TRPV1 CHANNEL BLOCKERS

Mª Jesús Pérez de Vega (1), Mª Teresa García-López (1), Laura Zaccaro (2), Miriam Royo (2), Fernando Albericio (2), Asia Fernández-Carvajal (3), Antonio Ferrer-Montiel (3), and Rosario González-Muñiz (1)

(1) Instituto de Química Médica-CSIC, Juan de la Cierva, 3, 28006 Madrid, Spain. (2) Parc Cientific de Barcelona, Josep Samitier 1-5, 08028 Barcelona, Spain (3) IBMC-UMH, Ed. Torregaitán, Av. de la Universidad s/n, 03202 Elche, Spain

TRPV1 (Transient Receptor Potential Vanilloid 1) is a non-selective, Ca2+ preferring, ion channel, activated by temperatures higher than 42°C, acidic pH, vanilloids such as capsaicin, and the endogenous cannabinoid receptor ligand anandamide. TRPV1 expression is up-regulated in a number of painful inflammatory disorders, and consequently is a promising therapeutic target for pain relief[1]. In this respect, numerous research programs have been dedicated to the search of TRPV1 modulators to be used as pharmacological tools for better understanding the pharmacology of these cation channels. In addition, several products from these programs have reached clinical trials for multiple therapeutic indications[1,2].

A few years ago, we found that dipeptide derivatives Xaa-Trp(NPS) and Trp(NPS)-Xaa (Xaa=Lys, Arg) inhibited the activation of TRPV1 in the micromolar range by blocking the pore entrance and avoiding calcium flow[3]. These dipeptides also acted as NMDA channel blockers, although with lower potency. In an attempt to fine-tune the potency/selectivity balance within this family of channel blockers, we have prepared and evaluated a new series of H-Trp(NPS)-Lys-NH_2 dipeptide derivatives of general formulae **1** and **2**, incorporating alkyl and guanidino moieties at the α–NH_2 group and at the Lys side-chain, respectively.

This communication deals with the solid-phase synthesis of the small library of Trp(NPS)-containing dipeptides **1** and **2**, and with the results of the evaluation of their channel blockade activity in TRPV1 and NMDA receptors, heterologously expressed in *Xenopus oocytes*.

We thank MICINN for the Consolider-Ingenio Grant (CSD2008_00005) to the Spanish Ion Channel Initiative (SICI; http://sici.umh.es/).

1. Patapoutian, A.; Tate, S.; Clifford J. Woolf, C. J. *Nature Rev Drug Discov*. **2009**, *8*, 55-68.
2. Gunthorpe, M. J.; Chizh, B. A. *Drug Discov. Today* **2009**, *14*, 56-67.
3. Bonache, M. A.; García-Martínez, C.; García de Diego, L.; Carreño, C.; Pérez de Vega, M. J.; García-López, M. T.; Ferrer-Montiel, A.; González-Muñiz, R. *ChemMedChem* **2006**, *1*, 429 – 438.

POSTER COMMUNICATIONS – PC.90

NANOCONJUGATES AS INTRACORPOREAL NEUTRALIZERS OF BACTERIAL ENDOTOXINS

María J. Vicent (1), Laura Cascales (2), Rodrigo J. Carbajo (3), Nuria Cortés (4), Àngels Messeguer (4) and Enrique Pérez Payá (2,5)

(1) Polymer Therapeutics Lab. Department of Medicinal Chemistry, Centro de Investigación Príncipe Felipe, Avda Autopista del Saler 16, E-46012 Valencia, Spain. (2) Peptide and Protein Lab. Department of Medicinal Chemistry, Centro de Investigación Príncipe Felipe, Avda Autopista del Saler 16, E-46012 Valencia, Spain. (3) Structural Biology Lab. Department of Medicinal Chemistry, Centro de Investigación Príncipe Felipe, Avda Autopista del Saler 16, E-46012 Valencia, Spain. (4) Department of Chemical and Molecular Nanotechnology, IQAC-CSIC, E-08034 Barcelona, Spain. (5) Instituto de Biomedicina de Valencia, IBV-CSIC, Jaume Roig 11, 46010 Valencia,

Sepsis is a leading cause of mortality that is most often provoked by endotoxins (i.e.lipopolysaccharides; LPS) released by gram-negative bacteria into the patient's bloodstream during infection. The therapeutic armory currently available for sepsistreatment is poor. We previously identified an LPS-neutralizing small molecule, PTD7. Here we tested the efficacy of novel PTD7-nanoconjugates in a murine model of sepsis. We found that PTD7-based nanoconjugates treated mice had improved survival that it was correlated with a marked decrease in proinflammatory cytokines in the blood. This proves that nanocojugate-based endotoxin neutralizers can function as intracorporeal neutralizers of bacterial endotoxins.

SULFORAPHANE AND ANALOGUES AS POTENT CHEMOPREVENTIVE AGENTS

Ana Alcudia (1), Siham Mallouk (2), Inmaculada Fernández (1), Franziska Lieder (3), Sabine Werner (3), and Noureddine Khiar (2)

(1) Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.
(2) Instituto de Investigaciones Químicas, C.S.I.C-Universidad de Sevilla, c/. Américo Vespucio, 49, Isla de la Cartuja, 41092 Sevilla, Spain.
(3) Department of Biology, Institute of Cell Biology, ETH Zurich, Zurich, Switzerland.

Sulforaphane **1**, firstly isolated in 1992 from broccoli extracts[1], is the main inducer of phase II detoxifying enzymes and is well documented as a powerful chemopreventive agent[2].

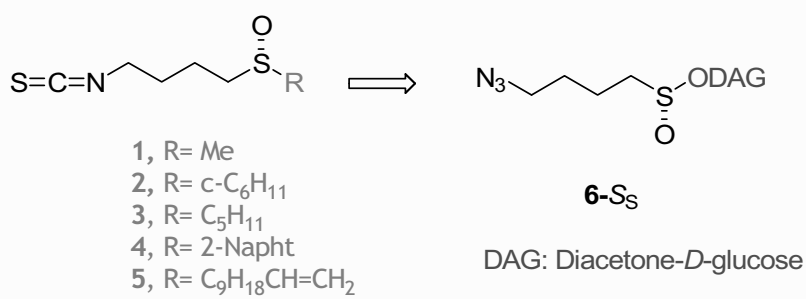
In this regard, animal studies on rats have established the chemopreventive activity of sulforaphane against colon cancer[3], while a recent preclinical and clinical studies have confirmed the chemopreventive activity of sulforaphane of women at risk for breast cancer[4].

Our continuing interest in the synthesis of optically pure sulfinylderivatives with biological and synthetic interests[5], led us to envisage a convergent and high yielding approach for the asymmetric synthesis of sulforaphane **1**, and some of its analogues differently substituted at the sulfinyl sulfur (**2-5**), Scheme 1. The key step of the synthesis is the diastereoselective preparation of sulfinate ester **6**-S_S using the *DAG-methodology*[6], developed in our research group.

The biological activity of the new compounds as inductors of phase 2 detoxifying enzyme has been studied by determining their ability to activate the cytoprotective transcription factor Nrf2.

Financial supports from the DGICyT (grant No. CTQ2006-15515-CO2-01 and CTQ2007-61185), the Junta de Andalucía (grant P06-FQM-01852 and P07-FQM-2774), and European Union "FEDER Fund" are gratefully acknowledged.

1. Zhang, Y.; Talay, P.; Cho, C. G.; Posner, G. H. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2399.
2. Juge, N., Mithen, R. F., Traka, M. *Cell. Mol. Life. Sci.* **2007**, *64*, 1105.
3. Chung, F. L.; Conaway, C.C.; Rao, C.V.; Reddy, B.S. *Carcinogenesis* **2000**, *21*, 2287.
4. Comblatt, B. S.; Ye, L.; Dikova-Kostova, A. T.; Erb, M.; Fahey, J. W., Singh, N. K., Chen, M-S.; Stierer, T., Garret- Mayer, E.; Argani, P.; Davidson, N. E., Talay, P.; Kensler, T.; Visvanathan, K. *Carcinogenesis* **2007**, *28*, 1485.
5. Fernández, I.; Khiar, N. *Chem. Rev.* **2003**, *103*, 3651.
6. Fernández, I.; Khiar, N.; Llera, J.M.; Alcudia, F. *J. Org. Chem.* **1992**, *57*, 6789.



Scheme 1

POSTER COMMUNICATIONS – PC.92

ISOCOMBRETASTATINS A. A HIGHLY POTENT FAMILY OF ANTIMITOTIC AGENTS

Raquel Álvarez, Concepción Álvarez, Rafael Peláez, Manuel Medarde

Department of Pharmaceutical Chemistry. Faculty of Pharmacy. University of Salamanca. "Campus Miguel de Unamuno". E-37007. SALAMANCA. Spain.

During our search of new antimitotic agents based on combretastatins and phenstatins we described some time ago the first example of 1,1-diarylethenes (Isocombretastatins A) as highly potent inhibitors of tubulin polymerization and cytotoxic agents[1]. These compounds were designed as the integration of non-isomerizable analogues of *cis*-combetastatins and *non*-ketonic analogues of phenstatins in the same structure.

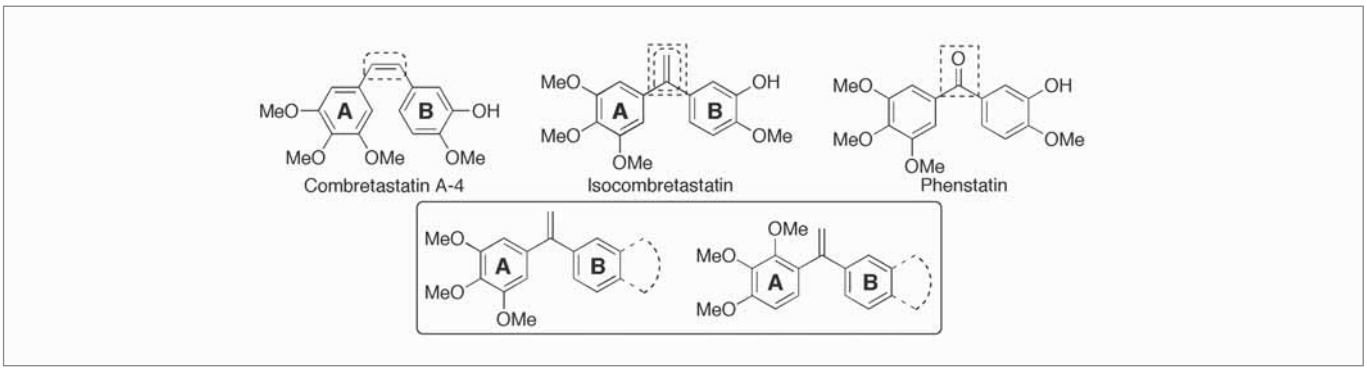
Recently, a patent[2] and a paper[3] on this family has appeared and a paper from our group accepted for publication[4]. In this communication we present a summary of these results and a comparison of the effect of 3,4,5-trimethoxyphenyl and 2,3,4-trimethoxyphenyl moieties (ring-A) while changing the structure of the other system (ring-B).

In brief, isocombretastatins are as potent as phenstatins in the inhibition of tubulin polymerization (ITP) assay (IC_{50} in the μM range), usually accompanied by a high cytotoxicity (IC_{50} tens nM). Compounds with the 2,3,4-trimethoxyphenyl moiety are less potent as ITP agents than 3,4,5-trimethoxyphenyl compounds, but they still maintain a noticeable cytotoxic effect (sub μM range).

These results suggest that the additional Hydrogen Bond between the carbonyl oxygen of phenstatins and the β-248-β-250 residues of tubulin, invoked as a pharmacophorc point for high ITP activity of these compounds[5], seems to be not responsible of high potency of the phenstatins-isocombretastatins family.

Authors thanks Spanish MICINN (SAF2008-04242), Junta de Castilla y León (SA067A09) and EU funds. RA and CA thank MEC for FPI predoctoral grants.

1. Concepción Álvarez. Ph. D. Thesis. University of Salamanca. July **2006**.
2. Alami, M.; Brion, J. D.; Provot, O.; Peyrat, J. F.; Messaoudi, S.; Hamze, A.; Giraud, A.; Bignon, J.; Bakala, J.; Liu, J- M. WO 2008122620, **2008**, 78 pp.
3. Messaoudi, S.; Trguier, B.; Hamze, A.; Provot, O.; Peyrat, J.F.; Rodrigo de Losada, J.; Liu, J-M; Bignon, J.;
Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J.; Brion, J.D.; Alami, M. *J. Med. Chem*., Article ASAP, jm900321u
4. Álvarez, R.; Álvarez, C; Mollinedo, F.; Sierra, B.G.; Medarde, M., Peláez, R. *Bioorg. Med. Chem.* (in press).
5. Nguyen, T. L.; McGrath, C.; Hermone, A. R.; Burnett, J. C.; Zaharevitz, D. W.; Day, B. W.; Wipf, P.; Hamel, E.; Gussio, R. *J. Med. Chem.* **2005**, *48*, 6107-6116



PORPHYRIN-QUINOLONE CONJUGATES: SYNTHESIS OF NEW DERIVATIVES WITH POTENCIAL PDT APPLICATIONS

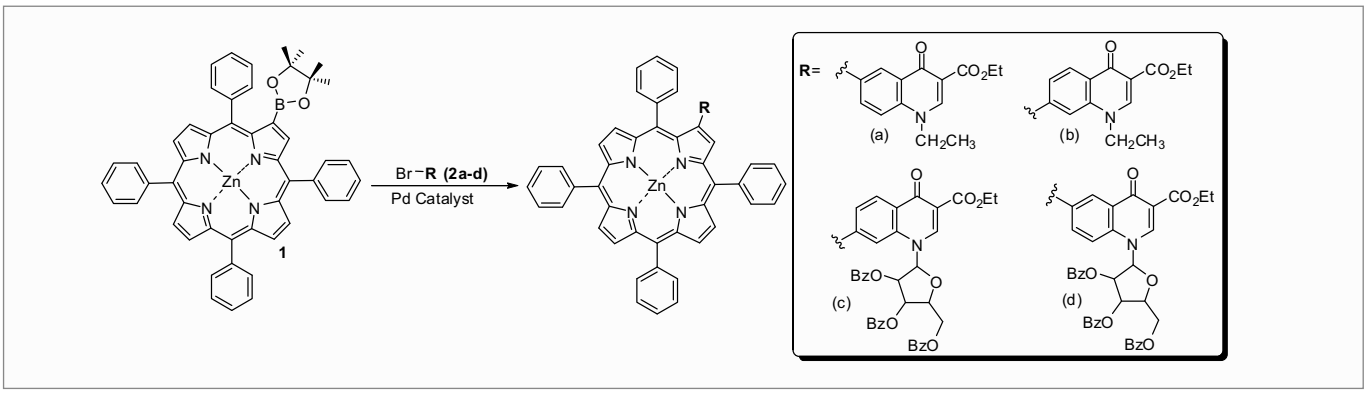
Ana T.P.C. Gomes (1), Anna C. Cunha (2), Maria G.P.M.S. Neves (1), Augusto C. Tomé (1), Artur M.S. Silva (1), Maria C.B.V. Souza (2), Vitor F. Ferreira (2), José A.S.Cavaleiro (1)

(1) University of Aveiro, Department of Chemistry and QOPNA, 3810-193 Aveiro, Portugal (2) Universidade Federal Fluminense, Departamento de Química Orgânica, Instituto de Quýímica, Outeiro de São João Baptista, 24020-141 Niterói, RJ, Brazil

Photodynamic Therapy (PDT) is an emerging therapy used in the treatment of several oncological and non-oncological diseases like wet age-related macular degeneration (AMD)[1]. In this therapy the combination of a photosensitizing drug, oxygen and visible light is able to produce lethal cytotoxic agents like singlet oxygen ($^1O_2$) and other reactive oxygen species that are responsible for the destruction of the diseased tissue[1,2]. Porphyrins have been used extensively as photosensitizers in PDT. In recent years, several groups have been envisaging new methods to modify the porphyrinic macrocycle aiming to get compounds with adequate photophysical and amphiphilic properties as potential new photosensitizers. Target-specific photosensitizers are also being developed for increasing the specificity for the tumour. The coupling of molecules with well-established pharmacological activities could represent an important approach for the discovery of new PDT drugs. Linking porphyrins to biologically active molecules might be considered a versatile method to new photosensitisers[3]. Such is the case with quinolone-porphyrin conjugates, since quinolones can act as antibiotic and anti-tumour agents[4]. In this communication we report a new approach to synthesise novel porphyrin- quinolone conjugates through Suzuki-Miyaura coupling reaction of borylated porphyrin **1** with several quinolone derivatives. Singlet oxygen generation studies of the final products will be also presented.

Thanks are due to the Universidade de Aveiro, Fundação para a Ciência e a Tecnologia (FCT), FEDER and the GRICES-CAPES collaborative programme for funding this work. ATPC Gomes and AC Cunha thank, respectively, FCT (SFRH/BD/38528/2007) and CAPES and for their research grants.

1. (a) Awan, M. A.; Tarin, S. A. *Surgeon*, **2006**, 4, 231-236; (b) Zheng, X.; Pandey, R. K. *Anti-Cancer Agents in Medicinal Chemistry*, **2008**, 8, 241-268.
2. Kormeili, T.; Yamauchi, P.S.; Lowe, N.J. *British Journal of Dermatology,* **2004**, 150, 1061–1069.
3. (a) Cavaleiro, J.A.S.; Tomé, J.P.C.; Faustino, M.A.F, *Topics in Heterocycl. Chem*. **2007**, 7, 179-248; (b) Santos, F. C.; Cunha, A. C.; Souza, M. C. B. V.; Tomé, A. C.; Neves, M. G. P. M. S.; Ferreira, V.F.; Cavaleiro, J. A. S. *Tetrahedron Lett.* **2008**, *49*, 7268 – 7270; (c) Srivastava, B. K.; Solanki, M.; Mishra, B.; Soni, R.; Jayadev, S.; Valani, D.; Jain, M.; Patel, P. R. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 1924–1929
4. (a) Gasparotto,V.; Castagliuolo, I.; Ferlin, M. G. *J. Med. Chem.* **2007,** *50,* 5509-5513; (b) Xia, Y.; Yang, Z.; Xia, P.; Bastow, K. F.; Tachibana, Y.; Kuo, S.; Hamel, E.; Hackl, T.; Lee, K. *J. Med. Chem.* **1998,** *41,* 1155-1162.



POSTER COMMUNICATIONS – PC.94

PYRIDONE DIARYL ETHER NON-NUCLEOSIDE INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

Joshua Kennedy-Smith (1), Nidhi Arora (1), Roland Billedeau (1), Jennifer Fretland (5), Julie Hang (4), Gabrielle Heilek (3), Seth Harris (2), Donald Hirschfeld (1), Hassan Javanbakht (3), Yu Li (4), Weiling Liang (1), Ralf Roetz (1), Mark Smith (1), Guoping Su (3), Judy Suh (1), Zachary Sweeney (1), Armando Villaseñor (2), Jeffrey Wu (1), Dennis Yasuda (1), and Klaus Klumpp (4)

(1) Department of Medicinal Chemistry, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94304; (2) Department of Discovery Technologies, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94304; (3) Department of Viral Disease Biology, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94304; (4) Department of Viral Disease Biochemistry, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94304; (5) Department of Non-Clinical Safety, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94304

New pyridone non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are potent inhibitors of HIV reverse transcriptase (HIVRT) polymerase activity were prepared. These compounds were also active inhibitors of wild-type and NNRTI-resistant HIV replication. Structure-based drug design was used to optimize the activity of the compounds against NNRTI-resistant mutants. The co-crystal structures of inhibitors in the NNRTI binding pocket of HIVRT are also described.

QSAR MODELS FOR QUINOXALINE 1,4-DI-*N*-OXIDE DERIVATIVES AS ANTITRYPANOSOMAL AGENTS

Esther Vicente (1,2), Pablo R. Duchowicz (2), Silvia Pérez-Silanes (1), Ignacio Aldana (1), Eduardo A. Castro (2), Hugo Cerecetto (3), Mercedes González (3) and Antonio Monge (1)

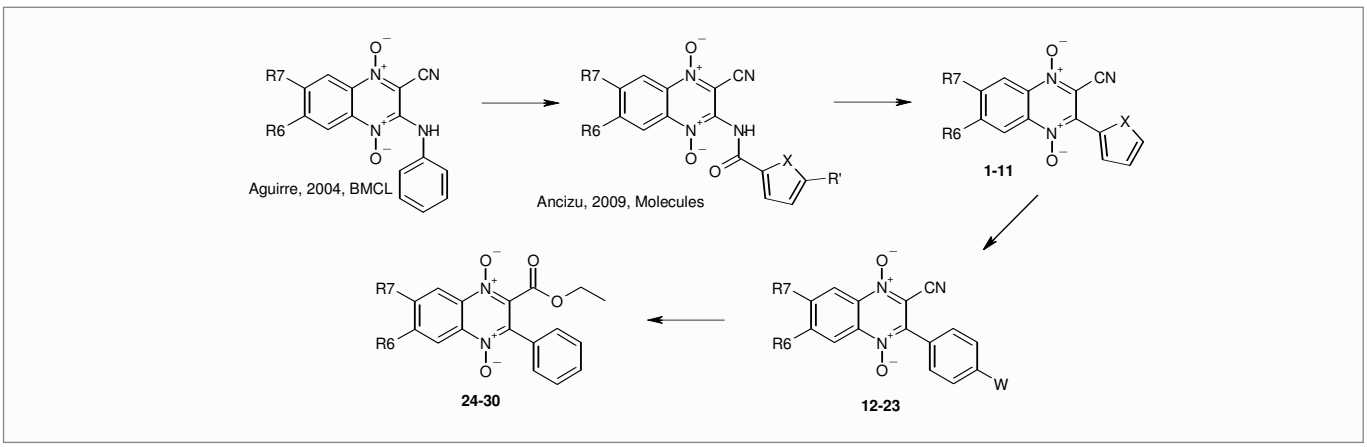
(1) Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, C/ Irunlarrea s/n, 31080, Pamplona. Spain. (2) División Química Teórica, Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata, Diag. 113 y 64, Suc.4, C.C. 16, La Plata, 1900. Argentina. (3) Departamento de Química Orgánica, Facultad de Química, Universidad de la República. Iguá 4225, 11400 Montevideo, Uruguay.

Chagas' disease or American trypanosomiasis is an important health problem that affects around 20 million people in Central and SouthAmerica. Around 2-3 million individuals develop the typical symptoms of this disease that results in 50 000 yearly deaths. The causative agent of this disease is the haemoflagellate protozoan *Trypanosoma cruzi* and the chemotherapy to control this parasitic infection remains undeveloped.

In a continuing effort to identify new active compounds to combat Chagas disease[1-2] and other neglected diseases, our research group prepared thirty 3-arylquinoxaline 1,4-di-*N*-oxide derivatives, **1-30**[3-5]. Six derivatives presented IC_{50} values of the same magnitude order than the standard drug nifurtimox (NFx), when tested *in vitro* against epimastigote forms of *T. cruzi*, making them valid new lead-compounds.

For the purpose of providing a rational guide for the synthesis of future compounds of this class, we established different Quantitative Structure-Activity Relationships (QSAR) with the available experimental biological data. First of all, we analyzed the effect of lipophilicity values on the activity of compounds through QSAR models, expressing the lipophilicity contribution with the octanol/water partition coefficient (logP). In addition, the molecular structure of twenty three quinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivatives was appropriately represented by 1497 theoretical descriptors, calculated with Dragon software[6], and the best linear regression models established with our variable subset selection algorithm were predictive[7]. Application of the QSAR equations developed now enables the proposal of new candidate structures that still do not have experimentally assigned biological data.

1. Aguirre, G. et al. *Bioorg. Med. Chem. Lett.* **2004**, 14(*14*): 3835-3839.
2. Ancizu, S. et al. *Molecules*. **2009**, 14(*6*): 2256-2272.
3. Vicente, E. et al. *Molecules*. **2008**, 13(*1*): 69-77.
4. Vicente, E. et al. *Eur. J. Med. Chem.* **2008**, 43(*9*): 1903-1910.
5. Vicente, E. et al. *Bioorg. Med. Chem*. **2009**, 17(1): 385-389.
6. DRAGON 5.0 Evaluation Version, http://www.disat.unimib.it/chm
7. Duchowicz, P. R. et al. *Chem. Phys. Lett.* **2005**, *412*, 376-380.



NOVEL CARBOHYDRATE-BINDING NON-PEPTIDIC MOLECULES AS POTENTIAL INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS REPLICATION

Paula Carrero (1), Virginia Lozano (1), Ana Ardá (2), Alejandra García-Alonso (3), Bart Hoorelbeke (4), Marleen Renders (4), Ernesto Quesada (1), Dolores Solís (3), Jesús Jiménez-Barbero (2), Jan Balzarini (4), María-José Camarasa (1), María-Jesús Pérez-Pérez (1) and Ana San-Félix (1)

(1) Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain. (2) Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain. (3) Instituto de Química Física Rocasolano (CSIC), Serrano 119, 28006 Madrid, Spain. (4) Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium

There exist several types of carbohydrate-binding agents for which anti-HIV activity has been reported. Amongst them, some lectins, proteins of natural origin that bind carbohydrates, show a potent activity against HIV. They predominantly target the glycans of the viral gp120 glycoprotein. Such interactions affect the conformation and/or efficient functioning of the envelope molecules thus preventing HIV entry into the host cell[1]. Furthermore, under the pressure of these lectins, mutated viruses have been isolated with deletions/modifications of glycans in their envelope[2]. Such a resistance spectrum is unique among the known HIV entry inhibitors and may have important implications for the future of anti-HIV therapy. Thus, appearance of mutations affecting the viral envelope might trigger an efficient immune response directed against the mutated virus since the previously hidden immunogenic epitopes of gp120 become exposed and then the host immune system may produce neutralizing antibodies against them. However, due to their high molecular weight and protein nature, lectins may be endowed with unfavourable pharmacokinetic properties that prevent their development as suitable drugs.

Our research efforts are focused on the synthesis of non-peptidic small molecules with carbohydrate-binding properties able to act through a mechanism similar to that of the natural lectins. The design, synthesis and biological activity of these molecules will be described.

1. J. Balzarini, H. Hatse, *et al*. *Antimicrob. Agents Chemother*. **2004**, 48, 3858-3870.
2. J. Balzarini, K. Van Laethem, *et al*. *Mol. Pharmacol*. **2005**, 67, 1556-1565.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW *p*-PIPERIDINYLALKYLPHENOXYSULFONYLUREA DERIVATIVES AS DUAL H_3 RECEPTOR ANTAGONISTS AND ANTIDIABETIC AGENTS

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

Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain.Email: ncastrillo@alumni.unav.es

The rapid increase in the prevalence of obesity and type 2 diabetes is a major global health problem. Obesity is the main risk factor for type 2 diabetes and by 2010, approximately 33 millions of Europeans will be suffering from diabetes. Because obesity and diabetes often go hand in hand, a new term describe current healthcare crisis: "the diabesity epidemic".

Due to this disease remains a difficult problem for the limited efficacy of available drugs, the introduction of new therapies to combat this global epidemic is for the great sake of medicinal chemistry.

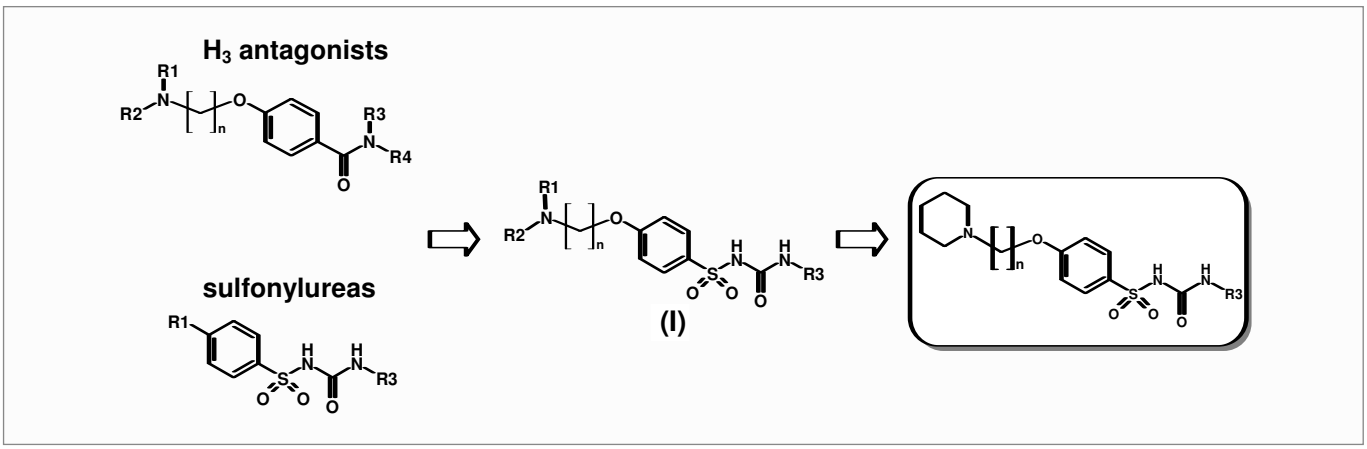
The histaminergic system represents a new biological target for treating obesity. H_3 receptor antagonists have been reported to be involved in the energy balance since high levels of hypothalamic histamine induces weight loss. Selective non-imidazole H3 receptor antagonists, as A-331440, reduce body weight in diet-induced obese mice[1].

The American Diabetes Association has suggested that sulfonylurea system is clinically significant for its antidiabetic properties. Glimepiride is the first third-generation sulfonylurea approved as oral antidiabetic drug[2].

Using this approach, we aim to identify new compounds for the treatment and prevention of diabesity. As part of the antiobesity research project, we have designed a novel structure **I** and synthesized new p-piperidinylalkylphenoxysulfonylurea derivatives in order to evaluate their dual H_3 antagonism and antidiabetic activities as well as their hERG affinity.

N. Castrillo was awarded a grant supported by the "Gobierno de Navarra". S. Galiano is grateful to the project given to the same supporter.

1. Hancock, A.A. *Curr. Opin. Investig.* **(2003)**, *4*, 1190-1197.
2. 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.98

NOVEL *p*-PYRROLIDINYLALKYL-PHENOXSULFONYLUREA DERIVATIVES WITH DUAL H_3 RECEPTOR ANTAGONISM AND ANTIDIABETIC ACTIVITIES

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

Unidad de Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain.Email: sgaliano@.unav.es

Obesity is one of today's major health problems throughout the world affecting 400 millions of people. Its alarming rising prevalence and the health risks associated with this disease warrant obesity as one of the most challenging therapeutic areas in the 21st century.

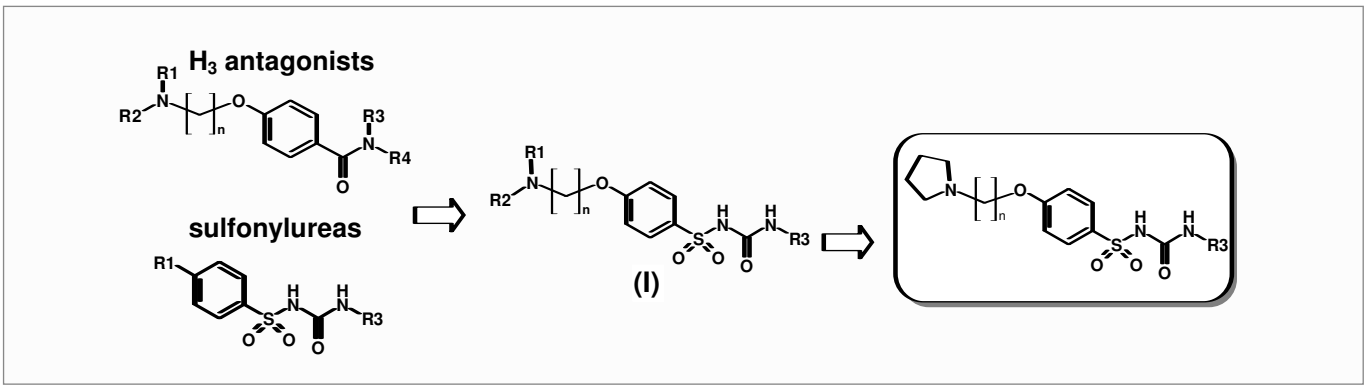
Closely linked to obesity is the rapid widespread of type 2 diabetes emerging in a new epidemic: the diabesity. The lack of efficacy drugs for this new disease makes this field one of the most attractive target.

One approach towards new antiobesity agents with antidiabetic properties is the synthesis of dual compounds combining the H_3 receptor antagonism and the sulfonylurea system. The histamine H_3 receptor is an important G protein-coupled receptor drug target that plays a role in obesity. Recently, it has been described the antiobesity effects of potent and selective non- imidazole H receptor antagonists which reduce weight gain in diet-induced obese mice[1]. Furthermore, the sulfonylurea system has been reported to show very interesting antidiabetic properties. Glibenclamide (INN), second generation sulfonylurea, is an oral anti-diabetic drug[2].

Based on the design of structure **I** and as a continuation of our research programm[3], novel series of p-pirrolidinylalkylphenoxysulfonylureas derivatives have been synthesized and evaluated for their dual activity as H_3 receptor antagonist and antidiabetic activity as well as their hERG affinity.

We wish to thank the Gobierno de Navarra for the project given to S.Galiano and the grant given to N. Castrillo.

1. Hancock, A.A., Bennani, Y.L., Bush, E.N. ; Esbenshade, T.A.; Faghih, R.; Fox, G.B.; Jacobson, P.; Knourek-Segel, V.; Krueger, K.M.; Nuss, M.E.; Pan, J.B.; Shapiro, R.; Witte, D.G.; Yao, B.B. *Eur. J. Pharmacol.* **(2004)**, *487*, 183–197.
2. American Diabetes Association. Diabetes Care **(2004)**, *27(1)*: S15-S34
3. Silvia Galiano, Javier Ceras, Nuria Cirauqui, Silvia Pérez, Laura Juanenea, Gildardo Rivera, Ignacio Aldana, Antonio Monge. *Bioorg. Med, Chem.* **(2007)**, *15*, 3896-3911



THEORETICAL STUDY OF A MULTI-STAGE HIV-1 REVERSE TRANSCRIPTASE

Natalya Toreeva (1), Maria Grishina (2), Vladimir Potemkin(2)

(1) Chelyabinsk State Universiti, (2) Chelyabinsk State Medical Academy

The multi-stage process of biological action has been studied using dataset of 2-(2,6-digalofenyl)-3-(substituted heteroaryl)-thiazolidin-4-one derivatives[1] HIV-1 reverse transcriptase inhibitors. The simulation of "pseudo-receptor – ligand" complexes with the CiS[2] algorithm shows, that the configuration isomerization takes place in the process of interaction with receptor. It has been shown that the configuration isomerization occurs through the deprotonation stage. Indeed, the compounds are CH- acids (the pK_a varies in the range 2.5÷3.2)[3]. The lower pK_a (and accordingly the free energy of the deprotonation) provides the greater biological activity. The figure shows the deprotonation process yielding in inversion of chiral center accompanying by proton addition to the opposite side of the center. The process is promoted by protolytic properties of medium and enzyme.

The CiS algorithm determined the conformers which are characterized by the largest probability of interaction with the receptor and the conformers which has the largest bioactivity.

It has been shown, that the most probable conformers taking part in the process of the intercalation into receptor cavity have S-configuration. Relationship between the bioactivity and the characteristics of their interaction with model receptor has the cross validation quality equal to 0.63. The most active conformers participating in receptor inhibition have R-configuration. Relationship between the bioactivity and the characteristics of their interaction with model receptor has the cross validation quality equal to 0.42.

The account of these two stages allows to obtain the equation of 0.99 cross validation quality. It has been determined the influence of each stage on whole process of the biological action. The contribution of the complex formation stage is 68%, while the interaction stage gives the increment 32%.

$pIC_{50}=a*BA_S+b*BA_R$, where BA_S and BA_R are bioactivity R- and S-configuration respectively.

The work is fulfilled under financial support of SKIF-GRID Supercomputer Initiative and RFBR (grants 07-03-96041, 07-04-96053).

1. Rawal R.K., Kumar A., Siddiqi M.I., Katti S.B. Molecular docking studies on 4-thiazolidinones as HIV-1 RT ingibitors// J Mol Model – 2007 – 13:155-161
2. Potemkin V., Grishina M. Principles for 3D/4D QSAR classification of drugs// Drug Discovery Today.- 2008.- V. 13.- No. 21/22.- P. 952 – 959.
3. Potemkin V.A., Pogrebnoy A.A., Grishina M.A. Technique for Energy Decomposition in the Study of "Receptor-Ligand" Complexes// J. Chem. Inf. Model., 2009, DOI: 10.1021/ci800405n.

NOVEL-GENERATION KINASE INHIBITORS: DEEP POCKET BINDERS BY FRAGMENT-BASED DESIGN

Gerhard Müller, Peter Sennhenn

Proteros fragments GmbH, Fraunhoferstr. 20, D-82152 Martinsried, Germany

Fragment-Based Lead Discovery has matured into an established and feasible alternative to e.g. high-throughput screening of traditional corporate libraries within the pharmaceutical industry, thus focusing the interest of medicinal chemists onto smaller fragment hits with high ligand efficiency. Fragment hits are most effectively evolved to traditional lead structures if high-resolution structure data become available within the course of the corresponding optimization campaigns.

This presentation highlights a novel kinase inhibitor design approach, notably the "retro-design concept" It takes advantage of a fragment-based lead assembly strategy that intentionally avoids the adenine binding region, which is in complete contrast to the majority of the traditionally pursued approaches towards kinase inhibitors. The main advantage of the retro-design strategy is that distinct binding kinetic attributes can be pre-engineered into the resulting lead structures on a fragment basis, thus yielding in kinase inhibitors with slow dissociation rates (k_{off}), i.e. long residence times on the respective target kinases. It has been systematically shown that the desired binding kinetic properties translate into candidates with high in-vivo efficacy. This presentation will introduce the underlying enzymology the design and application of a tailor-made fragment library, the medicinal chemistry routes for fragment confirmation and fragment evolution, as well as fragment-directed protein crystallography as one of the main enabling technologies for successful drug design.

NEW INHIBITORS OF CHOLINE KINASE AS ANTIPROLIFERATIVE AGENTS

Santiago Schiafino (1), Pablo Ríos (2), José M Jiménez López (2), María Paz Carrasco (2), Miguel A. Gallo (1), Carmen Marco (2), Antonio Espinosa (1), Antonio Entrena (1)

(1) Dpto. Química Farmacéutica y Orgánica, Facultad de Farmacia, Univ. Granada, 18071 Granada (Spain).
(2) Dpto. Bioquímica y Biología Molecular I, Fac. Ciencias, Univ. Granada, 18071 Granada (Spain).

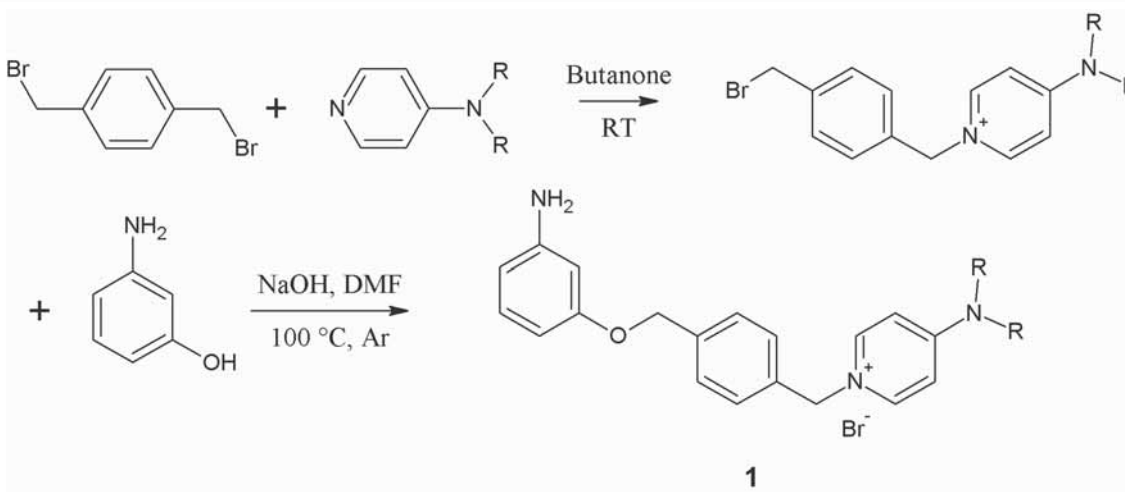
We have previously reported more than 250 symmetrical biscationic compounds, based on structural modifications of hemicholinium-3 (HC-3), as choline kinase inhibitors showing good antiproliferative activity[1].

The reported crystal structures of both *Caenorhabditis elegans*[2] and human Chok[3], the last one complexed with ADP or PCho, provides new opportunities to rationalize the knowledge regarding the inhibition of this enzyme.

Docking studies performed on a homology model of human Chok[4], based on the X-ray crystallographic structure of *C. elegans,* indicate that inhibitors can bind the binding sites of both substrates (ATP and Choline) simultaneously. 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 and appropriated spacer, can be designed.

In this communication the design, synthesis and biological evaluation of a series of 1-{4-[3-(aminophenoxy)methyl]benzyl}-4-(1-pyrrolidinyl)pyridinium bromides, represented by the general formula **1,** are described.

1. J. M. Campos, R. M. Sánchez-Martín, A. Conejo-García, A. Entrena, M. A. Gallo, A. Espinosa, Curr. Med. Chem. **2006**, *13*, 1231-1248.
2. D. Peisach, P. Gee, C. Kent, Z. Xu, Structure. **2003**, *11*, 703– 713.4.
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.



1

SYNTHESIS AND ANTITUMOR ACTIVITY OF NEW PYRROLO[2,1-c][1,4]BENZOXAZINES

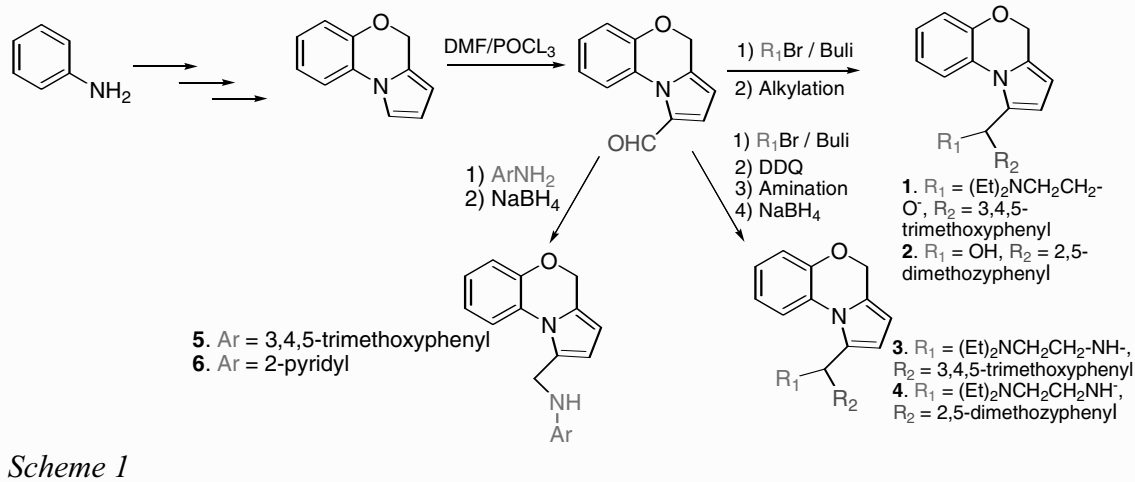
Giovanni Casula (1), Youssef Harrak (1), Pierre Renard (2), Daniel Henry Caignard (2), M. Dolors Pujol (1)

(1) Laboratori de Química Farmacèutica. Facultat de Farmàcia. Universitat de Barcelona. Av. Diagonal 643. 08028-Barcelona (Spain).
E-mail: mdpujol@ub.edu
(2) Les Laboratoires Servier. 3, Rue de la Republique. 92150-Suresnes (France).

The pharmacological and clinical profiles of the pyrrolo-benzoxazines have drawn the attention for medicinal chemists to develop a variety of derivatives in an effort to obtain better compounds such as anti-vascular, analgesics, SNC depressants and other agents. Nevertheless this heterocycle system is not described forming a part of compounds with antitumoral activity. We have tried the synthesis of compounds with the nucleus of pyrrolo-benzoxazine and with fragments like 3,4,5-trimethoxyphenyl, chains of dialkylaminoethyl in order to know his antitumoral properties. The pyrrolo[2,1-c][1,4]benzoxazine system was prepared by condensation of *2*-fluoroaniline with *2*,5-dimethoxytetrahydrofuran in glacial acetic acid, followed by the introduction of a formyl group at C-1 of the resulting condensed heterocyclic system. The formation of the benzoxazine ring involves an intramolecular nucleophilic displacement of the fluoride atom. The new formylation under Vilsmeier-Haak conditions and the treatment of the aldehyde with the appropriate lithium derivative gave the carbinol in acceptable yield. The *O*-substituted compounds were synthesized from the corresponding carbinol by alkylation. A series of diamines were prepared from the diarylketone by reductive amination (*Scheme 1*).

The synthesized compounds were evaluated for their antiproliferative activities against several human cancer cell lines. Compounds with trimethoxyphenyl group demonstrated strong cytotoxicity with mean IC50 values of 50-112 nM. Three different series of pyrrolo-benzoxazines were prepared and evaluated for anticancer activity. The synthesis and structure–activity relationships should be reported.

1. I. Sánchez, M. D. Pujol. *Tetrahedron* **1999**, *55*, 5593-5598.
2. X. Zhang, F. K. Habib, M. Ross, U. Burger, A. Lewenstein, K. Rose, J. C. Jaton. *J. Med. Chem*. **1995**, *38*, 735-741.



Scheme 1

NEW GLYCERYL DERIVATIVES OF RESORCINOL-ANANDAMIDE "HYBRIDS" AS CANNABINOID RECEPTOR LIGANDS

Antonella Brizzi (1), Alessia Ligrsti (2), Vittorio Brizzi (1), Federico Corelli (1), Vincenzo Di Marzo (2)

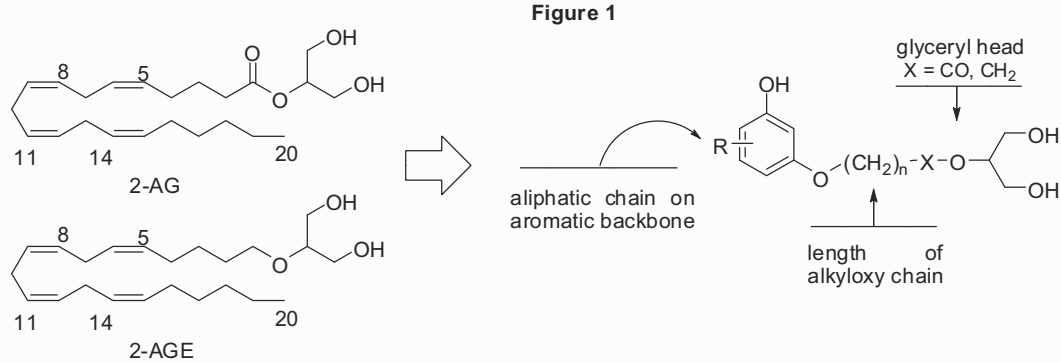
(1) Dipartimento Farmaco Chimico Tecnologico Università degli Studi di Siena Via A. De Gasperi 2 I-53100 Siena Italy,
(2) Istituto di Chimica Biomolecolare Consiglio Nazionale delle Ricerche Via Campi Flegrei 34 Comprensorio Olivetti I-80078 Pozzuoli Napoli Italy

Emerging evidence implicates endocannabinoid system in a wide variety of behaviours and diseases, promoting the development of new therapeutics that target the cannabinoid receptors or the enzymes involved in the synthesis, transport and degradation of endocannabinoids. AEA, the N-arachidonoyl-ethanolamine (anandamide), and 2-AG (figure 1), the 2-arachidonoyl-glycerol, are the two main and well studied endogenous ligands[1] for the CB1 and CB2 receptors, the two recognition sites for marijuana psychoactive principle (-)-Δ^9-tetrahydrocannabinol (THC). 2-AGE (figure 1)[2], the 2-glyceryl ether of arachidonyl alcohol (noladin ether), is the latest polyunsaturated fatty acid derivative to be proposed as the third endogenous ligand, having much higher affinity for CB1 than for CB2 receptors.

In previous studies[3], we have designed a structural model related to both THC and AEA, containing a rigid aromatic backbone and a flexible chain (figure 1), carrying an amidic head and on the basis of this pharmacophore model we have synthesized potent cannabinoid receptor ligands. With the aim to better investigate structure-activity relationships inside this new class of compounds and to explore their CB binding properties, we have introduced a glyceryl head in our pharmacophore model, designing and synthesizing both esters and ethers of glycerol (figure 1).

1. Devane, W. A. et al. *Science*, **1992**, *258*, 1946.
2. (a) Sugiura, T. et al. *Biochem. Biophys. Res. Commun.*, **1995**, *215*, 89. (b) Mechoulam, R. et al. *Biochem. Pharmacol.*, **1995**, *50*, 83.
3. (a) Brizzi, A. et al. *Bioorg. Med. Chem.*, **2007**, *15*, 5406-5416. (b) Brizzi, A. et al. *J. Med. Chem.*, **2009**, *52*, 2506-2514.

Figure 1



POSTER COMMUNICATIONS – PC.104

ANTIRETROVIRAL STRATEGY USING MULTI-TARGET AGENTS

Stefania Ferro (1), Laura De Luca (1), Sara De Grazia (1), Rosaria Gitto (1), Anna Maria Monforte (1), Maria Letizia Barreca (2), Frauke Christ (3) and Alba Chimirri (1)

(1) Dip. Farmaco-Chimico, Università di Messina, Viale Annunziata, I-98168, Italy; (2) Dip. Chimica e Tecn. del Farmaco, Università di Perugia, Via del Liceo 1, I-06123, Italy; (3) Div. of Molecular Medicine, Katholieke Univ. and IRC KULAK, Kapucijnenvoer 33, B-3000, Leuven, Belgium.

Despite the recent licensure of several new antiretroviral compounds, there is still a need to develop additional anti-HIV agents. In fact problems with tolerability, cross-resistance and pharmacokinetic as well as pharmacodynamic interactions still represent important obstacles to life-long control of HIV type-1 replication. Recently, the cellular protein lens epithelium-derived growth factor LEDGF/p75 has been identified as an important co-factor of HIV-1 integration and replication thus representing a very new actractive target for the development of anti-HIV drugs. A structure-based pharmacophore model for potential small-molecule inhibitors of protein- protein interactions (PPI) between HIV-1 Integrase (IN) and LEDGF/p75 was developed and used for virtual screening of our in-house chemical database CHIME. The search led to the identification of an interesting hit compound used as a starting point for rational design and synthesis of some derivatives[1].

Our studies resulted in the discovery of small molecules able to prevent PPIs between IN and cofactor LEDGF/p75 hopefully blocking HIV replication, through a different mechanism of action than all other drugs so far used in therapy.

Furthermore some derivatives were active also in the IN-inhibition test thus suggesting a possible *dual mode of action*.

This could be particularly important considering the requirement for combined therapy targeting the different steps of the HIV life cycle to stop the AIDS infection.

Research supported by THINC project (HEALTH-F3-2008-201032)

1. De Luca L, Barreca ML, Ferro S, Christ F, Iraci N, Gitto R, Monforte AM, Debyser Z, Chimirri A, *Chem Med Chem* **2009**, 000.

SYNTHESIS AND IMAO EVALUATION OF BROMO DERIVATIVE 3-PHENYLCOUMARINS

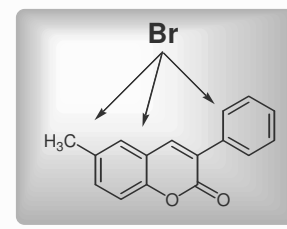
Maria Joao Matos (1,2), Dolores Viña (3), Carmen Picciau (2), Francisco Orallo (3), Fernanda Borges (4), Lourdes Santana (1) and Eugenio Uriarte (1)

(1) Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain
(2) Dipartimento Farmaco Chimico Tecnologico, Facoltà di Farmacia, Università di Cagliari, 09124, Cagliari, Italy
(3) Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain
(4) Departamento de Química, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

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 nucleus. This could be easily possible by two principal general methods: by a phenylation of a coumarin or by the construction of the coumarin's nucleus with the new ring already included on it. In the present work we describe the second method, by a classical Perkin reaction, perhaps the most direct and general method known for preparing the desired 3-phenylcoumarins[1]. A number of natural products and synthetic analogues featuring coumarin structural motif display wide-ranging biological properties[2].

In this work we developed synthetic methodologies that leads us to new series of 3- phenylcoumarins[1] with halogen atoms either in the phenyl ring in 3 position, in the benzenic ring of the coumarin or in an alkylic lateral chain. These molecules could have other substitutions – methyl and/or methoxy groups– in different positions. The introduction of halogens in the 3-phenylcoumarin´s structure makes them potential interesting pharmacological compounds, and also versatile precursors to a lot of substituted derivative analogues.

These bromo 3-phenylcoumarin´s derivatives proved to be very interesting molecules in iMAO assays. Some of them have IC_{50} in the picomolar range, and are selective to the isoform MAO- B. The present modifications, which we are studying more deeply, can direct these synthesized coumarins to improve the pharmacologic profile in the Parkinson's disease.



1. Matos, M.J., Viña, D., Quezada, E., Picciau, C., Delogu, G., Orallo, F., Santana, L., Uriarte, E. *Bioorganic & Medicinal Chemistry Letters*, **2009**, *19*, 3268–3270.
2. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Current Medicinal Chemistry,* **2005**, *12*, 887-916.

SYNTHESIS, EVALUATION, AND MOLECULAR MODELLING OF NEW OXOISOAPORPHINE DERIVATIVES: EXCELLENTS MAO-A SELECTIVE INHIBITORS

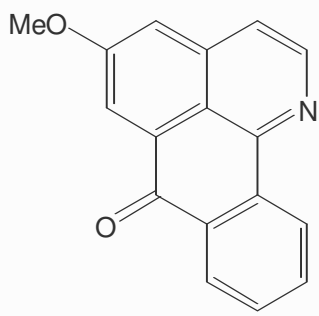
Lourdes Santana (1), Eduardo Sobarzo-Sánchez (2), Matilde Yáñez (3), Francisco Orallo (3), Eugenio Uriarte (1), Francesco Ortuso (4), Stefano Alcaro (4)

(1) Department of Organic Chemistry, (2) Department of Pharmacy and Pharmaceutical Technology and, (3) Department of Pharmacology, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain, (4) Dipartimento di Scienze Farmacobiologiche, Università di Catanzaro "Magna Graecia", "Complesso Ninì Barbieri", 88021 Roccelletta di Borgia (CZ), Italy.

The oxoisoaporphines (7*H*-dibenzo[*de,h*]quinolin-7-one) they are a small and unknown group of isoquinoline alkaloids that have been extracted of natural sources, specifically from roots of the *Menispermum dauricum* DC. Such compounds, isomers of the oxoaporphines (7*H*-dibenzo[*de,g*]quinolin-7-one), they have presented some pharmacological properties as anticarcinogenic[1] and with an interesting behaviour as selective acetylcholinesterase inhibitors[2]. These derivatives, depending of the substitution and aromatization degree on the aporphine skeleton, they have been evaluated *in vitro* as excellent and selective inhibitors of the human monoamine oxidase A (hMAO-A), in nanomolar concentration ranges.

In order to understand the affinity of these compounds on the active center of the hMAO-A receptor, it has been carried out molecular modelling of the three more active derivatives, being 5-methoxy-7*H*-dibenzo[*de,h*]quinolin-7-one (**1**) the most potent, with a IC_{50} value of 0.84 nM. Thus, this compound shows in the receptor model (Figure 1) an important interaction by hydrogen bond between the methoxyl and carbonyl groups of **1** and the near residuals amino acids. Therefore, through the molecular modelling, it was possible to conclude that the active center of the MAO-B receptor has an important steric hindrance that would explain the low affinity regarding **1**.

1. Tang, H.; Wang, X. -D.; Wei, Y. -B.; Huang, S. -L.; Huang, Zhi-Shu; Tan, J. –H.; An, L. –K.; Wu, J. –Y.; Sun- Chi Chan, A.; Gu, L. –Q. *Eur. J. Med. Chem.* **2008**, *43*(5), 973.
2. Tang, H.; Ning, F. –X.; Wei, Y. –B.; Huang, S. –L.; Huang, Z. –S.; Chan, A. S. –C.; Gu, L. -Q. *Bioorg. Med. Chem. Lett.* **2007**, *17*(13), 3765.



(1)

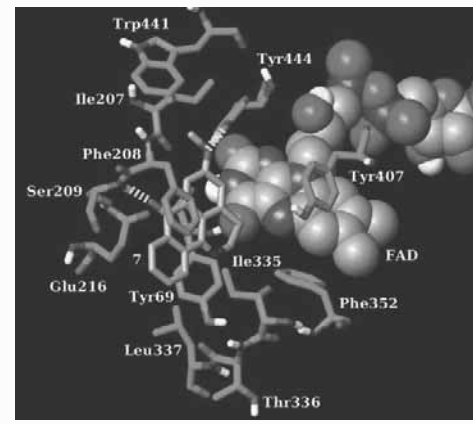


FIGURE 1

ADMET FILTERS FOR DRUGS METABOLISM PREDICTION

Nadezda Ivshina (1), Maria Grishina (2), Vladimir Potemkin (2) and Elena Afonkina (3)

(1) Institute of Organic Synthesis, Urals Branch of the Russian Academy of Sciences,
(2) Chelyabinsk State Medical Academy, (3) Chelyabinsk State University

Discriminant analysis for drug metabolism prediction has been carried out for the 1A1, 1A2, 2A6, 3A3, 3A4, 3A5, 3A7, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1 isoforms of cytochrom P450. It has been shown that metabolism defining characteristics are various energetic, geometrical and electronic parameters at the cytochrom P450 isoforms. High molecular weights (Mr=200-448 g/mol) are the characteristic features for substrates of the 3A4 isoform. Structures which are non-metabolising at the 3A4 isoforms have less molecular weights (Mr=131-294 g/mol). Besides the size and the form of molecules is important. It has been shown, that the substrates of the 3A4 isoform should have the minimal size along the principal inertial axis, less than 4.68-8.00 Å. The similar geometry provides the most effective contacts of atoms of the substrates with 3A4 isoform atoms, resulting to an opportunity of their metabolism. The 1A2 isoform substrates should have greater energy of the highest occupied molecular orbital in a contrast with non-metabolising molecules. Discriminant models have been created for drugs for a metabolism prediction on the basis of the received energetic, geometrical and electronic characteristics of structures. The part of these compounds are substrates of P450 cytochrom isoforms. ADMET filters have been used for metabolism prediction of drugs from databases TOSLAB and InterBioScreen. Theoretical study of electronic structures, conformational and tautomeric conditions of 3A4, 2C9 and 2D6 isoform of P450 cytochrom have been studied.

The work is fulfilled under financial support of SKIF-GRID Supercomputer Initiative and RFBR (grants 07-03-96041, 07-04-96053).

DOCKING STUDIES TO IDENTIFY THE BINDING MODE OF PROPAFENONES TO P-GLYCOPROTEIN

Freya Klepsch (1), Peter Chiba (2) and Gerhard F. Ecker (1)

(1) University of Vienna, Department of Medicinal Chemistry, Althanstraße 14, 1090, Vienna, Austria
(2) Medical University of Vienna, Institute of Medicinal Chemistry, Währinger Straße 10, 1090, Vienna, Austria

As a member of the transmembrane ABC transporter family, P-glycoprotein (P-gp, ABCB1, MDR1) is responsible for exporting a large number of xenotoxins out of the cell. When the protein is over expressed its high polyspecificity[1] can lead to the extrusion of structurally and functionally diverse therapeutic drugs. In cancer therapy this phenomenon is called multidrug resistance (MDR) and represents one major reason for the failure of anti-cancer chemotherapy. Propafenones constitute a substance class that is able to block this transmembrane transporter. Although the binding pocket of propafenones is still not precisely defined biological studies could restrict it to certain areas at the transmembrane domains of P-gp[2]. The protein is a pseudo homo-dimeric structure where each monomer consists of a transmembrane domain (TMD) and a nucleotide binding domain (NBD). The putative binding sites are proposed to be located in the TMD region of the protein.

In March 2009 a high resolution structure of ABCB1 was published[3] (PDB code: 3G5U, resolution: 3.80 Å, source: mouse) which, for the first time, enables to complement our numerous ligand based studies with target based approaches. Therefore docking studies were performed using the GOLD software package. Consensus scoring utilizing 11 different scoring functions (scoring functions implemented in GOLD, MOE, GLIDE, as well as those from XScore) largely reduced the number of potential binding poses to be considered. To group the remaining poses hierarchical RMSD clustering as well as clustering according to the protein ligand interaction fingerprint (PLIF in MOE) was performed. This finally led to a small number of binding mode hypotheses of propafenone-type inhibitors of P-glycoprotein.

This work was supported by the Austrian Science Fund, grant F03502

1. Loo TW, Clarke DM. Arch Biochem Biophys. 2008; 476:51-64. doi:10.1016/j.abb.2008.02.025.
2. Pleban K et al. Mol Pharmacol. 2005; 67:365-374. doi:10.1124/mol.104.006973.
3. Aller SG et al. Science 2009. doi:10.1126/science.1168750.

SYNTHESIS, EVALUATION AND SAR DATA OF A LIBRARY OF HISTONE DEACETYLASE INHIBITORS

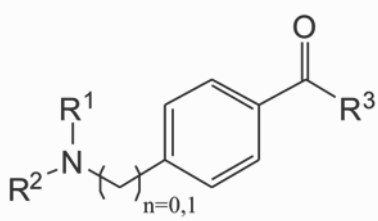
Cristina Roca (1), Marian Martínez-Balbás (2), Franck Dequiedt (3), Àngel Messeguer (1), and Jordi Bujons (1)

(1) Departments of Biological Chemistry and Molecular Modeling and of Chemical and Biomolecular Nanotechnology, Institute of Advanced Chemistry of Catalonia, Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain. (2) Institute of Molecular Biology of Barcelona, Consejo Superior de Investigaciones Cientificas, Parque Científico de Barcelona, Josep Samitier 1-5, 08028 Barcelona, Spain. (3) Departament of Cellular and Molecular Biology, FUSAGx, 13, Av Marechal Juin, bat92 B-5030 Gembloux, Belgium

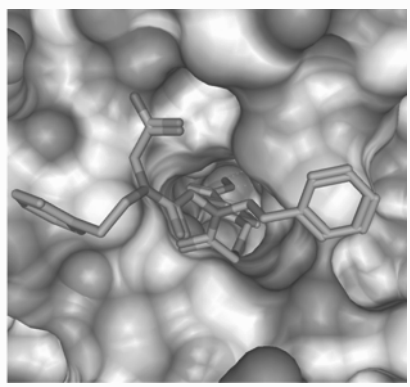
Modification of the acetylation state of the ε-amino group of specific lysine residues of histones plays a crucial role in the regulation of the transcriptional process and, thus, it is central to cell proliferation, differentiation and apoptosis[1]. Two families of enzymes, histone acetyl transferases (HATs) and histone deacetylases (HDACs), are involved in the regulation of this equilibrium. Recent studies show that inhibition of HDACs causes arrest of cell growth and induces cell differentiation. As a consequence, there is an increasing interest on the development of new HDAC inhibitors as potential anticancer drugs.

Using recently reported structural data on different isoforms of HDACs[2,3], we have conducted docking studies to design a library of potential inhibitor compounds based on scaffold **1**. These compounds have been synthesised using solid and liquid phase methodologies. Their antiHDAC activities have been evaluated *in vitro* against nuclear HeLa extracts and purified HDAC1, HDAC6 and HDAC8 isoforms to determine their selectivity. The effects on proliferation of transformed and cancer cells lines were also determined. From these studies, hits with submicromolar potencies and isoform selectivities have been identified.

1. Pennisi, E. Opening the way to gene activity. Science 1997;275:155-157.
2. Somoza, J.R. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. Structure 2004;12:1325-1334.
3. Wang, D-F., Helquist, P., Wiech, N.L., Wiest, O. Toward selective histone deacetylase inhibitor design: Homology modeling, docking studies, and molecular dynamics simulations of human class I histone deacetylases. J. Med. Chem. 2005; 48:6936-6947.



1



POSTER COMMUNICATIONS – PC.110

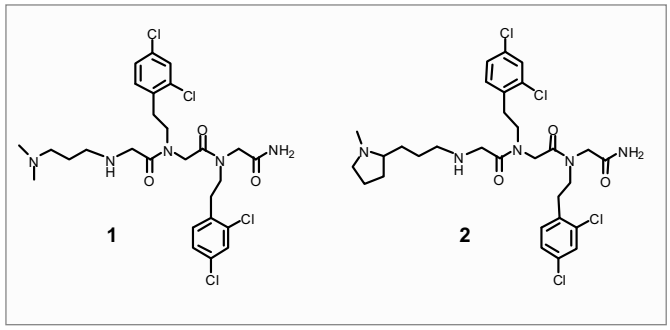
DISCOVERY OF A NEW CLASS OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 (TRPV1) ANTAGONIST: DESIGN, SYNTHESIS AND SAR DATA

Miquel Vidal (1), Asia Fernández Carvajal (2), Jordi Bujons (1), Antonio Ferrer-Montiel (2), and Àngel Messeguer (1)

(1) Instituto de Química Avanzada de Cataluña, Consejo Superior de Investigaciones Científicas, Jordi Girona 18-24, 08034 Barcelona, Spain. (2) Centro de Biología Molecular y Celular, Universidad Miquel Hernández, 03202 Alicante, Spain.

TRPV1 is a nonselective cation channel with high permeability to calcium, belonging to the superfamiliy of TRP channels. It is activated by exogenous agonist, such as capsaicin, as well as by physical and chemical noxius stimuli, such as heat or acids. In addition, it is known that TRPV1 is activated by different inflammatory mediators, like bradikinin and lipoxygenase products, and that it is related to several pathological conditions, such as inflammatory, diabetic and cancer pain. All together, the available data suggest that TRPV1 is an integrator of multiple pain-producing stimuli and, consequently, it is considered a biological target of interest for the discovery of novel analgesics.

Preliminary results from our group on the screening of a combinatorial library of trimers of N- alkylglycine (peptoids) lead to the identification of two noncompetitive TRPV1 antagonists (**1** and **2**) with activities in the low μM range[1]. More recently, different groups have identified compounds of peptoid or dipeptide nature which show similar blockade capacity[1-4]. However most of these compounds contain relatively flexible scaffolds that might complicate their consideration as lead compounds for further development. In our pursuit to discover new chemical classes of TRPV1 antagonists that can enter in further stages of optimization and development, we have evolved the structures of **1** and **2** into new cyclic-scaffold based compounds. Among them several blockers with submicromolar activities have been identified. Analysis of the SAR data has been used to build a predictive model for the activity of the compounds.



1. C. García-Martínez, M. Humet, R. Planells-Cases, A. Gomis, M. Caprini, F. Viana, E. De La Pena, F. Sánchez-Baeza, T. Carbonell, C. De Felipe, E. Pérez-Payá, C. Belmonte, A. Messeguer and A. Ferrer-Montiel, *Proc Natl Acad Sci U S A* **2002**, *99*, 2374-2379.
2. M. A. Bonache, C. García-Martínez, L. García de Diego, C. Carreno, M. J. Pérez de Vega, M. T. García-López, A. Ferrer-Montiel and R. González-Muniz, *ChemMedChem* **2006**, *1*, 429-438.
3. C. García-Martínez, A. Fernández-Carvajal, B. Valenzuela, A. Gomis, W. Van Den Nest, S. Ferroni, C. Carreno, C. Belmonte and A. Ferrer-Montiel, *J Pain* **2006**, *7*, 735-746.
4. M. Quintanar-Audelo, A. Fernández-Carvajal, W. Van Den Nest, C. Carreno, A. Ferrer-Montiel and F. Albericio, *J Med Chem* **2007**, *50*, 6133-6143.

CHEMOENZYMATIC SYNTHESIS OF STRUCTURALLY DIVERSE POLYHYDROXYLATED PYRROLIDINE DERIVATIVES AND THEIR GLYCOSIDASE INHIBITORY PROPERTIES

Jordi Calveras (1), Meritxell Egido-Gabás (1), Livia Gómez (1), Josefina Casas (1), Teodor Parella (2), Jesús Joglar (1), Jordi Bujons (1), and Pere Clapés (1)

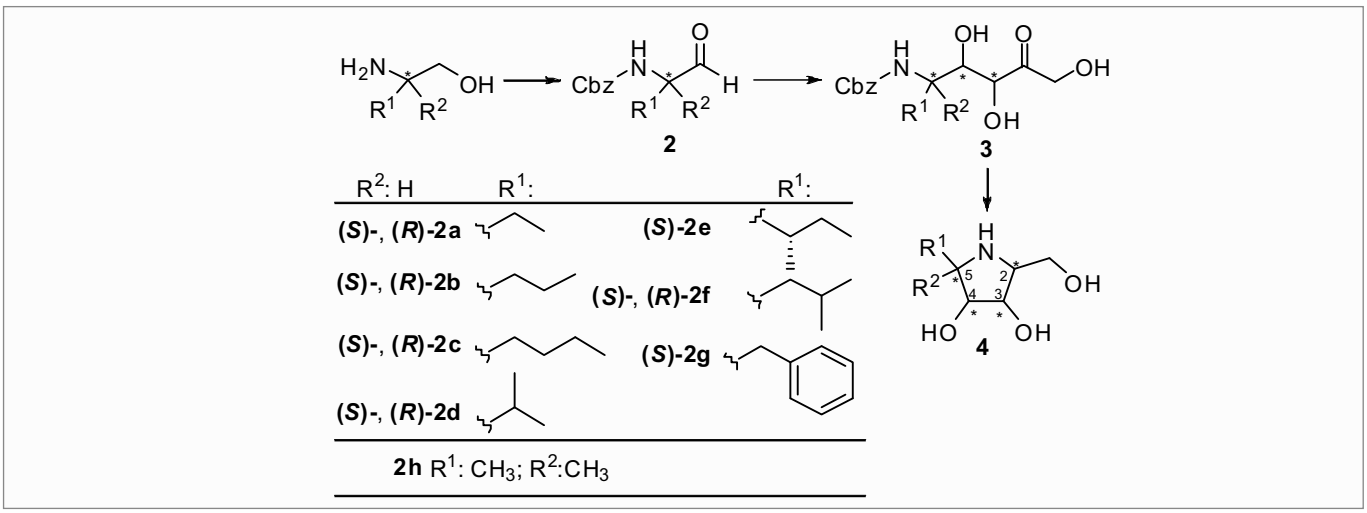
(1) Departments of Biological Chemistry and Molecular Modeling and of Biomedicinal Chemistry, Institute of Advanced Chemistry of Catalonia, Consejo Superior de Investigaciones Científicas, Jordi Girona 18, 08034 Barcelona, Spain. (2) Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, Bellaterra, Spain.

Iminocyclitols are naturally occurring polyhydroxylated alkaloid mimics of glycosides. Many of them are potent inhibitors of glycosidases and glycosyltransferases. Glycoprocessing enzymes are interesting therapeutic targets as they are responsible for the metabolism of complex carbohydrate structures involved in many biochemical recognition processes. Glycosilation disorders affect to cell-cell communication, cell-matrix interaction and immunological response and, therefore, they are related to diseases such as cancer, viral infections including HIV, hepatitis B and C, Gaucher and Fabry diseases, cystic fibrosis, carbohydrate deficiency syndrome (CDS), rheumatoid arthritis (chronic polyarthrithis) and IgA nephropathy (IgAN) among others.

We have recently described a novel chemo-enzymatic strategy to synthesize iminocyclitols of the piperidine and pyrrolidine type[1,2]. The key step of the strategy devised was the aldol addition of dihydroxyacetone phosphate (DHAP) to *N*-benzyloxycarbonyl-aminoaldehydes catalyzed by DHAP aldolases. Therefore, it was regarded as both promising and significant to investigate the synthetic possibilities of the aforementioned strategy towards the preparation of novel pyrrolidine derivatives with potential new inhibitory properties against different glycosidases.

The polyhydroxylated pyrrolidines generated were tested as inhibitors against seven glycosidases. Among them, good inhibitors of α-L-fucosidase (IC_{50} = 1-20 μM), moderate of α-L- rhamnosidase (IC_{50} = 7-150 μM) and weak of α-D-mannosidase (IC_{50} = 80-400 μM) were identified. The apparent inhibition constants (K_i) were calculated for the most relevant inhibitors and docking studies were performed to understand both the mode of interaction with the glycosidases and their binding capacity[3].

1. L. Espelt, J. Bujons, T. Parella, J. Calveras, J. Joglar, A. Delgado and P. Clapés, *Chem. Eur. J.* **2005**, *11*, 1392-1401.
2. L. Espelt, T. Parella, J. Bujons, C. Solans, J. Joglar, A. Delgado and P. Clapés, *Chem. Eur. J.* **2003**, *9*, 4887-4899.
3. J. Calveras, M. Egido-Gabás, L. Gómez, J. Casas, T. Parella, J. Joglar, J. Bujons, and P. Clapés, *Chem. Eur. J.* **2009**, in press.



POSTER COMMUNICATIONS – PC.112

GRIND, CoMFA AND CoMSIA STUDIES OF BENZOPYRAN TYPE INHIBITORS OF P-GLYCOPROTEIN

Ishrat Jabeen (1), Penpun Wetwitayaklung (1), Peter Chiba (2) and Gerhard F. Ecker (1)

(1) University of Vienna, Department of Medicinal Chemistry, Althanstraße 14, 1090, Vienna, Austria
(2) Medical University of Vienna, Institute of Medicinal Chemistry Wahringerstraße 10, 1090, Vienna, Austria

The ATP-binding cassette efflux transporter P-glycoprotein (P-gp) is a large membrane-bound protein initially noted to be present in certain malignant cells associated with the multidrug resistance (MDR) phenomenon[1]. A better understanding of the structural requirements of inhibitors of P-gp will aid in the prediction of the receptor's binding sites. Towards this goal, different 3D QSAR techniques were used to generate predictive models for a data set of benzopyran analogues.

Benzopyrano[3,4-b][1,4]oxazines were synthesized and pharmacologically tested for their ability to inhibit P-glycoprotein mediated daunomycin efflux in multidrug resistant CCRF-CEM vcr 1000 cells[2]. GRID- Independent molecular descriptors (GRIND), CoMFA and CoMSIA analysis was performed to study the main structural determinats for drug P-gp interaction. GRIND[3,4] studies show that two H-bond acceptors at a distance of 4.80-5.20 Å are beneficial for high biological activity. Shape descriptors also play an important role to the activity. GRIND studies on benzopyran analogues were compared with those on propafenone type inhibitors of P-gp. In both cases two H-bond donors in the molecules at a distance of 6.0-6.80 Å correlate with a decrease of activity. Analysis of the CoMFA and CoMSIA three-dimensional contour plots revealed a consistent picture of the structural features that are responsible for the observed variations in the activity. Steric contour maps show that a bulky substituent is preferred at the ester moiety as well as at the beta carbon of the extended chain of the analogues. Electrostatic contour maps suggest that the substitution which increases the positive charge on the amino nitrogen increases the activity. Configuration of the chiral centers near the H-bond donor regions in the molecules does not affect the biological activity, as the fields are located both above and belowe the plane of the benzopyran. In contrast, chirality at the amino acid part was shown to be of some influence, as the H-bond acceptor regions can be covered by most of the compounds only if L-configuration with the nitrogen out of the plane is present.

This work was supported by the Austrian Science Fund, grant F03502 and Higher Education Commission Pakistan

1. Wandell, C.; Kim, R.B.; Kajiji, S.; Guengerich, F.P.; Wilkinson, G.R.; and Wood, A.J.J. *Cancer* **1999,** 59, 3944–3948.
2. Chiba, P.; Ecker, G.; Schmid, D.m.; Drach, J.; Tell, B.; and Gekeler, V. *Mol. Pharmacol*. **1996**, 49, 1122 1130.
3. Pastor, M.; Cruciani, G.; McLay, Iain .; Pickett, S.; Clementi, S.*J. Med. Chem.* **2000,** *43,* 3233-3243
4. Durán, A.; Comesaña, G and Pastor M. *J. Chem Inf Mod.* **2008**

CYTOTOXICITY MECHANISMS OF PYRAZINO[1,2-*b*]ISOQUINOLINE-4-ONE DERIVATIVES AND SAR STUDIES

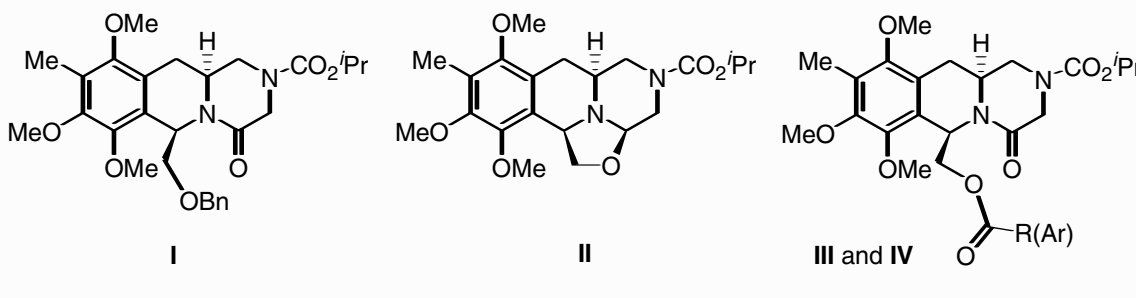
Irene Ortín (1), Juan Francisco González (1), Elena de la Cuesta (1), Cristina Manguán-García (2,3), Rosario Perona (1,2,3), and Carmen Avendaño (1)

(1) Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad Complutense, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain.
(2) Instituto de Investigaciones Biomédicas CSIC/UAM, Traslational Oncology Unit H. La Paz/UAM, C/Arturo Duperier, 4, 28029 Madrid, Spain.
(3) CIBER de Enfermedades Raras (CIBERER) Valencia, Spain.

The cytotoxicity showed by **I**, an interesting representant of the title compounds, for HT-29 human colon cancer cells (CI_{50} value of $1.95x10^{-7}M$)[1] has been related to the induced cell death at the G2 phase and not to DNA damage. This compound promotes the degradation of components of the G2/M checkpoint machinery, in particular cdc2, Cyclin B1 and Wee1, which represents a novel mechanism of cytotoxicity. Degradation of Wee1 seems to be mediated by proteasome activity but degradation of cdc2 has to occur through a different mechanism. The activity of **I** on G2 cell cycle components suggests that tumor cells that are arrested in G2/M by anticancer drugs like cisplatin could be targeted by compound **I**, increasing the apoptosis induction, and that their optimized analogs might be useful in the treatment of colon cancer through combination therapies with cisplatin or other anticancer drugs that affect the cytoskeleton integrity such as taxol® and taxotere®.

SAR studies with compounds obtained by manipulation of the N(2) and C(4)-functional groups and the C(6)-chain of compound **I** have confirmed the importance of these structural features in the *in vitro* antitumor activity. Fused oxazolidine derivatives as compound **II** were inactive, and the lack of activity found in the replacement of the C(4)-lactam by a cyanoamine function, as in compounds **III** and **IV**, could be explained considering that their all-*syn* relative configuration makes them too stable to generate alkylating iminium species.

1. Ortín, I.; González, J. F.; de la Cuesta, E.; Manguán-García, C.; Perona, R.; C. Avendaño. *Bioorg. & Medic. Chem.* **2008**, *16*, 9065.



POSTER COMMUNICATIONS – PC.114

DEVELOPMENT OF NEW ADENOSINE A_{2A} RECEPTOR AGONISTS: SYNTHESIS AND PHARMACOLOGICAL STUDIES

Anna M. Rodríguez (1), Gloria Rosell (2), José M. Brea (3), M. Isabel Loza (3), Ángel Guerrero (1) and M. Pilar Bosch (1)

(1) Department of Biological Chemistry and Molecular Modelling, IQAC (CSIC), Jordi Girona, 18-26, 08034 Barcelona (Spain), (2) Department of Pharmacology and Therapeutic Chemistry (Unity Associated to CSIC), Fac. of Pharmacy, University of Barcelona, Avda. Diagonal, s/n, 08028 Barcelona (Spain), (3) USEF, Department of Pharmacology, Fac.of Pharmacy, University of Santiago de Compostela, Campus Universitario Sur s/n, 15782 Santiago de Compostela (Spain)

Adenosine receptors (ARs) play an important role in many biological processes and therefore the development of potent and selective AR agonists and antagonists has been an important subject of research in Medicinal Chemistry for more than 30 years. Along this period, it has been demonstrated that selective A_{2A}AR modulators have potential applications in a wide range of therapeutic processes, particularly in cardiovascular, inflammatory and neurodegenerative diseases.

In this work and following a previous work from our group[1], we present the synthesis and biological evaluation of a number of new adenosine derivatives as potential agonists for the human A_{2A}AR. The compounds contain an ethyl-substituted tetrazole moiety at the 4'-position of the ribose and an amine group substituted with different phenyl ring systems at the 2-position of the adenine. The main aim of the work was to disclose the effect of the presence of several types of substituent at the *para*-position of the phenyl ring, as well as the relevance of the chirality at the vicinal position of the amine. Synthesis of the chiral amines has been achieved by enzymatic resolution methodologies. The biological activity of the potential agonists has been evaluated by radioligand binding assays using recombinant human ARs, and by determination of the cAMP production in these receptors expressed in transfected CHO (A_1AR), HeLa (A_{2A}AR, A_3AR) and HEK-293 (A_{2B}AR) cells. Some of the compounds displayed a high affinity and potency for A_{2A}AR (K_i 5-56 nM, EC_{50} 2-10 nM) and low or negligible affinity for A_1AR, A_{2B}AR and A_3AR.

1. Bosch M.P. et al., J. Med. Chem. 2004, 47, 4041-53.

KEY INTERACTIONS RESPONSIBLE OF MOLECULAR RECOGNITION PROCESS BETWEEN MKK-DERIVED D-SITE PEPTIDES AND MAPKs

Patricia Gómez-Gutiérrez and Juan J. Pérez

Dept of Chemical Engineering; Universitat Politècnica de Catalunya; Av. Diagonal, 647; 08028 Barcelona, Spain

Mitogen activated protein kinases (MAPKs) are Ser/Thr protein kinases involved in the regulation of several physiological processes including, gene expression, mitosis, movement, metabolism and programmed death. Like their substrates, MAPKs are regulated by phosphorilation, acting as substrates of the MAPK kinases (MKKs) that in turn, are substrates of the MAPK kinase kinases (MKKKs). There are three well characterized subfamilies of MAPKs in multicellular organisms: ERK1/2; JNK1/2/3; and P38s activated by different highly selective MKKs[1].

MAPKs increase their specificity for substrates, activators or scaffold proteins through a docking domain referred as the D-site that in MKKs is located in the N-terminus. The ability of MKK to recognize its cognate MAPK is highly dependent of docking interactions that contains a consensus sequence: $(Arg/Lys)_{2-3}$-$(X)_{1-6}$-ϕ- X-ϕ (being X an spacer and ϕa hydrophobic residue)[2].

We report in the present work the results of computational study aimed at characterizing the structural features involved in the specificity of the recognition between p38 by different MKKs. Specifically, we analyzed the crystal structure of the peptide domain of MKK3 docked on the D-site of p38 and from it, models of the complex of the binding peptides of MEK1, MEK2, MKK4, MKK6 and MKK7 were also constructed by homology modeling. Molecular dynamics simulations were run in order to understand the importance the different residue-residue interactions. Simulations were carried out using explicit solvent and analyzed using the MM/PSBA method to understand the free energy of the binding process. The energy decomposition of the interactions provides information about the key interactions involved in the molecule-molecule recognition. This information may be useful to design new specific kinase allosteric inhibitors.

1. Johnson, G.L. and Lapadat, R (2002). Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science*, 298, 1911-1912.

2. Bardwell A.J., Frankson E. and Bardwell, L (2009) Selectivity docking sites in MAPK kinases. *J. Biol. Chem.*, 284 (19), 13165-73.

DESIGN AND SYNTHESIS OF NOVEL MMP-2 INHIBITORS WITH HYDROXAMATE AND OXADIAZOLE UNITS AS ZINC BINDING GROUPS

Anna Puckowska (1, 2), Marta Bruczko (1, 3), José María Zapico (1), Pilar Serra (1), Sonsoles Martín-Santamaría (1), Beatriz de Pascual-Teresa (1), Ana Ramos (1)

(1) Department of Organic Chemistry, Faculty of Pharmacy, University of San Pablo CEU, Madrid, Spain,
(2) Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Bialystok, Poland,
(3) Department of Medicinal Chemistry, Medical University of Bialystok, Poland.

Matrix metalloproteinases are a family of zinc-dependent endopeptidases that mediate several physiological and pathological proceses, involved in inflammatory, malignant and degenerative diseases. Therefore they are promising biochemical targets, and the design of selective inhibitors of different types of MMPs, especially MMP-2, which plays major role in cancer, is the biggest challenge nowadays[1].

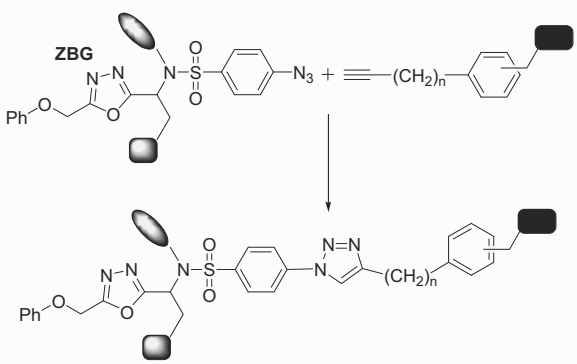
The requirements for a potent MMP inhibitor are: a functional group capable of chelating zinc ion (zinc binding group, ZBG), and one or more side chains undergoing effective van der Waals interactions with the enzyme subsites, mainly with the S1' subsite, responsible for MMP selectivity. Hydroxamic acid derivatives constitute the main family of selective MMP-2 inhibitors, but development of MMP inhibitors with non-hydroxamate ZBGs is necessary to improve the efficacy, pharmacokinetics and selectivity[2].

In order to identify the features which better define S1' selective side chains, a bibliographic search of selective hydroxamate MMP-2 inhibitors and docking studies were carried out. From the docking studies we have identified the most interesting side chains in order to gain activity and selectivity. These side chains have been connected to the hydroxamate ZBG through triazol linkers, by means of click reactions[3], leading to putative selective MMP-2 inhibitors.

We have also identified new heterocylic ZBGs derived from an oxadiazole system. Based on this novel ZBG, the design and syhthesis of a second generation of MMP-2 selective inhibitors has been undertaken (Scheme 1 and Figure 1).

Affinity assays and NMR experiments are currently in progress in order to characterize their binding properties.

1. Murphy, G.; Nagase, H. *Molec. Asp. Med.* 2008, *29*, 290-308.
2. Georgiadis, D.; Yiotakis, A. *Bioorg. Med. Chem.* 2008, *16*, 8781-94,
3. Kolb, C. K.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed*. 2001, *40*, 2004-2021.



Scheme 1. Click chemistry approach.

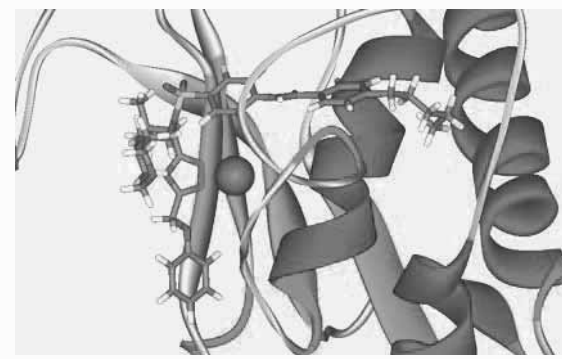


Figure 1. Docked structure of one of the designed compounds.

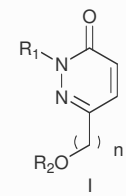
SYNTHESIS AND PHARMACOLOGICAL STUDY OF NEW 6- AND 2,6-SUBSTITUTED PYRIDAZIN-3(2H)-ONES

Tamara Costas (1), Pedro Besada (1), Carla Chessa (1), Alesandro Piras (1), Carmen Terán (1), Laura Acevedo (2), Francisco Orallo (2) and Dolores Viña (2)

(1) Departamento de Química Orgánica, Facultad de Química, Universidade de Vigo, 36310, Vigo, Spain.
(2) Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain.

Pyridazinone analogues have received an increasing interest in Medicinal Chemistry due to their pharmacological activities. Cardiovascular system is an important target for this class of compounds, and numerous substituted pyridazinone analogues have been reported with significant platelet aggregation inhibitory activity and/or antihypertensive activity[1,2]. In this communication, we describe the synthesis of a new series of 6- and 2,6-substituted-3(2H)-pyridazinones (**I**).

A new methodology developed in our laboratory has been followed for the preparation of piridazinones **I**[3,4]. The synthesis of a conveniently functionalized butenolide by oxidation with oxygen singlet of the corresponding furan, followed by reaction with hydrazine or methylhydrazine afforded the desired piridazinones. Finally, the piridazinone series are completed by modifications at the level of the oxygen atom in the side chain. All new compounds were tested for vasorelaxant activity and for platelet aggregation inhibitory activity. The study of the structure activity relationships of this series of compounds will provide important information for the design of new potent compounds.



I

We are grateful to the Xunta de Galicia (PGIDIT07PXIB) and University of Vigo for financial support.

1. Abouzid, K.; Hakeem, M. A.; Khalilb, O.; Makladc, Y. Bioorg Med Chem 2008, 16, 382-389.
2. Coelho, A.; Raviña, E.; Fraiz, N.; Yáñez, M.; Laguna, R.; Cano, E.; Sotelo, E. J. Med. Chem. 2007, 50, 6476-6484.
3. Pérez, M.; Canoa, P.; Gómez, G.; Terán, C.; Fall, Y. Tetrahedron Lett 2004, 45, 5207-5209.
4. Teijeira, M.; Suárez, P. L.; Gómez, G.; Terán, C.; Fall, Y. Tetrahedron Lett 2005, 46, 5889-5892.

POSTER COMMUNICATIONS – PC.118

QSAR STUDY OF ADENOSINE ANALOGUES AS AGONISTS OF THE HUMAN A3 ADENOSINE RECEPTOR USING 3D MOLECULAR DESCRIPTORS

Yunierkis Pérez-Castillo (1,2), Marta Teijeira (1), Pedro Besada (1) and Carmen Terán (1)

(1) Departamento de Química Orgánica, Universidad de Vigo, Campus Lagoas-Marcosende, Vigo, España.
(2) Molecular Simulations and Drug Design Group, CBQ, UCLV, Santa Clara, Cuba.

Adenosine is a primordial signalling molecule present in every cell of the human body that mediates its physiological functions. The A3 subtype adenosine receptor (A3AR) have been related with conditions like ischemia, inflammation and cancer and A3AR agonists are in clinical trials for the treatment of rheumatoid arthritis and colorectal cancer[1]. Quantitative Structure-Activity Relationship (QSAR) studies have been successfully applied to the design of new adenosine receptor agonist and have proven to be a useful methodology for the rational design of new drug candidates[2,3].

Here, we propose a QSAR model based on three dimensional (3D) molecular descriptors to predict the activity of adenosine analogues nucleosides as A3AR agonists. In brief, we compiled from literature adenosine analogues with Ki values measured against human HEK cells expressing the human A3AR using [125I]I-AB-MECA as reference pattern, 3D structure of molecules were generated using the CORINA software, molecular descriptors were calculated with the DRAGON package and the data was subject to cluster analysis and partitioned in training and test sets. These resulted in 102 and 23 compounds in training and test sets respectively.

Linear regression models were constructed using a genetic algorithm for variable selection as implemented in the MobyDigs software. For the best model found, the applicability domain was established and outlier compounds were identified. These resulted in a model with R^2=84.43 and Q^2loo=81.26 for training set and Q^2=74.83 for the external test set. The information obtained from the model was used to design novel compounds to be synthesized and assayed as potential agonist of the human A3AR.

We are grateful to the Xunta de Galicia (PGIDIT07PXIB) and University of Vigo for financial support.

1. Gessi, S.; Merighi, S.; Varani, K.; Leung, E.; Mac Lennan, S.; Borea, P. A. The A3 adenosine receptor: An enigmatic player in cell biology. Pharmacology & Therapeutics 2008, 117, 123-140.
2. González, M. P.; Terán, C.; Teijeira, M.; Helguera, A. M. Quantitative structure activity relationships as useful tools for the design of new adenosine receptor ligands. 1. Agonist. Curr Med Chem 2006, 13, 2253-66.
3. González, M. P.; Terán, C.; Teijeira, M.; Besada, P.; González-Moa, M. J. BCUT descriptors to predicting affinity toward A3 adenosine receptors. Bioorg Med Chem Lett 2005, 15, 3491-5.

HOMOLOGY MODELING, DOCKING AND MM-GBSA RESCORING OF BENZODIAZEPINE RECEPTOR LIGANDS: STRUCTURAL FEATURES REGULATING α1- VERSUS α5-SUBTYPE SELECTIVITY

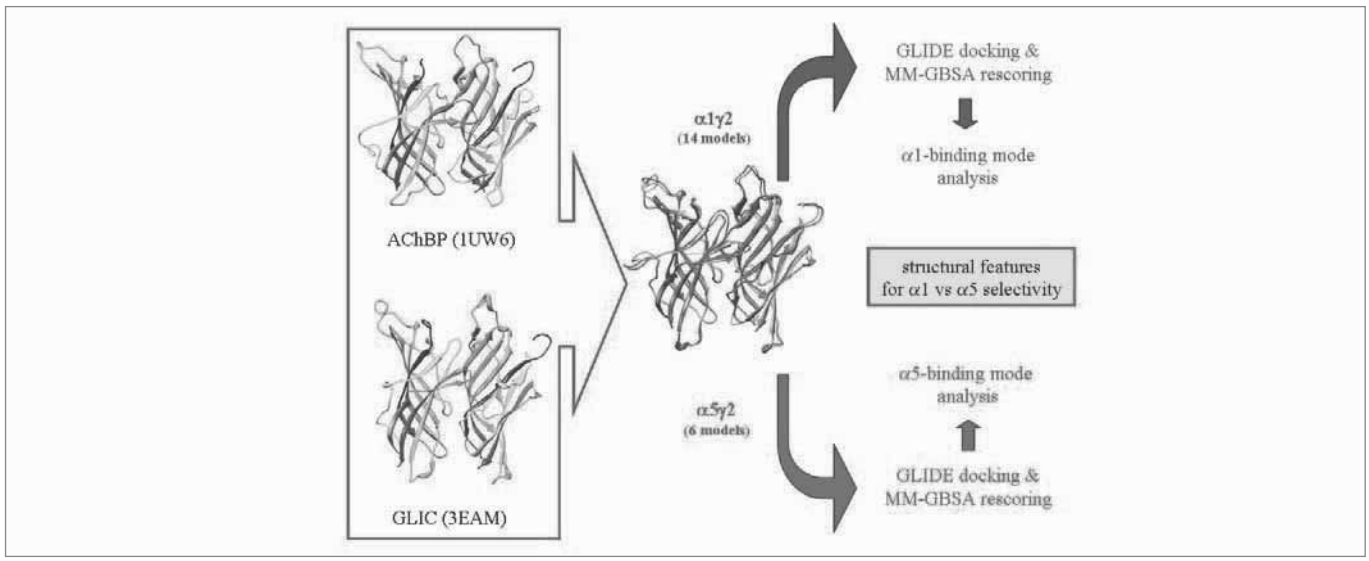
Claudia Bonaccini, Chiara Guarino, Matteo Chioccioli, Silvia Selleri and Paola Gratteri

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, Italia

Since their discovery, many efforts were spent to understand the structural features of $GABA_A$ receptors because of the multiplicity of their involvement in sedative/motor effects, anxiolytic activity, alcohol response and memory processes[1]. $GABA_A$ receptors belong to the superfamily of ligang gated ion channel and are generally composed of α, β and γ subunits. The binding site for GABA and competitive ligands is located within the extracellular domain, at the interface between β and α chains. Most $GABA_A$ receptors also contain a recognition site for prototypic benzodiazepines which is found at the interface between γ2/γ3 and either an α1, α2, α3 or α5 subunit; of these subtypes, the α1β2γ2 combination predominates in the brain and is mainly involved in sedation1, while the α5-subtype constitutes around 25% of the total $GABA_A$ receptors within the hippocampus and it has been identified as an attractive target for potential cognition enhancers[2]. The study of the structural requirements for addressing ligands' α-subtype selectivity represents a great challenge to obtain drugs with the desired pharmacological activity avoiding side effects.

In the present work, we homology modelled the benzodiazepine binding sites of α1 and α5 $GABA_A$ subtypes using available information on the closest homologs of the receptor. After an extensive validation of the obtained models we used them for binding mode investigation and affinity prediction on a series of benzodiazepine ligands showing different pharmacological profiles with respect to the studied subtypes.

1. Rudolph U. and Möhler, H. (2006) *Curr Opin Pharmacol.*, 6(1), 18-23.
2. Chambers, M.S. et al. (2004) *J. Med. Chem.*, 47(24), 5829-32.



POSTER COMMUNICATIONS – PC.120

VINYL SULFONES AS CASPASE INHIBITORS

Maria M. M. Santos (1), Ana S. Newton (1), Isabel Coutinho (2), Paulo M. C. Glória (1), Lucília Saraiva (2) and Rui Moreira (1)

(1) iMed.UL, Faculty of Pharmacy, University of Lisbon, Portugal,
(2) REQUIMTE/CEQUP, Faculty of Pharmacy, University of Oporto, Portugal

Apoptosis, or programmed cell death, has received increasing attention for its critical role in several disorders. Excessive neuronal apoptosis, which can be triggered by a number of stimuli, leads to a variety of acute disorders such as stroke, as well as progressive diseases including Alzheimer's, Huntington's and Parkinson's diseases, amyotrophic lateral sclerosis, multiple sclerosis, and spinal muscular atrophy[1]. Among the more studied players are cysteinyl-aspartate-specific proteases (caspases). They are characterized by their specific cleavage of an aspartic acid residue from their respective peptide substrates and known to play essential and distinct roles in apoptotic cell death[2]. Thus, caspases are targets for drug therapy. One type of irreversible cysteine proteases inhibitors that has received special attention in the last few years are those based on Michael acceptor scaffolds[3]. This class of inhibitors includes vinyl sulfones, alfa,beta-unsaturated carbonyl derivatives, and miscellaneous derivatives. They inhibit cysteine proteases by forming covalent bonds with the active site thiol of cysteine proteases.

Here, we present the synthesis of a series of vinyl sulfones (Figure 1) and the evaluation of caspase inhibition using yeast cells expressing the human caspase-3.

This work was supported by the Fundação para a Ciência e a Tecnologia (Lisbon, Portugal) by the award of a doctoral fellowship to A.S.N. (SFRH/BD/41276/2007) and postdoctoral fellowship to P.M.C.G. (SFRH/BPD/22631/2005).

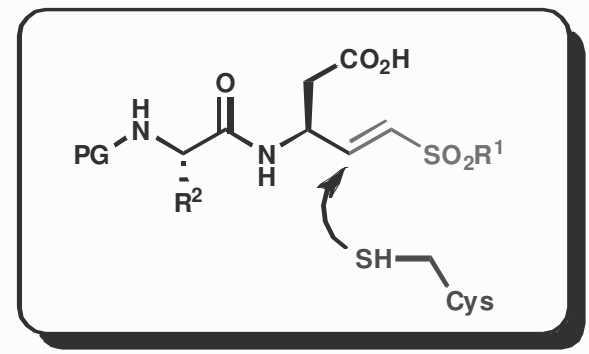


Figure 1 – Vinyl sulfones inhibitors.

1. R. C. Taylor, S. P. Cullen, S. J. Martin, "Apoptosis: controlled demolition at the cellular level", Nature Reviews Molecular Cell Biology, **2008**, 9, 231-241.
2. J.-B. Denault, G. S. Salvesen, "Caspases: Keys in the Ignition of Cell Death", Chem. Rev., **2002**, 102 , 12, 4489-4500.
3. M. M. M. Santos, R. Moreira, "Michael Acceptors as Cysteine Protease Inhibitors", Mini-Reviews in Medicinal Chemistry, **2007**, 7, 1040-1050.

DUAL SRC/IGF-1R INHIBITORS BASED ON 2-(1*H*-INDOL-3-YL)-2-OXOACETOHYDRAZIDE SCAFFOLD

Stefanie Schmidt (1), Frank Totzke (2), Christoph Schächtele (2), Michael H.G. Kubbutat (2), Thomas Lemcke (3), Lutz Preu (1) and Conrad Kunick (1)

(1) Institut für Pharmazeutische Chemie, Technische Universität Braunschweig, Beethovenstraße 55, 38106 Braunschweig, Germany, (2) ProQinase GmbH, Breisacher Str. 117, 79106 Freiburg, Germany, (3) Institut für Pharmazie, Universität Hamburg, Bundesstraße 45, 20146 Hamburg, Germany

The receptor tyrosine kinase IGF-1R is expressed on vascular smooth muscle cells and plays an essential role in various cell signalling pathways. SRC, a member of the largest group of non receptor tyrosine kinases, is expressed in most tissues. It is a key messenger in a variety of cell signalling events like cell growth, cell differentiation, cell death and cell migration. IGF-1R and SRC are biochemically connected, because SRC mediates downstream effects of receptor tyrosine kinases like IGF-1R. Hyperactivity of both SRC and IGF-1R are associated with cancer development and progression. Recently it has been shown that concomitant inhibition of tumour-relevant kinases is a promising approach to improve efficacy of cancer therapy as well as to avoid resistance. In an effort to discover novel inhibitors of the c-MET tyrosine kinase we identified a new kinase inhibitor chemotype based on a 2-(1*H*-indol-3-yl)-2-oxoacetohydrazide parent scaffold. A structure modification campaign revealed not only congeners with enhanced c-MET inhibitory activity, but also potent dual SRC/IGF-1R inhibitors. The postulated binding mode as well as the syntheses and the kinase inhibition profiles of the novel compounds will be presented in the poster.

DISCOVERY OF STRUCTURALLY SIMPLE, HIGHLY POTENT AND SELECTIVE A_3 ADENOSINE ANTAGONISTS

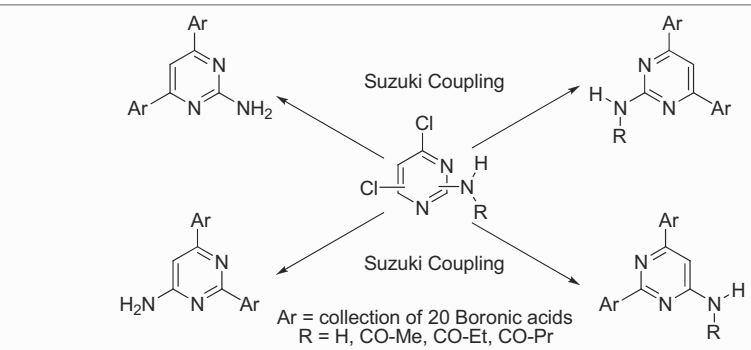
Vicente Yaziji (1), Alberto Coelho (1), José Brea (2), M. Isabel Loza (2), Hugo Gutiérrez-de-Terán (3), David Rodríguez (3) and Eddy Sotelo (1)

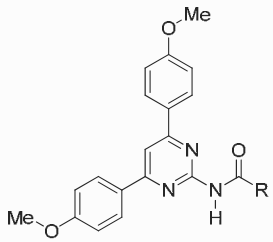
(1) Combinatorial Chemistry Unit (COMBIOMED) and (2) Screening Unit (USEF), Institute of Industrial Pharmacy (IFI). University of Santiago de Compostela. Santiago de Compostela. 15782-Spain. (3) Fundación Pública Galega de Medicina Xenómica. Complexo Hospitalario Universitario de Santiago. A Choupana s/n. Santiago de Compostela. Spain.

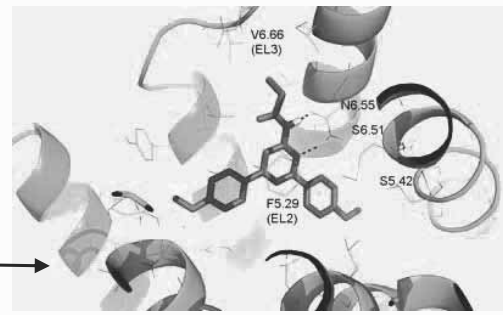
Adenosine receptor antagonists constitute an emerging class of therapeutic agents that promise conceptually new strategies to address significant unmet medical needs in diverse therapeutic areas[1]. From a structural point of view, the best known classes of adenosine receptor antagonists encompass highly diverse families of bi-, tri-, tetra- and, to a lesser extent, monocyclic nitrogen-containing aromatic scaffolds[1]. While the structural manipulation of diverse scaffolds has permitted the identification of potent and selective antagonists, the search for structurally simpler ligands with improved pharmacokinetic profiles remains a challenge. In the present communication we describe the design, synthesis, biological evaluation as well as the most salient features of the SAR of a library of 160 diarylpyrimidines that enabled the identification of several structurally simple, highly potent and selective A_3 adenosine receptor antagonists. In order to shed light on the particular selectivity profile exhibited by some promising compounds the binding modes of representative compounds will be also discussed.

Part of this work has been financially supported by the Fondo Europeo de Desarrollo Social (FEDER). E. Sotelo is the recipient of a Consolidation Group Research Grant from the Conselleria de Educación (Xunta de Galicia). E. Sotelo, A. Coelho and H. Gutiérrez-de-Terán are researchers of the Isidro Parga Pondal program (Xunta de Galicia, Spain). J. Brea is recipient of an Isabel Barreto contract from Xunta de Galicia.

1. Baraldi, P. G., Aghadeh, M., Tabrizi, M. A., Gessi, S., Borea, P. A., *Chem. Rev.*, **2008**, *108*, 238–263. Jacobson, K. A., Goao, Z., *Nature Reviews Drug Discovery*, **2006**, *5*, 247–264. Moro, S., Gao, Z. G., Jacobson, K. A., Spallouto, G., *Med. Res. Rev.*, **2006**, *26*, 131–159.







Code	R	hA_1 [¥]	hA_{2A} [¥]	hA_{2B} [¥]	hA_3 [¥]
ISVY074	Et	5	1	3	3.56
ISVY071	Pr	1	4	1	11.00

[¥] Adenosine binding data is expressed as K_i (nM) in hA_3 or as % of displacement at 0.1 μM (hA_1, hA_2 receptors).

PREPARATION AND VALIDATION OF PEPTIDOMIMETIC LIBRARIES ATTACHED TO MICROPLATES

Gloria Navarro-Vendrell (1), Joaquim Messeguer (1), Nuria Cortés (1), Nuria García-Sanz (2), Aarón Navarro (2), Antonio Ferrer-Montiel (2), and Àngel Messeguer (1)

(1) IQAC-CSIC, Jordi Girona 18, 08034 Barcelona.
(2) Instituto de Biología Molecular y Celular, Universidad Miguel Hernández. 03202 Elche.

A method for to the construction of microarray libraries of discrete compounds attached to solid supports is described. The validation of the libraries was performed by the identification of actives against selected targets.

A first library of 512 *N*-alkylglycine trimers containing an aspartic acid residue as linker was prepared. Optimization of the immobilization was performed using a model compound. Then, a representative sample of the library was attached to the microplate surface.

The validation of the system was accomplished using an assay for the identification of trypsin inhibitors (Fig 1). Positive controls for the analysis should be also as attached compounds. To this end, two types of compounds bearing COOH were selected. The first one was antipain, a commercial small peptidomimethic. The second type involved the most active compounds identified from a library of *N*-alkylglicine pentamers synthesized previously[1] (prepared again with an aspartic acid).

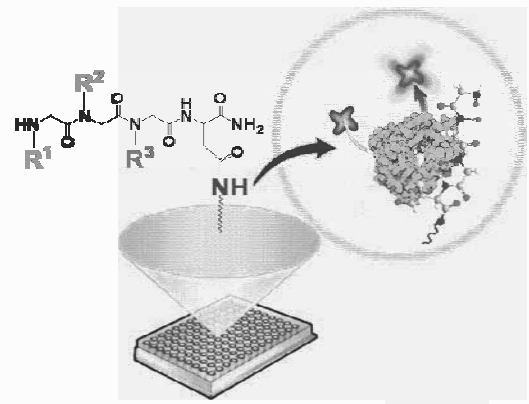


Figure 1

Results obtained on the identification of trypsin inhibitors pointed out the importance of the linker structure when using the microarray format.

Complementarily, a second library of 48 heterocyclic derivatives was constructed employing a longer linker. After optimization with a suitable model derivative, immobilization of the library components was carried out successfully. This library was validated by means of the trypsin inhibition assay. Potent inhibitors were identified from an initial screening. Enzymatic selectivity was evaluated (also on functionalized microplate) by using an assay for elastase inhibition. Our first results identified 12 compounds active at low concentrations and selective for trypsin. Finally, the inhibition assays of trypsin for these compounds were also performed in solution to confirm the results obtained with the microplate system.

Once the validation of the library was accomplished, we are extending the screening to pharmaceutical targets with higher complexity degrees and results obtained along this line will be also presented.

1. Messeguer, J.; Cortés, N.; García-Sanz, N.; Navarro-Vendrell, G.; Ferrer-Montiel, A.; Messeguer, A. *J. Comb. Chem.*, **2008**, 10, 974-980.

IDENTIFICATION OF A PEPTOID THAT BLOCKS AXONAL CHEMOREPULSION AND ENHANCES AXON REGENERATION

Marisol Montolio (2), Joaquim Messeguer (1), Isabel Masip (1), Patricia Guijarro (2), Rosalina Gavín (2), José Antonio del Río (2), Eduardo Sorianol (2), and Àngel Messeguer (1)

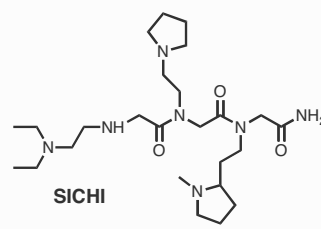
(1) Dept. of Chemical and Biomolecular Nanotechnology, IQAC-CSIC, Jordi Girona 18, 08034 Barcelona.
(2) IRB Barcelona, Department of Cell Biology, University of Barcelona, and CIBERNED (ISCIII), Barcelona Science Park , Baldiri Reixac 10, E-08028 Barcelona, Spain.

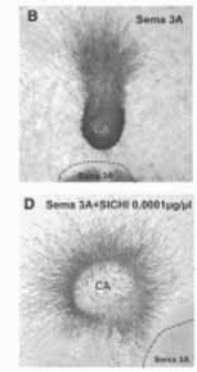
Secreted Semaphorins are a large group of extracellular proteins involved in a variety of processes during development, including neuronal migration and axon guidance. Class III Semaphorins are vertebrate secreted proteins with crucial roles during the development of the nervous system. Most secreted Semaphorins mediate axon-growth inhibition at a distance, thereby promoting axonal chemorepulsion in both the central and peripheral nervous system. In the adult CNS, severed axons fail to regenerate beyond the lesion site. The failure of axon regeneration is mainly attributable to the environment encountered by injured axons. It has also been shown that class III Semaphorins, including Sema 3A, are expressed in the adult brain and spinal cord, where they are regulated by synaptic activity and after lesions. Therefore, the identification of molecules that block Semaphorin functions could be of great interest for axon regeneration.

We present our results on the screening of a peptoid combinatory library, constructed under the positional scanning format, to search for Semaphorin 3A inhibitors. We identified a peptoid (SICHI: Semaphorin Induced CHemorepulsion Inhibitor) that blocks Semaphorin 3A- chemorepulsion and growth-cone collapse in axons at millimolar concentrations. SICHI inhibits the binding of Semaphorin 3A to its receptor complex (Neuropilin 1/Plexin A1) and Semaphorin 3A-induced phosphorylation of GSK3. SICHI promotes also neural regeneration of damaged axons.

1. M. Montolio et al., *Chem. Biol*. 2009, 0000.

SICHI inhibits the chemorepulsion induced by Class III Semaphorin





DIHYDROSPHINGOSINE RELATED COMPOUNDS HIGHLY ACTIVE AGAINST MULTI-DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

Esther del Olmo (1), Luis A. Bustos (1), José L. López-Pérez (1), Gloria M. Molina-Salinas (2), Salvador Said-Fernández (2), Arturo San Feliciano (1)

(1) Departamento de Química Farmacéutica 1, Facultad de Farmacia - CIETUS, Campus Unamuno, 37007-Salamanca, España
(2) Centro de Investigaciones Biomédicas del Noreste, CIBIN-IMSS, Monterrey, NL, México.

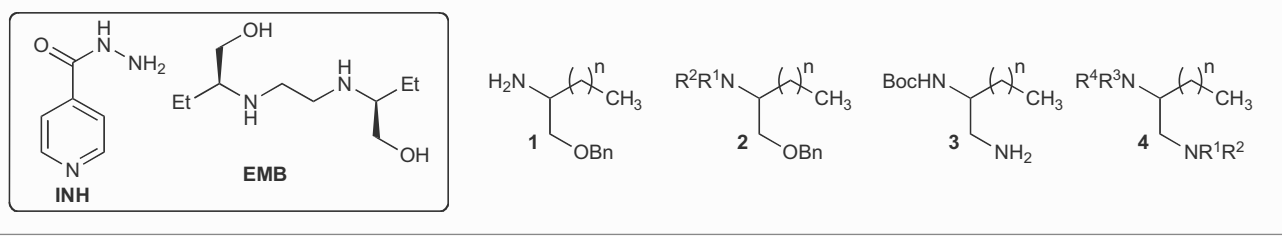
Tuberculosis (TB), malaria and HIV/AIDS kill 6 million people every year; nearly 2 million deaths are finally caused by TB. 5000 people per day. There are estimated 8 million of new cases each year, 95% of which occur in developing countries[1]. Drugs such as isoniazid (INH), Rifampicin (RMP) and ethambutol (EMB) have historically been successful in the treatment of TB infections. In the recent past, however, poor compliance with the prolonged and complicated dosage regimens currently used to treat the disease, in conjunction with the advent of the AIDS epidemic and the increased mobility of populations, has led to the emergence of numerous multi-drug-resistant (MDR) and even extremely-resistant (XDR) strains of *Mycobacterium tuberculosis*[2]. Resistance to the frontline agents, most notably INH and RMP, results in the treatment of patients with second-line agents. Among these second-tier drugs, the old drug thiacetazone, being used in Africa and South America for the treatment of MDR-TB, has shown to be a very effective when administered in combination with INH.[3]

Our research group had previously prepared certain aminoalcohol and diamine sphingoanalogues which were tested *in vitro* on sensible H37Rv and MDR strains of *M. tuberculosis,* with very promising results[4a]. After that we prepared certain cyclic analogues, to analyse the influence of conformational restriction on the anti-MTB activity[4b]. Here we analyse the influence of the aliphatic chain length on the activity.

The synthetic procedure started with the preparation of α-aminoacids, with four different chain lengths, according to a previous report[5]. The amino group was Boc-protected and the carboxyl function reduced to hydroxyl, through mixed anhydride formation followed by $NaBH_4$ reduction. The alcohol was then protected as benzyl ether, and the Boc group removed, to provide compounds **1** (n = 9, 13, 15, 17). The amine was then alkylated or acylated to obtain compounds **2**. Compounds **1** were also mesylated, transformed into the corresponding azides and then reduced to the Boc-protected diamine derivatives **3**. The unprotected primary amino group of compounds **3** was then alkylated or acylated, and the Boc group eventually removed, to give compounds **4**. Compounds **3** and **4** were tested *in vitro* on cultured H37Rv and MDR strains of *Mycobacterium tuberculosis*. Several new compounds showed IC50 values under 3 µg/mL. The influence of the size chain on the anti-MTB potency will be analysed

LAB *thanks Banco Santander (Spain) for a research fellowship. Research supported by grants MEC AGL2005-02168/GAN and FIS PI-060118 (Spain); performed under the auspices of CYTED-Project X.11: PIBATUB.*

1. (a) World Health Organization. *WHO report* **2009,** WHO/HTM/TB/2009.411. (b) Korenromp E.L., Bierrenbach A.L., Williams B.G., Dye C. *Int J Tuberc Lung Dis*. **2009**.,*13*, 283-303.
2. (a) LoBue P. *Curr Opin Infect Dis*. **2009**, *22*, 167-73. (b) Hah N.S., Wright A., Bai G., Barrera L., Boulahbal .F, Martín-Casabona N., et al. *Emerg Infect Dis* **2007**, *13*, 380—7.
3. Houston, S.; Fanning, A. *Drugs,* **1994**, *48*, 689.
4. (a) Patent Applic. OPE: 200602940. Nov. 17, 2006. (b) WO2008059014 A2. May 22, 2008.
5. del Olmo E., Alves M., López J.L., Inchaustti A., Yaluff G., Rojas de Arias A., San Feliciano A. *Bioorg Med Chem Lett*. **2002**, *25*, 12, 659-62.



POSTER COMMUNICATIONS – PC.126

5-HT_4-RECEPTOR LIGAND INTERACTION AND ACTIVATION THROUGH MINOR AND MAJOR REARRANGEMENTS OF SIDE CHAINS AND HELICES

Jessica Sallander (1), Lucie Pellissier (2), Sylvie Claeysen (2), Aline Dumuis (2), Mercedes Campillo (1), Joël Bockaert (2), Leonardo Pardo (1)

(1) Laboratori de Medicina Computacional, Unitat de Bioestadistica, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. (2) CNRS, UMR 5203, Instiut de Génomique Foncionelle, Montpellier, France.

G protein-coupled receptors (GPCRs) are allosteric molecules in equilibrium with many different conformational states. GPCRs adopt at least one inactive (R) and several active (R*) states. R* states can be reached, even in the absence of agonists or mutations (basal constitutively active R* (R*basal)). Inactive R state caused by the binding of a full inverse agonist can be called the R ground state (Rg). One of the main questions in GPCR molecular pharmacology is to understand the structural arrangements of the seven transmembrane helices (TM) that occur to stabilize either Rg or the different R* states. It is noteworthy that some main arrangement and rearrangement events during activation seem to be common, at least in family A GPCRs. The recent crystal structure of the ligand-free opsin, which contains several features characteristic of R* states, has shown that the intracellular part of TM6 is tilted outward by 6 to 7 Å, whereas TM5 moves toward TM6 by 2 to 3 Å. In addition, conformational changes occurring to generate either Rg or R* states are accomplished by the rearrangements of side chains forming different networks of interactions between helices.

With the help of site-directed mutagenesis studies and by molecular modeling simulations[1], we have been able to characterize some key steps of receptor activation: starting at the binding site, working it down through the helices. Here we show mayor and minor rearrangement of the helices and side chain interactions, in order for them to as a final point activate the G protein. As these mechanisms involve residues highly conserved within GPCRs, it is expected to be shared by other members of this family.

This can be useful to understand the mechanism of how different ligands interact with the 5-HT_4 receptor and may prove valuable for structure-based drug discovery efforts and facilitate the design of more effective and selective pharmaceuticals.

1. Pellissier#, LP.; Sallander#, J.; Campillo, M.; Gaven, F.; Queffeulou, E.; Pillot, M.; Dumuis, A.; Claeysen, S.; Bockaert, J.; Pardo, L.; *Mol. Pharmacol.* **2009**, 75, 982-90.

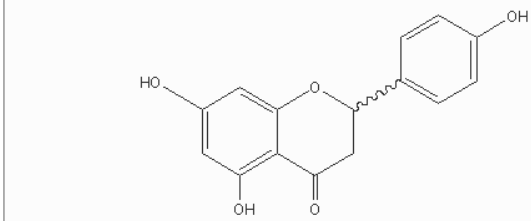
PREPARATIVE ENANTIOSELECTIVE HPLC OF RACEMIC NARINGENIN AND BIOLOGICAL PROFILE INVESTIGATION OF PURE ENANTIOMERS

Raffaella Gaggeri (1), Daniela Rossi (1), Simona Collina (1), Marcel Baierl (2), Markus Juza (2), Ornella Azzolina (1)

(1) Department of Pharmaceutical Chemistry, University of Pavia, Viale Taramelli, 12, I-27100 Pavia, Italy
(2) DSM Nutritional Products, Analytical Development SIL, Hauptstrasse 4, CH-4334 Sisseln, Switzerland

Naringenin [5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one] **1** is a chiral flavanone present in several fruits juices[1]. It is a well-known antioxidant, having a beneficial effect on human health as radical scavenger[2]. Additionally, an interesting anti-inflammatory activity of (±)-Naringenin has been reported recently[3]. Since the two enantiomers may have different biological properties as well as a different toxicological profile, in the present work we focus on their resolution and pharmacological properties investigation. To the best of our knowledge, the biological activity of Naringenin enantiomers has not been studied yet. The resolution of commercially available (±)-Naringenin via enantioselective HPLC has been selected by us as a promising strategy for provinding a quick and easy access to g-amounts of Naringenin enantiomers, necessary to support biological and toxicological studies. Firstly, an exhaustive screening of the commercially available chiral stationary phases has been performed on analytical scale[4]. Successively, the optimal experimental conditions were scaled-up to preparative scale in order to obtain the desired pure enantiomers in quantities suitable for biological evaluation. Biological properties of each enantiomer were evaluated and compared to those of (±)-Naringenin.

The chiral resolution of (±)-Naringenin via HPLC and biological investigation of pure enantiomers will be presented and discussed.



(±)-**1**

1. Yanez JA, et al. *Biopharm Drug Dispos*, 2008; 29: 63-82.
2. Galati G, et al. *Toxicology*, 2002; 177: 91-104.
3. Bodet C, et al. *J Periodont Res*, 2008; 43: 400-407.
4. Juza M,"*Optimization of Chiral Separations in HPLC*" in S. Kromidas (Editor), HPLC Made to Measure - A Practical Handbook for Optimization, Wiley-VCH, Weinheim, 2006, 427pp.

POSTER COMMUNICATIONS – PC.128

NOVEL PKC α LIGANDS: RATIONAL DESIGN, PREPARATION AND BIOLOGICAL INVESTIGATION

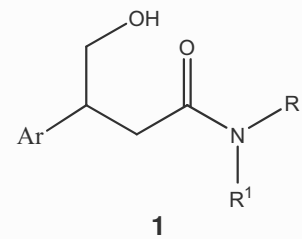
Anna Caterina Carnevale Baraglia (1), Daniela Rossi (1), Marialaura Amadio (2), Alessia Pascale (2), Christian Laggner (3), Jari Yli-Kauhaluoma (4), Raimo K. Tuominen (5), Ornella Azzolina (1), Simona Collina (1)

(1) Department of Pharmaceutical Chemistry, University of Pavia, V.le Taramelli, 12, I-27100 Pavia, Italy.
(2) Department of Experimental and Applied Pharmacology, University of Pavia, V.le Taramelli 14, I-27100 Pavia, Italy.
(3) Department of Pharmaceutical Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, A-6020 Innsbruck, Austria.
(4) Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland.
(5) Division of Pharmacology and Toxicology, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland.

Modulation of mRNA decay is an efficient post-transcriptional way of controlling gene expression. Among the different players, the RNA-binding proteins (RBPs) can affect the processing of selected transcripts, their transport and sub-cellular localization in the cytoplasm, their stability and translatability. The ELAV (Embryonic Lethal Abnormal Vision) proteins are RBPs which preferentially bind to adenine and uridine-rich elements (AREs) found in the 3'-untranslated region (3'-UTR) of a subset of mRNAs, increasing mRNA cytoplasmic stability and rate of translation[1,2]. Evidence in the literature indicates that the ARE-dependent mRNA decay can be affected by the Protein Kinase C (PKC) pathway. Particularly, the involvement of PKC α isozyme in ELAV protein activation has been recently demonstrated by us[3].

With the aim to identify a new class of PKC α ligands, we built an homology model of the PKC α binding site using the crystal structure of the PKC δ C1b domain complexed with phorbol-13-*O*-acetate as a template[4]. Successively, new molecules based on the 3-aryl-4-hydroxy butirramidic scaffold **1** were identified as potential PKC α ligands by means of docking experiments. A compound library was finally mapped, taking into account the synthetic feasibility and the molecular modeling suggestions.

Molecular modeling study, synthetic procedures applied for the preparation of designed compounds and preliminary binding data will be presented.



1

1. Keene, J. D. *Proc. Natl. Acad. Sci. U S A.* **1999**, *96*, 5-7.
2. Nagai, K.; Oubridge, C.; Ito, N.; Avis, J.; Evans, P. *Trends Biochem Sci.* **1995**, *20*, 235-240.
3. Pascale, A.; Amadio, M.; Scapagnini, G.; Lanni, C.; Racchi, M.; Provenzani, A., Govoni, S.; Alkon, D.L.; Quattrone, A. *Proc. Natl. Acad. Sci. U S A.* **2005**, *102*, 12065-12070.
4. Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917–924.

NEW *MYCOBACTERIUM TUBERCULOSIS* DNA GYRASE INHIBITORS: NAPHTHYRIDONE SERIES AGAINST TB

Ana Guardia, David Barros, Modesto J. Remuiñán

GlaxoSmithKline, DDW, Tres Cantos, Spain.

Tuberculosis has become one of the most extended diseases around the world. In spite of the efforts to find the best available drug regime, new therapies are necessary in order to reduce the current extensive duration of the treatment. Furthermore, multidrug-resistance and long- term persistence are tasks that still need to be addressed by scientists. Herein we present new DNA Gyrase inhibitors with a different MOA to the well known quinolones, which are currently used in the treatment of TB.

The starting point of MGI was the *in vitro* evaluation of 2000 compounds covering the existing diversity of bacterial topoisomerase inhibitors whithin GSK's compound collection against *Mycobacterium tuberculosis* (MIC determination). The initially selected hits, compounds whith general structure A (Figure 1) showed good *in vitro* potency but unfortunately they had develovability problems probably associated to its high lipophilicity. SAR studies were undertaken to find new chemical series with remarkable potency, improved physicochemical properties and less synthetic complexity. Aiming to get the best balance among potency, safety and exposure, a wide range of compounds were prepared. These approches afforded compounds with remarkable *in vitro* profile and significant *in vivo* efficacy, as it will be presented in this poster.

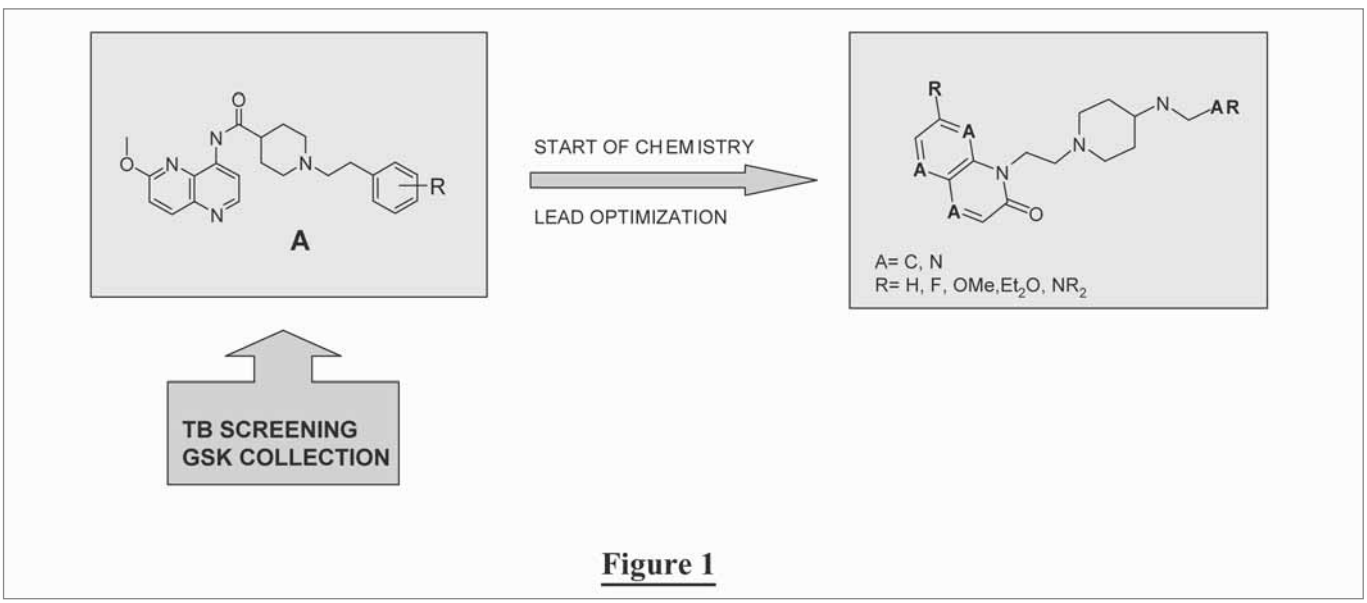


Figure 1

POSTER COMMUNICATIONS – PC.130

OPTIMIZATION OF INHIBITORS FOR HUMAN GLUTAMINYL CYCLASE BY STRUCTURE BASED DESIGN AND BIO-ISOSTERIC REPLACEMENT

Mirko Buchholz (1), Wolfgang Brandt (2), Torsten Hoffmann (1), Stephan Schilling (1), Hans-Ulrich Demuth (1) and Ulrich Heiser (1)

(1) Departments of Medicinal Chemistry, Enzymology and Preclinical Pharmacology, Probiodrug AG, Weinbergweg 22, 06120 Halle, Germany; (2) Department of Bioorganic Chemistry, Leibnitz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle, Germany

The inhibition of human Glutaminyl Cylase (hQC) has come into the focus as a new potential approach for the treatment of Alzheimer's Disease. The hallmark of this principle is the prevention of the formation of $Aß_{3(pE)-40,42}$, as these Aß-species were shown to be of elevated neuro-toxicity[1] and likely to act as a seeding core[2] leading to an accelerated formation of Aß-oligomers and fibrils. The further development of the first hQC inhibitors is presented. Starting from 1-(3-(1H-imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)thiourea[3] the exchange of the thiourea scaffold by bio-isosteric replacements led to the development of new classes of potent inhibitors whereas the optimization of the metal-binding group was achieved utilizing a hQC homology model and afforded a first insight into the probable binding mode of the inhibitors in the hQC active site. The efficacy of the resulting hQC inhibitors was assessed in a cell culture assay, directly monitoring the inhibition of $Aß_{3(pE)-40,42}$ formation.

1. Schilling, S. et al.; J. Neurochem. 2008, 106, 1225-1236.; Schilling, S. et al.; Nat. Med. 2008, 14, 1106-1111.; Russo, C. et al. ; J. Neurochem. 2002, 82, 1480-1489.
2. Miravalle, L. et al.; Biochemistry 2005, 44, 10810-10821.; Schilling, S. et al.; Biochemistry 2006, 45, 12393-12399.
3. Buchholz, M. et al.; J. Med. Chem. 2006, 49, 664-677.

SOLID PHASE SYNTHESIS OF MULTIFUNCTIONAL GLUTAMIC ACID DERIVATIVES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

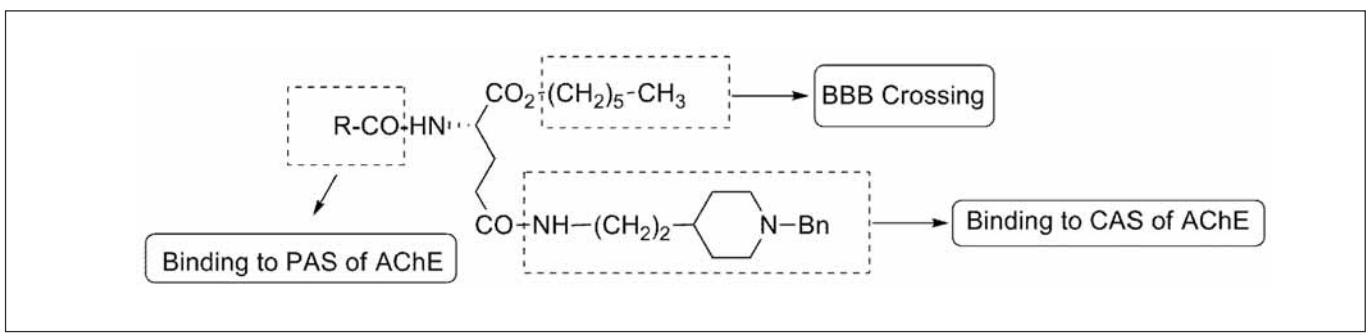
Mariana P. Arce (1), María Isabel Rodríguez-Franco (1), Carmen Gil (1), Gema C. González- Muñoz (1), Concepción Pérez (1), Beatriz López (1), Mercedes Villarroya (2), Manuela G. López (2), Antonio G. García (2), Santiago Conde (2)

(1) Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid. (2) Instituto Teófilo Hernando, Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo 4, 28029 Madrid.

Solid phase synthesis was employed to obtain series of L-glutamic acid derivatives that exhibit attractive *in vitro* biological activities for the treatment of Alzheimer's disease[1]. At submicromolar concentrations, they efficiently inhibit acetylcholinesterase (AChE), displace propidium from the peripheral anionic site (PAS) of the AChE, and protect neurons against damage caused by both exogenous and mitochondrial free radicals. They show no toxicity and able to cross the blood brain barrier (BBB). Consequently, it is expected that these multifunctional compounds could be able to improve cognitive impairment, diminish the oxidative damage caused by free radicals, including those of mitochondrial origin, and delay the degenerative process related to the excessive deposition of amyloid β-peptide (Abeta) in patients suffering Alzheimer's disease.

To Ministerio de Ciencia e Innovación (SAF 2006-01249) and Comunidad de Madrid (S-SAL/0275/2006). The fellowships to M. P. A. and G. C. G. from MEC and CSIC respectively are also acknowledged.

1. Conde, S.; Rodríguez-Franco, M. I.; Arce, M. P.; González-Muñoz, G. C.; Villarroya, M.; López, M. G.; García, A. G. Nuevos derivados de aminoácidos dicarboxílicos y su aplicación en el tratamiento de enfermedades neurodegenerativas. ES Pat. P200703264 /PCT ES200807022.



POSTER COMMUNICATIONS – PC.132

HYDROXY DERIVATIVES OF 3-ARYLCOUMARINS AS TYROSINASE INHIBITORS

Saleta Vázquez (1), Elías Quezada (1), Santiago Vilar (1), Giovanna Delogu (2), Lourdes Santana (1), Antonella Fais (3), Benedetta Era (3)

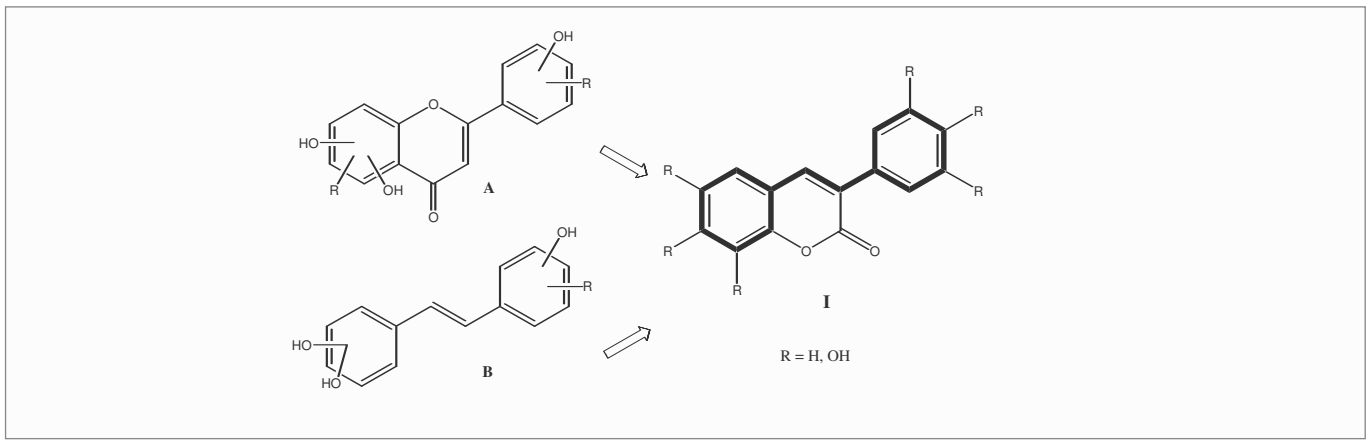
(1) Departamento Química Orgánica, Facultad de Farmacia, Universidad Santiago de Compostela, Spain
(2) Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Cagliari, Italy
(3) Dipartimento Scienze Applicate ai Biosistemi, Università degli Studi di Cagliari, Italy

Tyrosinase inhibitors have been tested as therapeutic agents for treatment of melanine hyperpigmentation. Many efforts have been addressed to the search for effective and safe tyrosinase inhibitors, and flavonoids (A) are one of the most best-studied group of polyphenols[1]. On the other hand, very recently have been found that stilbene-type oxi-resveratrol analogs (B) shown significant inhibitory activity[1]. In addition, some coumarin derivatives like umbelliferone have appeared as inhibitor of the mentioned oxidase[2].

On the basis of this information, we synthesized new hydroxylated coumarin-resveratrol hybrids (**I**) structurally related to the previously mentioned, in order to study the inhibition mechanisms to provide the basis for development new effective inhibitors.

To investigate the structure-activity relationships, the IC_{50} values of these compounds were measured. The preliminary biological studies showed that these compounds exhibited an interesting inhibitory activity on the tyrosinase.

1. Likhitwitayawuid, K. *Curr. Sci.* **2008**, *94*, 44-52.
2. Sollai, F.; Zucca, P.; Sanjust, E.; Steri, D.; Rescigno, A. *Biol. Pharm. Bull.* **2008**, *31*, 2187-2193.



BIOLOGICAL EVALUATION OF NEW TACRINE ANALOGUES FROM 4-AMINOPYRROLE-3-CARBONITRILE

Patrícia Silva (1), Francisco Peixoto (2), Abdelattif Salaheldin (3), Ana M.F. Oliveira-Campos (3), Lígia M. Rodrigues (3) and M. Manuel Oliveira (1)

(1) Centro de Química-Vila Real, UTAD, Quinta de Prados, 5001-801 Vila Real Portugal
(2) CECAV, UTAD, Quinta de Prados, 5001-801 Vila Real Portugal
(3) Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The Alzheimer's disease (AD) is recognized as one of the most severe conditions affecting the aged and it is life-threatening for this group of people. The disease is characterized by neuronal loss, synaptic damage, vascular plaques and a deficit in neurotransmitter acetylcholine (ACh) that leads to a progressive impairment in memory, cognitive functions and behavioral disturbances.

In order to increase the ACh level in the synapse, the inhibition of acetylcholinesterase (AChE) represents the currently employed approach for the treatment of AD. Tacrine was the one of first AChE inhibitor introduced in this therapy. The reduced selectivity of this drug for AChE and the need of increasing concentrations during long periods of treatment result in hepatoxic side-effects.

Many efforts have been made by different research groups on the synthesis of several tacrine analogues and their screening for biological activity[1-3].

The aim of this work was the study of inhibitory activities of the synthesized tacrine analogues from 4-aminopyrrole-3-carbonitrile against AChE. The study was performed by determining the rate of hydrolysis of acetylthiocholine (ATCI) in comparison with the reference compound tacrine using the method of Ellman *et al*[4].

IC_{50} values for compounds with AChE from *Electrophorus electricus* and using brain homogenates will be presented.

1. P. Valenti, A. Rampa, A. Bisi, V. Andrisano, V. Cavrini, L. Fin, A. Buriani, A. Giusti, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2599.
2. A. Martinez-Grau, J. L. Marco, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3165.
3. L. M. Rodrigues, C. S. Francisco, A. M. Campos, A. M. Salaheldin, *Synth. Commun.* **2008**, 38, 4369.
4. G. L. Ellman, K. D. Courtney, B. Jr. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **1961**, *7*, 88.

POSTER COMMUNICATIONS – PC.134

SYNTHESIS OF BIOACTIVE PRODUCTS USING ONE-POT CHEMICAL PROCESSES WHICH COUPLE RADICAL AND IONIC REACTIONS

Alicia Boto, Rosendo Hernández, and Javier Miguélez

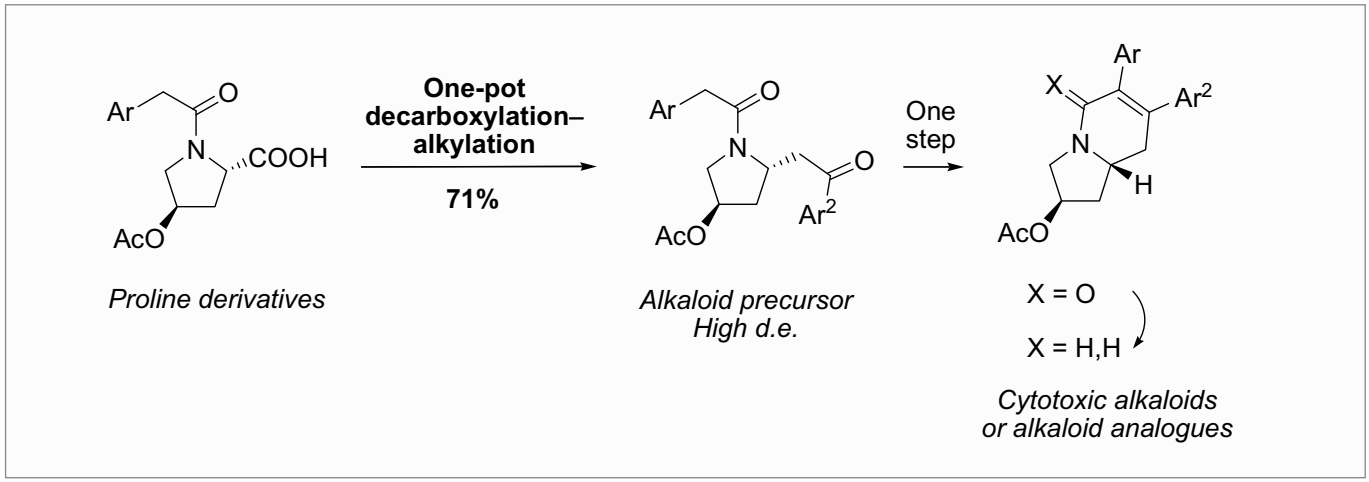
Instituto de Productos Naturales y Agrobiología CSIC, 38206-La Laguna, Tenerife, Spain.

The development of one-pot chemical processes, which couple radical and ionic reactions, has been carried out in our group to prepare bioactive products or their analogues[1]. These methodologies use relatively cheap starting materials such as sugar, amino acid or peptide derivatives, which are transformed into a variety of products (such as nucleoside analogues, alkaloid precursors, modified peptides, etc). In these processes, which couple radical and ionic reactions[2], the reaction intermediates were not isolated, but converted in situ into the next products. These strategies saved time and materials, and reduced the waste; besides, the reaction conditions were mild and high yields were usually achieved.

In this communication, we describe a stereoselective one-pot process which transformed proline derivatives into precursors of cytotoxic phenantroindolizidine alkaloids in good yields.

1. (a) As examples of these transformations, see: C. Saavedra, R. Hernández, A. Boto, E. Álvarez, *J. Org. Chem.* **2009**, *74*, 4655-4665. (b) A. Boto, D. Hernández, R. Hernández, *J. Org. Chem.* **2008**, *73*, 5287-5297; (c) A. Boto, J. A. Gallardo, D. Hernández, R. Hernández, *J. Org. Chem.* **2007**, *72*, 7260-7269; (d) A. Boto, D. Hernández, R. Hernández, E. Álvarez, *J. Org. Chem.* **2007**, *72*, 9523-9532; (e) A. Boto, D. Hernández, R. Hernández, *Org. Lett.* **2007**, *9*, 1721-1724; (f) J. Saavedra, R. Hernández, A. Boto, E. Álvarez, *Tetrahedron Lett.* **2006**, *47*, 8757-8760; (g) A. Boto, Y. León, J. A. Gallardo, R. Hernández, *Eur. J. Org. Chem.* **2005**, 3461-3468.

2. (a) For a review on the modification of amino acids and carbohydrates through radical chemistry, see: S. G. Hansen, T. Skrydstrup, *Top. Curr. Chem.* **2006**, *264*, 135-162; (b) For reviews on other methods coupling radical and ionic reactions, see: E. Godineau, Y. Landais, *Chem. Eur. J.* **2009**, *15*, 3044-3055.

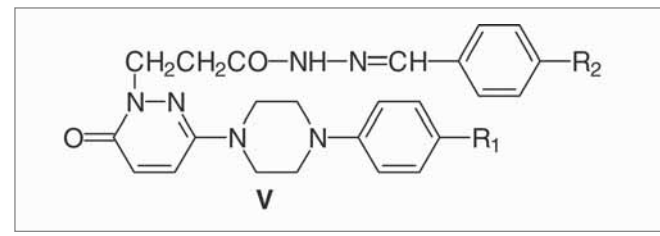


SYNTHESIS AND ANTIMICROBIAL ACTIVITY 6-SUBSTITUTED-3(*2H*)-PYRIDAZINONE-2-PROPYL-3-(*p*-SUBSTITUTED/NONSUBSTITUTED BENZAL)HYDRAZONE DERIVATIVES

Mehtap Gökçe, Berna Özçelýk, Fatma Kaynak, M. Fethý Þahýn

(1) Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry,
(2) Gazi University, Faculty of Pharmacy, Department of Microbiology, Beþevler, 06330-Ankara-TURKEY

The pyridazinone nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs. Furthermore a number of hydrazide-hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial-antifungal[1], anticonvulsant[2], antiinflammatory[3], antimalarial[4], analgesic[5,6], antiplatelets[7], antituberculosis[8] and anticancer activities[9]. Aroylhydrazide-hydrazones containing hetero-ring such as pyridine[3,10], indole[11], 1,2,4-oxadiazole[5], 1,2,3-triazole[6] and imidazo[2,1-b]thiadiazole ring[8] have attracted special attention. A few of pyrazole carbohydrazide hydrazone derivatives have also been reported[12,13]. These results led us to design new structurally related derivatives, keeping the 6-(substitute arylpiperazinyl)-3(2H)-piridazinone framework and modifiying the substitutent on the 2 position of the pyridazinone ring. As part of a program aimed at developing simple and efficient syntheses of pharmacologically useful pyridazinones, we synthesized new 6-substituted-3(*2H*)- pyridazinone-2-propyl-3-(p-substituted/nonsubstituted benzal)hydrazone **V** derivatives. All the targets compounds were identified by spectroscopic data and confirmed by elemental analysis. In order to determine the antibacterial activity of the title compounds derivatives two Gram positive, two Gram negative bacteria species and clinical isolates were screened. Also two candida species were used for antifungal activity. The assessment of the antimicrobial activities of the synthesized compounds was performed using the broth microdilution test.



V

1. Loncle, C.; Brunel, J.M.; Vidal, N.; Dherbomez, M.; Letourneux, Y. Eur. J. Med. Chem. **2004**, 39, 1067-1071.
2. Küçükgüzel, Þ.G.; Mazi, A.; Sahin, F.; Öztürk, S.; Stables J. Eur. J. Med. Chem. **2003**, 38, 1005-1013.
3. Todeschini, A.R.; Miranda, A.L.P.; Silva, K.C.M.; Parrini, S.C.; Barreiro, E.J. Eur. J. Med. Chem. **1998**, 33, 189-199.
4. Melnyk, V.; Leroux, P.; Sergheraert C.; Grellier P., Bioorg. Med. Chem. Lett., **2006**, 16 31-35.
5. Leite, L.F.C.C.; Ramos, M.N.; da Silva, B.P. ; Miranda, A.L.P.; Fraga, C.A.M.; Barreiro, E.J. Farmaco. **1999**, 54, 747-757.
6. Lima, P.C.; Lima, L., da Silva, M. K.C.M.; Le´da, P.H.O.; Miranda, A.L.P. de; Fraga, C.A.M.; Barreiro, E.J. Eur. J. Med. Chem. **2000**, 35, 187-203.
7. Cunha, A.C.; Figueiredo, J.M.; Tributino, J.L.M.; Miranda A.L.P. Castro, H.C.; Zingali R.B.; Fraga, C.A.M.; de Souza, M.C.B.V.; Ferreira V.F.; Barreiro, E.J. Bioorg. Med. Chem. **2003**, 11, 2051-2059.
8. Koçyiðit, B.; Oruç, E., Unsalan, S.; Kandemirli, F.; Shvets, N.; Rollas S.; Anatoly D. Eur. J. Med. Chem. **2006**, 41, 1253-1261.
9. Terzioglu, N.; Gürsoy, A. Eur. J. Med. Chem. **2003**, 38, 781-786.
10. Galic, N.; Peric, B.; Kojic-Prodic, B.; Cimerman, Z. J. Mol. Struct. **2001**, 559, 187-194.
11. Kaynak, F.B.; Öztürk, D.; Özbey, S.; Çapan, G. J. Mol. Struct. **2005**, 740, 213-221.
12. Rostom, Sh.A.F.; Shalaby, M.A.; El-Demellawy, M.A. Eur. J. Med. Chem. **2003**, 38, 959-974.
13. Bernardino, A.M.R.; Gomes, A.O.; Charret, K.S.; Freitas, A.C.C, Eur. J. Med. Chem.41, **2006**, 80-87.

POSTER COMMUNICATIONS – PC.136

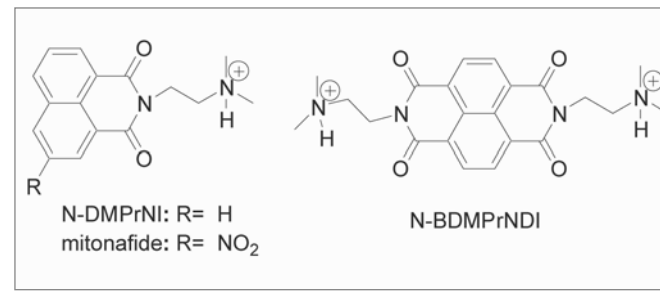
DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF SUBSTITUTED NAPHTHALENE IMIDES AND DIIMIDES AS ANTICANCER AGENTS

Vincenzo Tumiatti (1), Andrea Milelli (1), Anna Minarini (1), Marialuisa Micco (1), Anna Gasperi Campani (2) Laura Roncuzzi (2,4), Daniela Baiocchi (2), Maddalena Zini (3), Claudio Stefanelli (3) and Carlo Melchiorre (1)

(1) Department of Pharmaceutical Sciences, Via Belmeloro 6, (2) Department of Experimental Pathology, Via S. Giacomo 14, (3) Department of Biochemistry "G. Moruzzi", Via Irnerio 48, Alma Mater Studiorum-University of Bologna, 40126 Bologna, Italy. (4) Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, Bologna, Italy.

The search for novel chemotherapeutic agents and approaches to cancer treatment is an active research field stimulated by the discovery of new biological targets and by the possibility of obtaining new drugs without serious and undesirable side effects. Small molecules able to reversibly interact with the DNA structure represent a prolific research area for new potential anticancer agents. Such molecules, known as intercalators, are typically characterized by a planar heterocyclic moiety of approximately the size and shape of a DNA base pair. In the literature, there are several examples of naphthalimide (NI) and1,4,5,8-naphtalentetracaboxylic diimide (NDI) derivatives as intercalating agents[1]. Two important prototypes of these series are represented by N-DMPrNI and N-BDMPrNDI[2], which showed the ability to intercalate into steps containing at least one G:C base pair. These considerations prompted us to design derivatives using NI and NDI scaffolds as molecular building blocks. These new NI and NDI derivatives contain one or two basic polymethylene chains, respectively, in their structures, whose length may have relevance for the anticancer activity.

They were evaluated by in vitro assays for their antiproliferative activity in human breast cancer (SKBR-3) and leukemia (CEM) cell lines,



and the cytotoxic activity of the most interesting compounds, was investigated at molecular level in comparison to mitonafide.

This research was supported by grants from MIUR, Rome (PRIN), University of Bologna (RFO) and Polo Scientifico-Didattico di Rimini.

1. Brana, M. F.; Ramos, A. *Curr. Med. Chem. Anticancer Agents* **2001**, *1*, 237-255.
2. Liu, Z. R.; Hecker, K. H.; Rill, R. L. *J. Biomol. Struct. Dyn.* **1996**, *14*, 331-339.

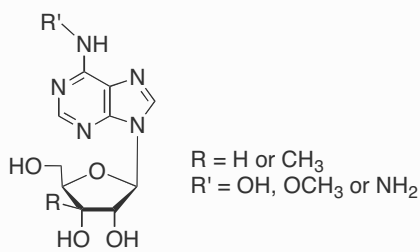
NEW N^6-SUBSTITUTED ADENOSINE AND 3′-*C*-METHYL- ADENOSINE DERIVATIVES AS ANTITUMOR AGENTS

Loredana Cappellacci (1), Riccardo Petrelli (1), Patrizia Vita (1), Palmarisa Franchetti (1), Praveen Kusumanchi (2), Mohineesh Kumar (2), Hiremagalur N. Jayaram (2) and Mario Grifantini (1)

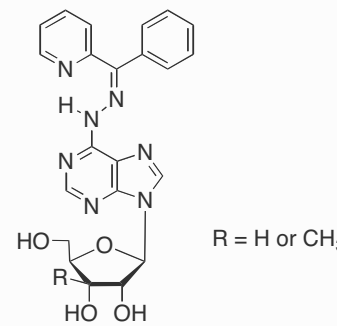
(1) Dipartimento di Scienze Chimiche, Università di Camerino, 62032 Camerino, Italy
(2) Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, U.S.A.

Many analogues of the natural nucleosides modified at nucleobase or at sugar moiety have been developed on the basis of their therapeutic potential as antitumor, antiviral and antiprotozoal agents. Among the N^6-modified adenosine analogues, N^6-hydroxy, N^6-methoxy and N^6-amino derivatives have proved to be potent cytotoxic agents acting through letal mutagenesis. Moreover, modification at the ribose moiety of purine nucleosides resulted in potent antitumor agents, such as in the case of 3′-*C*-methyladenosine (3′-Me-Ado), recently developed by us as a mechanism-based ribonucleotide reductase (RR) inhibitor, that displayed a significant cytotoxicity against a panel of human leukemia and carcinoma cell lines[1]. We now report on the synthesis and antitumor activity of a series of 3′-*C*-methyladenosine derivatives substituted at N^6 with a hydroxy, methoxy or amino group. Furthermore, azinyl hydrazones containing a N*-N*-N* structural motif able to inhibit RR were also prepared starting from N^6- amino-adenosine or *N*6-amino-3′-*C*-methyladenosine. The stereochemistry of these compounds was established to be Z by means of NMR spectroscopy. The antiproliferative activity of the substituted purine nucleosides against a panel of human tumor cell lines wiil be presented.

1. (a) Franchetti, P.; Cappellacci, L.; Pasqualini, M.; Petrelli, R.; Vita, P.; Jayaram, H. N.; Horvath, Z.; Szekeres, T.; Grifantini, M. *J. Med. Chem.* **2005**, *48*, 4983–4989. (b) Cappellacci, L.; Franchetti, P.; Vita, P.; Petrelli, R.; Lavecchia, A.; Jayaram, H. N.; Saiko, P.; Graser, G.; Szekeres, T.; Grifantini, M. *J. Med. Chem*. **2008**, *51*, 4260-4269.



R = H or CH_3
R' = OH, OCH_3 or NH_2



R = H or CH_3

POSTER COMMUNICATIONS – PC.138

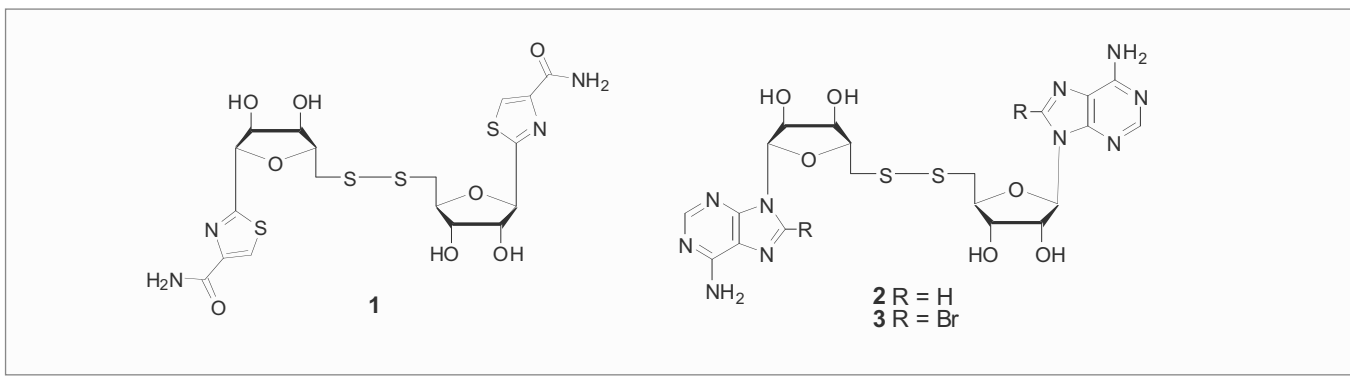
SELECTIVE INHIBITION OF NICOTINAMIDE ADENINE DINUCLEOTIDE KINASES (NADKs) BY COMPACT NAD MIMICS

Riccardo Petrelli (1,2), Krzysztof Felczak (1), Liqiang Chen (1), Patrizia Vita (2), Loredana Cappellacci (2), Palmarisa Franchetti (2), Mario Grifantini (2), Michele Di Stefano (3), Giulio Magni (3), and Krzysztof W. Pankiewicz (1)

(1) Center for Drug Design, University of Minnesota, Minneapolis, MN 55455, USA, (2) Dipartimento di Scienze Chimiche, Università di Camerino, 62032 Camerino, Italy, (3) Instituto di Biotecnologie Biochimiche, Università Politecnica delle Marche, 60131 Ancona, Italy

We have examined sequence diversity of 147 co-crystal structures of proteins utilizing NAD as a cofactor or as a substrate. Most of these proteins bind NAD in an *extended* conformation. However, there are clear exceptions to this general trend. Several bacterial reductases bind NAD in an extremely *folded* conformation and NADKs bind NAD in *bent* (compact) conformations. Although NAD can be accommodated in all of the above conformations, it is likely that compact NAD mimics should not fit to the binding domains of majority of NAD-utilizing enzymes that require an extended conformation of NAD.[1] It may be expected, therefore, that such compounds could be selective against NADKs. Tiazofurin adenine dinucleotide (TAD) is a potent inhibitor (K_i = 100 nM) of NAD-dependent IMP-dehydrogenase (IMPDH). This NAD mimic binds at the cofactor binding domain in the extended conformation. As a proof of concept, herein we report the synthesis of tiazofurin-5′-yl-adenosine-5′-yl disulfide[1] prepared by replacing the pyrophosphate (-O-P-O-P-O-) linkage by a short (-S-S-) disulfide bridge. We found that this compact disulfide analogue of TAD lost its activity against IMPDH and became a moderate inhibitor of *M. tuberculosis* (IC_{50} = 80 μM) NADK. We also found that di-(adenosine-5′-yl) disulfide (**2**), a by-product in the synthesis of **1**, showed slightly better activity against mycobacterium (IC_{50} = 45 μM) as well as human (IC_{50} = 87 μM) NADK. Introduction of bromine at the C-8 of adenine ring results in restriction of conformation of 8-bromo-adenosine to the *syn* conformation. We prepared the corresponding 8-bromo disulfide (**3**) and found further improvement in the inhibitory activity against human (IC_{50} = 6 μM) and mycobacterium (IC_{50} = 14 μM) NADKs. Compound **3** is the most potent inhibitor of NADKs reported so far. Using a simple method for preparation of disulfide **2** we synthesized a number of nucleobase and sugar modified analogues that have been evaluated against some NAD-dependent enzymes.

1. Chen, L.; Petrelli, R.; Felczak, K.; Gao, G.; Bonnac, L.; Yu, J.S.; Bennett, E.M,.; Pankiewicz, K.W. *Curr. Med. Chem.*, **2008**, *15*, 650-670.



MULTI-TARGET-DIRECTED LIGANDS AS ACETYLCHOLINESTERASE INHIBITORS AND NICOTINIC MODULATORS FOR ALZHEIMER'S DISEASE

Michela Rosini (1), Manuela Bartolini (1), Elena Simoni (1), Cecilia Gotti (2), David W. McClymont (3), Maria L. Bolognesi (1), Anna Minarini (1), Vincenzo Tumiatti (1), Vincenza Andrisano (1), Ian R. Mellor (3), Carlo Melchiorre (1)

(1) Department of Pharmaceutical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy
(2) CNR, Institute of Neuroscience, Milano, Italy, (3) School of Biology, University of Nottingham, U.K.

Alzheimer's disease (AD) is a complex neurological affliction, which is clinically characterized by loss of memory and progressive deficits in different cognitive domains. Numerous studies have highlighted that the cholinergic system is crucial for cognitive processes and that acetylcholine (ACh) function is profoundly compromised in AD. On this basis, a dominant strategy for AD treatment has focused on enhancing ACh-dependent neurotransmission, mainly by means of acetylcholinesterase inhibitors (AChEIs). Recently, however, growing interest has been devoted to the identification of neuronal nicotinic receptor (nAChRs) modulators that, in addition to their involvement in the cholinergic transmission, are actually considered to play important roles in neuroprotection, with particular relation to amyloid-β induced toxicity[1]. On these bases, the aim of the present study was to provide new multi-target-directed ligands, i.e. single molecules that can simultaneously exhibit a variety of pharmacological properties, leading to an effective and synergic treatment of AD. In particular, we wanted to enhance the cholinergic tone both by prolonging the action of endogenously released ACh through AChE inhibition, and by increasing the nicotinic transmission. To this purpose, we synthesized a series of compounds where a 1-(2-methoxybenzyl)-piperazine function, selected on the basis of the structural similarity between the ethyl-(2- methoxybenzyl)-amine group of the potent AChEI memoquin[2] and the 1-(2,4-dimethoxyphenyl)-piperazine moiety of the α7 nAChR agonist SEN10/WAY-264620[3], was linked with different length spacers to a carbazole moiety, which is shown to exert anti-amyloidogenic properties[4] and to interact with AChE gorge[5].

This research was supported by grants from MIUR, Rome (PRIN), University of Bologna (RFO).

1. Buckingham S. D., Jones A. K., Brown L. A., Sattelle D. B. Pharmacol. Rev. (61), 39-61, 2009.
2. Cavalli A., Bolognesi M., Capsoni S., Andrisano V., Bartolini M., Margotti E., Cattaneo A., Recanatini M., Melchiorre C. Angew. Chem. Int. Chem. Ed. (46), 3689-92, 2007.
3. Gaviraghi G., Ghiron C., Bothmann H., Roncarati R., Terstappenn G. C. PCT Int. Appl. (2006), W O 2006008133.
4. Howlett D.R., Gorge A.R., Owen D.E., W ard R.V., Markwell R.E. Biochem. J. (343), 419-23, 1999.
5. Rosini M., Simoni E., Bartolini M., Cavalli A., Ceccarini L., Pascu N., McClymont David W ., Tarozzi A., Bolognesi M.L., Minarini A, Tumiatti V., Andrisano V., Mellor I.R., Melchiorre C. J. Med. Chem. (51), 4381-84, 2008.

POSTER COMMUNICATIONS – PC.140

NOVEL INHIBITORS OF FATTY ACID SYNTHASE (FASN) WITH ANTICANCER ACTIVITY

Bellinda Benhamú (1), Carlos Turrado (1), Silvia Ortega-Gutiérrez (1), Teresa Puig (2), Joana Relat (3), Gemma Casals (2), Gloria Oliveras (2), Pedro F. Marrero (3), Diego Haro (3), Silvia Cufí (4), Helena Aguilar (4), Ander Urruticoechea (4), Ramón Colomer (2,5) and María L. López-Rodríguez (1)

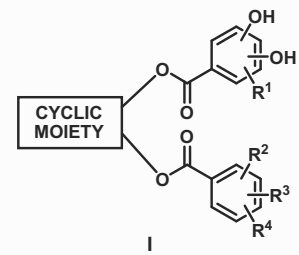
(1) Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain, (2) Institut Català d'Oncologia and Institut d'Investigació Biomèdica de Girona, E-17007 Girona, Spain, (3) Departament de Bioquímica i Biologia Molecular, Institut de Biomedicina, Universitat de Barcelona, E-08028 Barcelona, Spain, (4) Laboratori Translacional, Institut Català d'Oncologia, Hospital Duran i Reynals, E- 08907 L'Hospitalet de Llobregat (Barcelona), Spain, (5) Centro Oncológico MD Anderson España, E-28033 Madrid, Spain

Fatty acid synthase (FASN) has recently emerged as a promising target for antitumor drug development, since it 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.

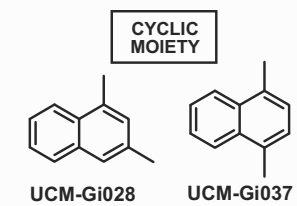
Targeting FASN inhibition, in the present work we have designed and synthesized a new series of compounds of general formula **I**, that were evaluated for their cytotoxic capacity in a panel of human breast cancer cell lines (SK-Br3, MCF-7 and MDA-MB-231) and in two types of non-malignant cells (fibroblasts and adipocytes), as well as for their effect in FASN activity[2].

The biological evaluation has resulted in the identification of two potent FASN inhibitors –UCM-Gi028 and UCM-Gi037 (90% and 70% inhibition of FASN activity)–, that are highly cytotoxic against SK-Br3 cells (IC_{50} = 21 and 29 μM) without affecting the growth of non- malignant cells. Importantly, the compounds do not stimulate carnitine palmitoyltransferase-1 (CPT-1) activity *in vitro* (89% and 90% activity of control) and they do not decrease body weight *in vivo* (97% and 95% of control), in contrast to previously reported FASN inhibitors (such as C75).

Altogether, our results show that synthesized compounds **I** represent a novel class of FASN inhibitors with *in vitro* 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 antitumor drugs. We are currently exploring their full potential with the synthesis of a larger structural field focused on an in-depth structure-activity relationship (SAR) study, as well as assessing their *in vivo* therapeutic properties in breast cancer animal models. These results will enable the definitive validation of FASN as a therapeutically useful option for cancer treatment.



I



UCM-Gi028 **UCM-Gi037**

$R^1=R^2=R^3=R^4=OH$

This work was supported by grants from Ministerio de Educación y Ciencia (MEC, SAF-2007/67008-C02-01), Instituto de Salud Carlos III (FIS PI04/1417, RD06-0020-0028, ISCIII-RETIC RD06), Comunidad Autónoma de Madrid (CAM, S-SAL-249-2006) and the Susan G. Komen Breast Cancer Foundation (PDF-0504073). The authors are grateful to MEC for Ramón y Cajal (SO-G) and FPU (CT) fellowships.

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 PCT/EP/2008/058099.

STRUCTURAL REQUIREMENTS OF P-GLYCOPROTEIN INHIBITION BY DIHYDRO-ß-AGAROFURAN SESQUITERPENES

Iván R. Torrecillas (1), Carolina P. Reyes (2), Cristina R. Mendoza (2), Leonardo Pardo (1), Santiago Castanys (3), Isabel L. Bazzocchi (2), Francisco Gamarro (3), Ignacio A. Jiménez (2), and Mercedes Campillo (1)

(1) Laboratori de Medicina Computacional, Unitat de Bioestadistica, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallés, Barcelona. (2) Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna and Instituto Canario de Investigación del Cáncer, Avenida Astrofísico Francisco Sánchez, 2, 38206 La Laguna, Tenerife. (3) Instituto de Parasitología y Biomedicina "López-Neyra", Consejo Superior de Investigaciones Científicas, Parque Tecnológico de Ciencias de la Salud, Avda. del Conocimiento s/n, 18100 Armilla, Granada, Spain.

P-glycoprotein (P-gp) is a member of ATP-Binding Cassette (ABC) transporters family which translocates a surprisingly broad spectrum of amphiphilic substrates across cellular membrane. This transmembrane transporter pumps out of the cell xenobiotics and drugs causing a multidrug-resistance (MDR) phenotype. Drug resistance has emerged as a major problem to chemotherapy and the treatment of infectious diseases. This mechanism is one of the main reasons cancer cells become resistance to chemotherapy drugs. During the last 20 years much research has focused in P-glycoprotein inhibition to permit success chemotherapy of cancer.

Dihydro-beta-agarofuran sesquiterpenes from Celastraceae family of plants, are natural compounds previously shown to reverse MDR in several tumor cell lines and Leishmania strains[1]. A set of 79 sesquiterpenes has been tested on a multidrug-resistant resistant Leishmania tropica line overexpressing P-glycoprotein to determine their ability to revert the resistance phenotype and to modulate the intracellular accumulation of the classical P-gp substrate daunomycin.

We have performed a three-dimensional quantitative structure-activity relationship (3D-QSAR) model using *Accelrys Discorery Studio*[2]. Since P-glycoprotein is thought to interact with substrates from inner leaflet the membrane, subsets of sesquiterpenes were performed according to their solvation energy, and the pharcophore hypothesis obtained were evaluated in order to elucidate the common features required to interact with the protein. The modeling of P-glycoprotein with the new structural data available[2,3], and the pharmacophores obtained suggest the minimum hydrophobic and hydrogen-bond acceptor features necessary for P-glycoprotein inhibition by sesquiterpenes.

1. Cortés-Selva F, Jiménez IA, Munoz-Martínez F, Campillo M, Bazzocchi IL, Pardo L, Ravelo AG, Castanys S, Gamarro F. Curr Pharm Des. 2005;11(24):3125.
2. Accelrys, San Diego, CA,USA. (http://accelrys.com)
3. Ward A, Reyes CL, Yu J, Roth CB, Chang G. Proc Natl Acad Sci U S A. **2007**, 104(48):19005.
4. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G. Science. 2009; 323:1718.

POSTER COMMUNICATIONS – PC.142

FUNCTIONAL CHARACTERIZATION OF DIAMINOPYRIMIDINES AS HISTAMINE H_4 RECEPTOR LIGANDS

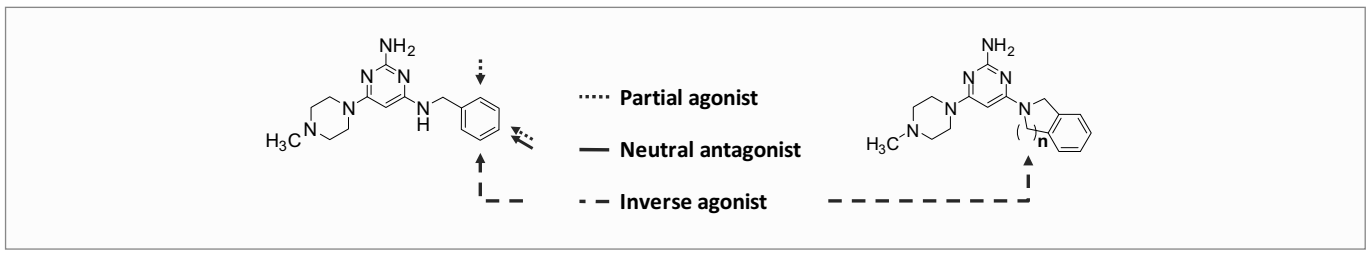
Kerstin Sander (1), Tim Kottke (1), Yusuf Tanrikulu (2), Ewgenij Proschak (2), Erich H. Schneider (3), Roland Seifert (4), Gisbert Schneider (2) and Holger Stark (1)

(1) Johann Wolfgang Goethe-University, Institute of Pharmaceutical Chemistry, ZAFES/Liff/CMP, Max- von-Laue-Str. 9, 60348 Frankfurt am Main, Germany
(2) Johann Wolfgang Goethe-University, Institute of Organic Chemistry and Chemical Biology, ZAFES/Liff/CMP, Siesmayerstr. 70, 60323 Frankfurt am Main, Germany
(3) University of Regensburg, Department of Pharmacology and Toxicology, Universitätsstr. 1, 93053 Regensburg, Germany
(4) Medical School of Hannover, Institute of Pharmacology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

The human histamine H4 receptor (hH$_4$R) is predominantly expressed on hematopoietic mononuclear cells. It is involved in immune and inflammatory responses as histamine signaling induces cell shape change and chemotaxis of mast cells as well as eosinophils, mast cell migration, and upregulation of adhesion molecules on monocytes. From these physiological reactions one can deduce several potential therapeutic indications for hH$_4$R antagonists/inverse agonists in the broad field of anti-inflammatory therapy and (neuropathic) pain[1].

By combining modern screening methods, i.e. ligand-based virtual screening[2] and pseudoreceptor modeling[3], and classical approaches in medicinal chemistry, i.e. Topliss scheme, we developed a series of *N*^{4}-benzyl-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamines (Figure) resulting in hH$_4$R ligands with affinities in the nanomolar concentration range. Some compounds showed moderate selectivity over the other histamine receptor subtypes (hH$_1$R, hH$_2$R, hH$_3$R)[4]. Here, we report structure-efficacy relationships of these new ligands: interestingly, slight structural changes on substitution pattern and conformational restriction evoke extensive differences in functionality and lead to either partial agonists or antagonists/inverse agonists.

1. Zampeli, E.; Tiligada, E. The role of histamine H4 receptor in immune and inflammatory disorders. *Br. J. Pharmacol.* **2009**, *157*, 24-33.
2. Schneider, G.; Neidhart, W.; Giller, T.; Schmid, G. "Scaffold-hopping" by topological pharmacophore search: a contribution to virtual screening. *Angew. Chem. Int. Ed.* **1999**, *38*, 2894-2896.
3. Tanrikulu, Y.; Proschak, E.; Werner, T.; Geppert, T.; Todoroff, N.; Klenner, A.; Kottke, T.; Sander, K.; Schneider, E.; Seifert, R.; Stark, H.; Clark, T.; Schneider, G. Homology model adjustment and ligand screening with a pseudoreceptor of the human histamine H4 receptor. *ChemMedChem* **2009**, *4*, 820-827.
4. Sander, K.; Kottke, T.; Proschak, E.; Tanrikulu, Y.; Schneider, E.; Seifert, R.; Schneider, G.; Stark, H. Development of diaminopyrimidines as histamine H4 receptor ligands - virtual screening and scaffold optimization. *XXXVIIIth Meeting of the European Histamine Research Society*, Fulda, Germany **2009**, O27.



SYNTHESIS OF NEW nNOS PDZ LIGANDS

Ana María Pérez López (1), José Francisco Domínguez Seglar (1), Rafael Salto (2), María Dolores Girón (2), Natalia Sevillana (2), José Antonio Gómez Vidal (1)

(1) Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia (Universidad de Granada), Campus de Cartuja, s/n 18071 Granada (E-mail: jagvidal@ugr.es)
(2) Departamento de Bioquímica y Biología Molecular II, Facultad de Farmacia (Universidad de Granada), Campus de Cartuja, s/n 18071 Granada

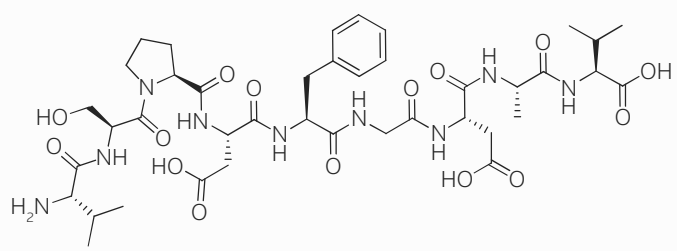
The signalling molecule nitric oxide (NO) plays important roles these including nervous system. There are three different isoforms of nitric oxide synthase (NOS) which account for NO production: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). nNOS and eNOS are constitutively expressed and their activities are calcium dependent. Of the three isozymes, nNOS is the predominant source of NO in neurons[1].

Active nNOS is in a dimeric form. Each monomer consists of an oxygenase domain (N-terminal) and a reductase domain (C-terminal) both separated by a calmodulin binding motif. The oxygenase domain binds the substrate L-Arginine and catalyzes its oxidation to citrulline and NO. Also, a PDZ domain is located at the N-terminal which mediates protein-protein interactions. Many proteins can interact directly with the nNOS PDZ domain this influencing subcellular localization of nNOS, and eventually the regulation of its functions.

The nNOS PDZ domain participates in linking nNOS to N-methyl-D-aspartate receptor (NMDA) via PSD95 (a scaffolding protein). It has been established that inhibitors of protein-protein interactions mediated by the nNOS PDZ domain could behave as neuroprotective drugs[2].

The peptide VSPDFGDAV has been described as a potent ligand of the nNOS PDZ domain[3]. A new solid phase synthesis of analogues of this peptide and the affinity determination to the nNOS PDZ domain is discussed.

1. Zhou, Li; Zhu, D. Neuronal nitric oxide synthase: Structure, subcellular localization, regulation, and clinical implications. *Nitric Oxide* **2009**, *20*, 223-230.
2. Cao, J.; Viholainen, J. I.; Dart, C.; Warwick, H. K.; Leyland, M. L.; Courtney, M. J. The PSD95-nNOS interface: A target for inhibition of excitotoxic p38 stress-activated protein kinase activation and cell death. *J. Cell Biol.* **2005**, *168(1)*, 117-126.
3. Stricker, N. L.; Christopherson, K. S.; Yi, B. A.; Schatz, P. J.; Raab, R. W.; Dawes, G.; Bassett, D. E., Jr.; Bredt, D. S.; Li, M. PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. *Nat. Biotechnol.* **1997**, *15(4)*, 336-342.



VSPDFGDAV

IDENTIFICATION OF NEW LIGANDS FOR σ RECEPTORS. DESIGN AND SYNTHESIS OF A β–AMINOKETONIC DRUG DISCOVERY LIBRARY

Daniela Rossi (1), Maria Valeria Raimondi (2), Alice Pedrali (1), Ornella Azzolina (1) Simona Collina (1)

(1) Department of Pharmaceutical Chemistry, University of Pavia, Viale Taramelli, 12-27100 Pavia, Italy (2) Department of Medicinal Chemistry and Technology, University of Palermo, Via Archirafi, 32-90123 Palermo, Italy

In the last years our research has been focused on the design, synthesis and pharmacological evaluation of new potential σ ligands based on arylalkenyl- and arylalkyl-aminic and (N-alkylaminoalkyl-substituted)arylalkenylamidic scaffolds[1,2]. Generally, binding assays showed significant σ1 affinity and interesting selectivity for the σ1 compared to the σ2 receptor. A moderate affinity for σ2 receptor was also observed for some compounds. In Figure 1 the most interesting ligands with their affinities are reported[1].

With the aim to deeply investigate the molecular features involved in σ receptor binding and to discover new σ ligands, we addressed our efforts to the design and preparation of a new class of potential ligands. Our approach consisted in designing a discovery library based on a new β-aminoketonic scaffold. The library design was carried out taking into account the synthetic feasibility, the commercial availability of building blocks as well as molecular modeling suggestions.

Thus, an efficient and easy-to-use protocol, based on combined Microwave Assisted Organic Synthesis (MAOS), Polymer Assisted Solution Phase Synthesis (PASPS) and Solid Phase Extraction (SPE) was developed. Interestingly, the optimized procedure led us to prepare the designed library compounds with high purity, suitable for biological screening.

Synthetic procedures applied for the preparation of desired compounds as well as preliminary binding data will be presented and discussed.

1. Collina S, et al. *Bioorg Med Chem*, 2007; 15: 771.
2. Urbano M, et al. *Lett. Drug Des. Disc.,* 2007, 4 605.

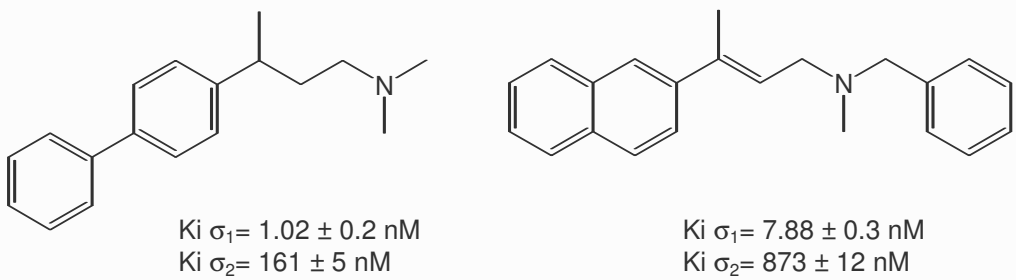


Figure 1

CHROMONE-BASED ADENOSINE LIGANDS AS A NEW CHALLENGE IN DRUG DEVELOPMENT FOR THE TREATMENT OF NEUROLOGICAL DISORDERS: THE STORY SO FAR

Alexandra Gaspar (1), Tiago Silva (1), Elsa Reis (1), Nuno Milhazes (2), Manuela Garrido (3), Jorge Garrido (3), Fernanda Borges (1), Eugenio Uriarte (4), Nasir Khan (5), Inês Seabra (5), Jorge Gonçalves (5), Jorge Oliveira (5)

(1) CIQ/Departamento de Química, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; (2) Instituto Superior de Ciências da Saúde-Norte, Gandra, PRD, Portugal; (3) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal; (4) Departamento de Química Orgânica, Facultad de Farmacia, Universidad de Santiago de Compostela, Espana; (5) REQUIMTE, Serviço de Farmacologia, Faculdade de Farmácia da Universidade do Porto, Portugal

One of the most exciting processes in drug discovery is the finding of new targets and the discovery of new leads. In fact, in the neurodegenerative area adenosine receptors (AR) of A_2 type are increasingly viewed as a prolific and druggable class of targets. It has been shown that A_2AR are abundantly expressed in striatal neurons, a characteristic which prompted to examine the effects of specific agonists/antagonists to this receptor in the context of disorders where communication to or from striatal neurons is disturbed, such as Parkinson's and Huntington's disease. The rationale is that pharmacological modulation of A2A-receptors holds the potential for improved symptomatic control and possibly slowdown of disease progression, thus prompting the development of novel, selective and potent AR receptor ligands.

Lead discovery and optimization, guided by structure-activity-relationships (SAR) and quantitative-structure-activity relationships (QSAR), of new A2AR ligands based on chromone scaffold is the aim of the present project. Accordingly, a library of novel chromone derivatives was obtained through the application of innovative parallel and solid phase synthetic strategies (PCT/IB2008/050674). The compounds have been screened towards different targets (see the other communication of the research group) and the affinity of each compound for adenosine receptors subtypes was determined by radioligand binding assays, namely evaluating their ability to displace [^{3}H]-DPCPX, [^{3}H]- ZM241385, [^{3}H]-DPCPX, and [^{3}H]-NECA from cloned human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors. As a first indication of a putative therapeutic window, *in vitro* neurotoxicity assays were conducted in order to ascertain the concentration range between significant receptor binding and the occurrence of neuronal damage. In this communication some of the results obtained so far will be presented.

The authors thank financial support from FCT (Portugal)-Project PTDC/QUI/70359/2006.

1. Diniz C, Borges F, Santana L, Uriarte E, Oliveira JM, Gonçalves J, Fresco P., *Curr Pharm Des.* **2008**,*14*,1698;
2. Baraldi PG, Romagnoli R, Preti D, Fruttarolo F, Carrion MD, Tabrizi MA.*Curr Med Chem.* **2006**, *13*, 3467;
3. Jacobson KA, Gao ZG. *Nat Rev Drug Discov*. **2006**, 5, 247.

POSTER COMMUNICATIONS – PC.146

PYRAZOLOCHLORIN DERIVATIVES: SYNTHESIS AND PHOTOPHYSICAL PROPERTIES

Nuno M. M. Moura (1), Francesca Giuntini (1), Maria A. F. Faustino (1), Maria G. P. M. S. Neves (1), Augusto C. Tomé (1), E. M. Rakib (2), A. Hannioui (2), S. Abouricha (2), Beate Röder (3), José A. S. Cavaleiro (1)

(1) QOPNA and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
(2) Equipe d´Hetérochimie Organique, Facultés des Sciences et Tecnhiques, BP 523, 2300 Beni-Mellal, Maroc
(3)Institut für Physik, Photobiophysik, Humboldt Universität, Newtonstr. 15, D-12489 Berlin, Germany e-mail: nunomour@gmail.com

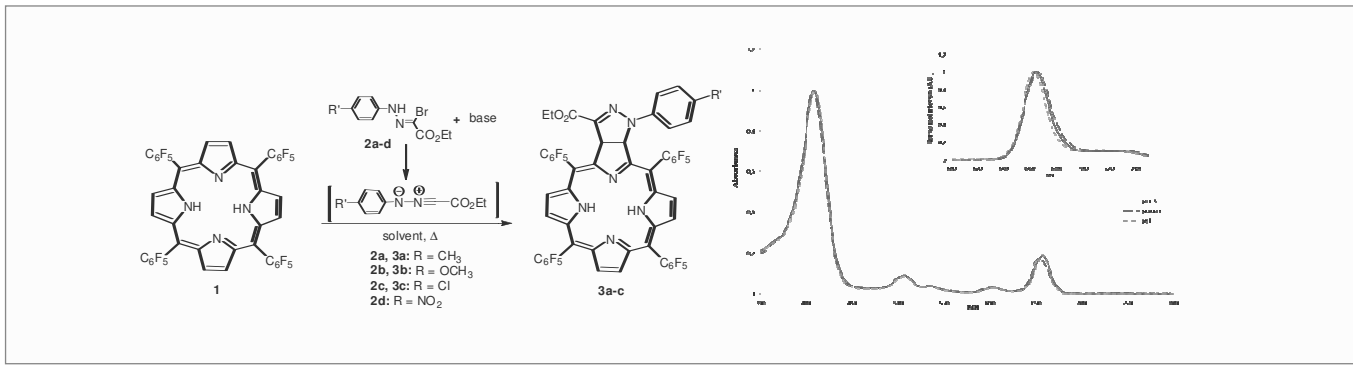
The development of new synthetic methods leading to reduced porphyrins (mainly chlorins and bacteriochlorins) is an area of huge interest. This is due to the significant potentialities of this type of compounds as efficient photosensitizers for photodynamic therapy (PDT) namely their intense absortion band in the red region[1]. PDT combines three components: light, molecular oxygen and a photosensitizer (PS). The PS plays an important role on the efficiency of the photodynamic process and must fulfill some properties. Regardless of other requisites, all PS must have good absorption capacity at the wavelength of the spectral region where the light have better penetration in the tissue and show good efficiency to generate singlet oxygen[1].

Our group has shown that *meso*-tetraarylporphyrins can participate in 1,3-dipolar cycloaddition reactions with a variety of 1,3-dipoles leading to chlorins, bacteriochlorins and isobacteriochlorins in good yields[2]. Nitrile imines (propargyl-allenyl type 1,3-dipoles) have been extensively used in 1,3-dipolar cycloaddition reactions with alkenes to synthesize pyrazolines and pyrazoles[3].

Following our interest on the preparation of tetrapyrrolic derivatives with potential application in PDT[2], we prepared chlorin derivatives **4** by 1,3-dipolar cycloaddition of nitrile imines to 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin[4]. In this communication we will present and discuss the synthesis and the photophysical properties of the new chlorin derivatives.

Thanks are due to the University of Aveiro, "Fundação para a Ciência e a Tecnologia" (FCT) and FEDER for funding the Organic Chemistry Research Unit. NMM Moura also thanks FCT for his PhD grant SFRH/BD/44630/2008. MAFF thanks DAAD for funding her stay at Humboldt-Universität zu Berlin.

1. Sternberg, E. D.; Dolphin, D. *Tetrahedron* **1998**, 54, 4151.
2. Cavaleiro, J. A. S.; Neves, M. G. P. M. S.; Tomé, A. C. *Arkivoc*, **2003**, (xiv), 107.
3. a) Wang, Y.; Rivera Vera, C. I.; Lin, Q. *Org. Lett.* **2007**, 9 (21), 4155; b) Atir, R.; Mallouk, S.; Bougrin, K.; Soufiaoui, M. *Synth. Commun*. **2006**, 36, 111.
4. Moura, N. M. M., F. Giuntini, Maria A. F. Faustino, Maria G. P. M. S. Neves, Augusto C. Tomé, Artur M. S. Silva, E. M. Rakib, A. Hannioui, S. Abouricha, Beate Röder, José A. S. Cavaleiro *Arkivoc*, 2010, (v), 24.



BIOLOGICAL INVESTIGATIONS OF NSAID METAL DERIVATIVES

Kerstin Bensdorf and Ronald Gust

Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, 14195 Berlin

Cyclooxygenases (COX) are the key enzymes in the production of prostaglandins which do not exhibit only physiological effects. Since COX are overexpressed in certain tumor cells they play an important role in tumor development and progression. Increased levels of prostaglandins cause a greater aromatase expression and thus intensified growth of hormone dependent cancers. Furthermore apoptosis is inhibited by increase of the Bcl-2 expression and lowered caspase-3 activity. The COX effects can be regulated by COX inhibition as well as by influencing the COX expression. NSAIDs and some metals are known for their COX inhibiting effects. Metal containing NSAID derivatives are tested for their effects on cancer cells with regards to their influence on the cellular prostaglandine E_2 levels and on isolated cyclooxygenases. The effects on apoptosis will be investigated in a caspase-3 and in a Bcl-2 assay. The influence on COX expression mediated by NFκB will be determined in western blot analyses.

SYNTHESIS AND INVESTIGATION ON THE MODE OF ACTION OF (4R,5S)/(4S,5R)- AND (4R,5R)/(4S,5S)- 2,4,5-TRIARYL-4,5-DIHYDRO-1H-IMIDAZOLE DERIVATIVES

Sabrina Elsner, Ronald Gust

Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, 14195 Berlin

Almost 60% of mammary carcinoma are hormone dependent. The tumor cells contain estrogen receptors and require estradiol for proliferation. Selective estrogen receptor modulators (SERMs) are frequently used in therapy because they have antiestrogenic effects in breast tissue but estrogenic effects in bones and the cardiovascular system.

In recent years our group synthesised a lot of imidazole derivatives, which meet the structural requirement for interactions with estrogen receptors. The compounds are currently investigated for their effects against human hormone (in)dependent tumor cells. In vitro compounds with lipophilic substituents show cytotoxicity while OH groups prevent these effect. Whether cell death is caused by necrotic or apoptotic processes has to be resolved in LDH-release- and caspase-3-assays. An interaction with estrogen receptors is only found for the hydroxylated derivatives. Receptor binding and the influence of the compounds on receptor density will be investigated in different cell based assays and with isolated receptors. Further compounds have to be synthesised performing SAR studies which should reveal the essential structural elements for cytotoxic and (anti)estrogenic effects. For this purpose we are now establishing new synthesis methods using a microwave.

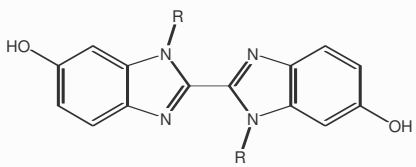
SYNTHESIS OF 1,1′-DIALKYL- AND 1,1′-ALKYL-BRIDGED- 2,2′-BISBENZIMIDAZOLE DERIVATES FOR VISUALISING THE ESTROGEN RECEPTORS IN HUMAN CANCER CELLS

Kathrin Szymanowitz, Anja Wellner and Ronald Gust

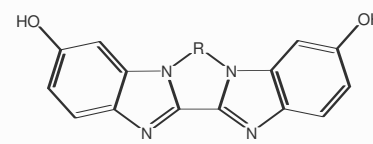
Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, 14195 Berlin

Breast cancer is one of the most frequent cancers in women. About 60 percent of this tumor entity are hormone dependent, which means they contain estrogen receptors and require estradiol for growing. Therefore the differentiation between hormone dependent and hormone independent cancers plays an important role with regards to the individual therapy. Presently radioactive methods are used for diagnostic. In recent years promising fluorescent 1,1′-dialkyl- and 1,1′-alkyl-bridged-2,2′-bisbenzimidazole derivates have been synthesised in our group. These compounds shall be used for visualising the estrogen receptor in a non-radioactive method. Furthermore compounds with a potential (anti)estrogenic effect have been developed.

All of these compounds meet the structural requirements to interact with estrogen receptors. Currently methods are set up to determine their binding quality to the estrogen receptors in whole cell assays and with isolated receptors. The (anti)estrogenic effect and the influence on the receptor density of these compounds are investigated in transactivation assays and western blots.



1,1′-dialkyl-2,2′-bisbenzimidazol-6,6′-diol



1,1′-alkyl-bridged-2,2′-bisbenzimidazol-6,6′-diol

HORMONALLY ACTIVE COMPOUNDS AND THEIR EFFECTS IN HUMAN TUMOR CELLS

Anja Wellner and Ronald Gust

Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, 14195 Berlin

At this time it is known that almost 60% of breast cancers are hormone dependent. Hormone dependence is given by the existence of hormone receptors such as estrogen receptors. Effects mediated via these receptors are important for proliferation. The differentiation between estrogen receptor subtypes alpha and beta is of significance since the subtypes differ in tissue distribution as well as in mediated effects. In recent years our group synthesised a large number of compounds, such as 2-imidazolines, that meet the structural requirements for interactions with estrogen receptors. To which extent they cause cytotoxic effects and whether these effects are specific for hormone dependent tumor cells, we examine in crystal violet assays using different human cell lines. Receptor binding will be determined in chemiluminescence assays and with radioactive methods. The influence of the compounds on receptor density and whether they are able to subtype specifically activate or block the estrogen receptor will be investigated in cell based assays.

NEW INSIGHTS INTO EPOTHILONE-TUBULIN INTERACTIONS. FIRST DETERMINATION OF THE CONFORMATION OF EPOTHILONE B BOUND TO DIMERIC TUBULIN

A. Canales (1), L. Nieto (2), K.H. Altmann (3), F. Díaz (2), J. Jiménez-Barbero (2)

(1) Dpto. Química Orgánica I, Facultad de Ciencias Químicas, Univ. Complutense, E-28040, Madrid, Spain ma.canales@quim.ucm.es; (2) Centro de Investigaciones Biológicas. CSIC, Madrid, Spain; (3) Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland

During the last years, great efforts have been made to obtain the bioactive conformation of epothilones. The solid state (deduced by either X-ray or solid state NMR) structure of free epothilone B is known[1,2], as well as its conformations in organic solvents[3]. However, its tubulin bound bioactive conformation is still a controversial issue.

According to the NMR analysis in non polar solvents, the most populated conformer of epothilone in the free state is indeed very similar to the x-ray conformer (crystal obtained from dichloromethane).[2] This conformer is characterized for a *gauche+* disposition around the C2-C3-C4-C5 dihedral angle and an *anti*-periplanar geometry for the C16-C17-C18-C19 torsion.

The first epothilone tubulin-bound conformation was determined by solution state NMR, using an unpolymerized soluble form of $\alpha\beta$ tubulin complexed to epothilone A[4]. Almost immediately, the tubulin/epothilone A complex was also studied through electron-crystallography, using tubulin sheets[5]. The conformation of the tubulin-bound epothilone was strikingly different in the two studies, suggesting the need for further investigation. As this discrepancy may reflect the dependence of the epothilone binding mode on the tubulin polymerization state, or the presence of different binding sites in the protein, further studies are required.

The aim of the present work is to determine the conformation of epothilones A and B bound to dimeric tubulin in solution by NMR to gain insight into the epothilone-tubulin recognition processes.

1. Lange, A., Schupp, T., Petersen, F., Carlomagno, T. & Baldus, M. (2007) High-resolution solid-state NMR structure of an anticancer agent, *Chem.Med.Chem. 2*, 522-7.
2. Höfle G, B. N. & Steinmetz, H. (1996) Epothilone A and B Novel 16 Memebered Macrolides with Cytotoxic Activity: Isolation, Crystal Structure, and Conformation in Solution, *Angew. Chem. Int. Ed .Engl. 35*, 1567-1569.
3. Taylor, R. E. & Zajicek, J. (1999) Conformational properties of epothilone, *J. Org. Chem. 64*, 7225-7228.
4. Carlomagno, T., Blommers, M. J., Meiler, J., Jahnke, W., Schupp, T., Petersen, F., Schinzer, D., Altmann, K. H. & Griesinger, C. (2003) The high-resolution solution structure of epothilone A bound to tubulin: an understanding of the structure-activity relationships for a powerful class of antitumor agents, *Angew. Chem. Int. Ed. Engl. 42*, 2511-5.
5. Nettles, J. H., Li, H., Cornett, B., Krahn, J. M., Snyder, J. P. & Downing, K. H. (2004) The binding mode of epothilone A on alpha,beta-tubulin by electron crystallography, *Science,305*, 866-9.

POSTER COMMUNICATIONS – PC.152

SYNTHESIS, BINDING AFFINITY AND SAR OF NEW *N*-CONTAINING HETEROCYCLIC ANALOGUES OF HALOPERIDOL AS POTENTIAL ATYPICAL ANTIPSYCHOTICS

Enrique Raviña (1), María Barceló (1), Christian F. Masaguer (1), María Isabel Loza (2), José Brea (2), Manuel Pastor (3) and Ferran Sanz (3)

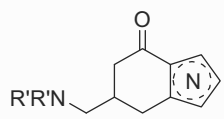
(1) Department of Organic Chemistry, and (2) Department of Pharmacology. Faculty of Pharmacy. University of Santiago de Compostela. 15782-Santiago de Compostela, Spain. (3) Computer-Assisted Drug Design Laboratory, Research Unit on Biomedical Informatics (GRIB), IMIM, Universitat Pompeu Fabra, Barcelona, Spain

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 newer 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].

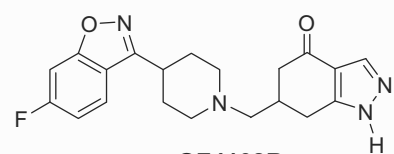
In this communication, we will describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D_2 antagonists for potential use as treatments for schizophrenia. A typical bioisosteric replacement of the benzene in the aminobutyrophenone pharmacophore by a five-membered heterocycle (I) containing at least one *N* (pyrazole, isoxazole, oxazole, thiazole) has been applied. From this work, compound **QF4108B** has emerged as a new lead because of its favourable pharmacological profile.

We thank the MEC (Ref SAF2005-08025-C03) for the financial support. M. B. thanks the Spanish Ministerio de Educación y Cultura for a predoctoral Fellowship.

1. Meltzer, H. Y.; Matsubara, S.; Lee, J. *J. Pharm. Exp. Ther.* **1989**, *251*, 238.
2. (a) Brea, J.; Rodrigo, J.; Carrieri, A.; Sanz, F.; Cadavid, M. I.; Enguix, M. J.; Villazón, M.; Mengod, G.; Caro, Y.; Masaguer, C. F.; Raviña, E.; Centeno, N. B.; Carotti, A.; Loza, M. I. *J. Med. Chem.* **2002**, *45*, 54; (b) Dezi, C.; Brea, J.; Alvarado, M.; Raviña, E.; Masaguer, C. F.; Loza, M. I.; Sanz, F.; Pastor, M. *J. Med. Chem.* **2007**, *50*, 3242; (c) Aranda, R.; Villalba, K.; Raviña, E.; Masaguer, C. F. Brea, J.; Areias, F.; Domínguez, E.; Selent, J.; López, L.; Sanz, F.; Pastor, M.; Loza, M. I. *J. Med. Chem.* **2008**, *51*, 6085



I



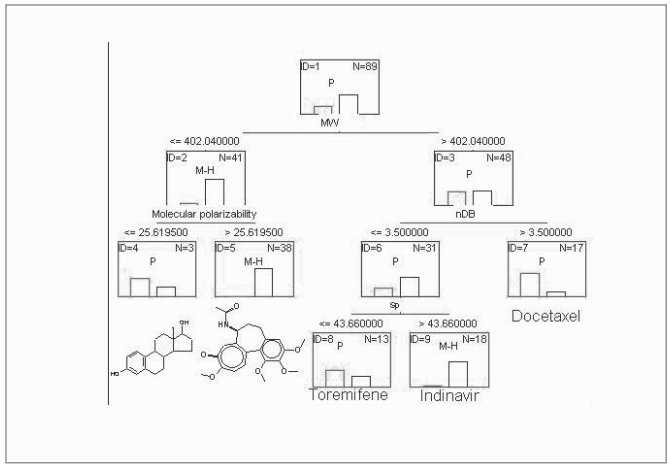
QF4108B

COMPUTATIONAL MODELS TO EXPLAIN THE RELATIONSHIP AMONG P-GLYCOPROTEIN, CYP3A4 AND BIOAVAILABILITY FROM MOLECULAR STRUCTURE

Miguel Ángel Cabrera-Pérez (1), Daimel Castillo-González (1,2), Isabel González-Álvarez (3) and Marival Bermejo-Sanz (3)

(1) Molecular Simulation & Drug Design Group, Chemical Bioactive Center, Central University of Las Villas, Santa Clara, Villa Clara, C.P. 54830, Cuba; (2) Department of Pharmacy, Chemistry and Pharmacy Faculty, Central University of Las Villas, Santa Clara, Villa Clara, C.P. 54830, Cuba; (3) Department of Ingeniery, Area of Pharmacy and Pharmaceutical Technology, Miguel Hernández University, 03550 Sant Joan d'Alacant, Alicante, Spain

Cytochrome P450 (CYP3A4) and P-glycoprotein (P-gp) are present, at high levels, in the small intestine enterocytes and liver. This suggests that bioavailability (F) of Pgp-CYP3A4 substrates can be reduced by the simultaneous action of both processes. In order to study this relationship a dataset of 292 substrates and non-substrates of P-gp and CYP3A4, with human F values reported, was used. Different molecular descriptors based on chemical functional groups, atom-centred fragments and molecular properties were calculated. Classification Tree Analysis were applied to identify, through simple hierarchical rules, those structural features responsible for poor F values among Pgp and CYP3A4 substrates, and non-substrates. A first model evidenced that molecular weight (MW), polarizability and number of double bond were able to explain the 96% of Pgp substrates with poor F values while for non-substrates, the electronegativity, partition coefficient and the number of acceptor atoms for H-bonds identified the 85% of cases with poor F. For Pgp and CYP3A4 substrates the appearance of C=O groups in the structures and ranges of MW explained the 95% of cases while for Pgp and CYP3A4 non-substrates, the number of atoms and the hydrophilic factor were able to explain the 90% of compounds with moderate-poor bioavailability. The quality of the models were validated by an external set of 22 compounds with F values reported (13 Pgp substrates and 9 Pgp non-substrates). The global predition for each model was 76.9%, 84.6%, 66.7% and 77.8% respectively. This suggests that previous rules may help chemist to planning the synthesis focused on compounds with increased oral bioavailability, being a useful tool during the drug discovery process.



1. Hou, T., Wang, J., Zhang, W., Xu, X.et al., ADME Evalaution in Drug Discovery.7. Prediction of Oral Absorption by correlation and Classification. J Chem Inf Model 2007, 47, 208-18.

2. Norinder, U., Bergstrom, CAS, Prediction of ADMET Properties. Chem Med Chem 2006, 1, 920-37.

POSTER COMMUNICATIONS – PC.154

THIOBARBITURATE INHIBITORS OF NAD+-DEPENDENT HISTONE DEACETYLASES (SIRTUINS)

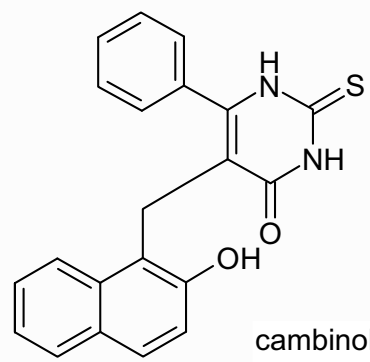
Manfred Jung (1), Martin L. Schmitt (1), Jörg Schemies (1), Tobias Rumpf (1), Julia Wagner (1), Urszula Uciechowska (2), Wolfgang Sippl (2)

(1) Institute of Pharmaceutical Sciences, University of Freiburg, Albertstr. 25, 79104 Freiburg, Germany; (2) Department of Pharmaceutical Chemistry, Martin-Luther Universität Halle-Wittenberg, Wolfgang- Langenbeckstr. 4, 06120 Halle/Saale, Germany.

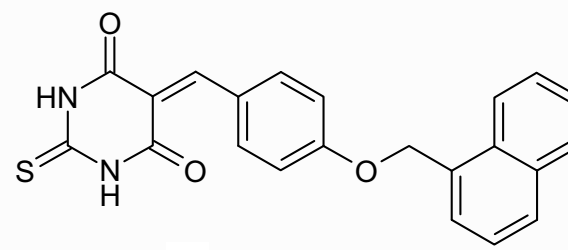
NAD+-dependent histone deacetylases (sirtuins) are enzymes which cleave acetyl groups from lysines in histones and other proteins and regulate central cellular functions[1]. Potent selective sirtuin inhibitors may be future drugs for the treatment of cancer or neurodegenerative diseases.

Starting from the sirtuin inhibitor cambinol[2] (see Scheme 1) and the X-ray structure of the human sirtuin subtype SIRT2 we had identified novel sirtuin inhibitors with a thiobarbiturate structure[3]. Subsequent in-vitro enzyme testing on newly synthesized compounds and analogues from commercial libraries have now resulted in structure-activity relationships that will be presented. E.g. compounds such as (**1**) were identified that show increased potency (IC_{50} values on SIRT1 1.5 µM, on SIRT2: 1.0 µM). Hyperacetylation of tubulin was shown in MCF7 breast cancer cells was shown for selected examples without general toxicity which may make them interesting candidates for studies on neuroprotective properties.

1. Milne JC, Denu JM. Curr Opin Chem Biol 2008;12:11-17.
2. Heltweg B, et al. Cancer Res 2006;66:4368-4377.
3. Uciechowska U, et al. ChemMedChem 2008;3:1965-1976.



cambinol



(1)

Scheme 1. Sirtuin inhibitors.

DESIGN OF NEW GRB2-SH2 DOMAIN INHIBITORS BASED ON A PHARMACOPHORE MODEL

Mireia Olivella (1), Iván Rodriguez Torrecillas (2), Leonardo Pardo (2), Mercedes Campillo (2)

(1) Departament de Biologia de Sistemes. Universitat de Vic, 08500 Vic, Spain, (2) Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Growth factor receptor bound protein 2 (GRB2) plays an important role in signal transduction. The inhibition of the Src homology 2 (SH2) domain blocks the activation of the epidermal growth factor receptor 2 (HER2 or ErbB-2), which is implicated in several types of cancer. Thus, GRB2 is an interesting target for anticancer drugs. High affinity peptidic inhibitors have been described in the literature[1-5]. Although these inhibitors present activities in the picomolar range, its peptidic nature is a limitation in terms of bioavailability and membrane transport.

This study combines 3D structural from X-ray crystallography data of the complexes between GRB2 and its peptidic ligands, flexible ligands superimposition of the GRB2 inhibitors from the literature lacking of a crystallized structure and molecular modeling techniques to generate a pharmacophore model for the GRB2-SH2 binding site. The key residues implicated in the binding pocket of GRB2 were identified. The model has been used to computationally design new high affinity non-peptidic compounds without a phosphate group as potential therapeutic agents.

1. Schoepfer, J.; Fretz, H.; Gay, B.; Furet, P.; García-Echeverría, C., End, N.; Caravatti, G. *Bioorg Med Chem Lett*. 1999, 18;9(2):221-6.
2. Furet, P.; García-Echeverría, C.; Gay, B.; Schoepfer, J.; Zeller, M.; Rahuel, J. *J. Med. Chem.* 1999, *42*, 2358-2363.
3. Shi, Z.-D.; Lee, K.; Liu, H.; Zhang, M.; Roberts, L. R.; Worthy, K. M.; Fivash, M. J.; Fisher, R. J.; Yang, D.; Burke, T. R. *Biochem. Biophys. Res. Comm.* 2003, *310*, 378-383.
4. Liu, W.-Q.; Vidal, M.; Olszowy, C.; Million, E.; Lenoir, C.; Dhôtel, H.; Garbay, C. *J. Med. Chem.* 2004, *47*, 1223-1233.
5. Kang, S.-U.; Choi, W. J.; Oishi, S.; Lee, K.; Karki, R. G.; Worthy, K. M.; Bindu, L. K.; Nicklaus, M. C.; Fisher, R. J.; Burke, T. R. *J. Med. Chem.* 2007, *50*, 1978-1982.

IDENTIFICATION OF POTENT CARBONIC ANHYDRASE INHIBITORS: SYNTHESIS, BIOLOGICAL EVALUATION AND ENZYME-LIGAND X-RAY STUDIES

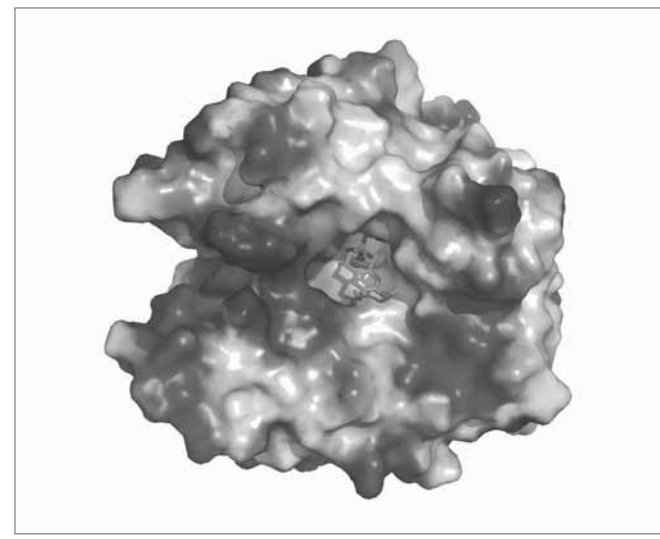
Rosaria Gitto (1), Stefano Agnello (1), Stefania Ferro (1), Claudiu T. Supuran (2), Jiri Brynda (3, 4), Pavel Mader (4), and Alba Chimirri (1)

(1) Dipartimento Farmaco-Chimico, Università di Messina, Messina, Italy; (2) Laboratorio di Chimica Bioinorganica, Università di Firenze, Firenze, Italy; (3) Institute of Organic Chemistry and Biochemistry AS CR, Structural Biology, Prague; (4) Institute of Molecular Genetics AS CR, Laboratory of Structural Biology, Prague, Czech Republic

The carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes regulating a broad range of physiological functions. There are several human CA (hCA) isoforms with different tissue distribution, expression levels, and subcellular locations. They are responsible for different biological effects and some of these isozymes constitute valid targets for the development of anticancer, antiglaucoma, antiobesity, or anticonvulsant drugs[1]. However, their diffuse localization in many tissues and organs limits potential clinical applications. So the development of CA inhibitors possessing high potency and selectivity against some specific isoforms represents an attractive strategy to obtain new pharmacological tools thus avoiding side effects and improving therapeutic safety.

Recently we identified some 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides that proved to inhibit some CA isoforms[2]. Now we extended this study to a new series of 3,4-dihydroisoquinoline-2(1*H*)-sulfonamides containing some structural modifications searching the enhancement of potency as well as selectivity.

The synthesis of target compounds was easily accomplished in microwave conditions and the screening against some relevant CA isoforms was performed. We found some derivatives inhibiting hCAII, hCAIX and hCAXIV isoforms at nanomolar concentrations. Moreover, we recently determined the X-ray crystal structures of new compounds in complex with hCAII, providing structural information on the inhibitor binding mode and inhibitor-protein interactions



Research supported by Università di Messina (PRA2006) and project no. AV0Z50520514 (Academy of Sciences of the Czech Republic).

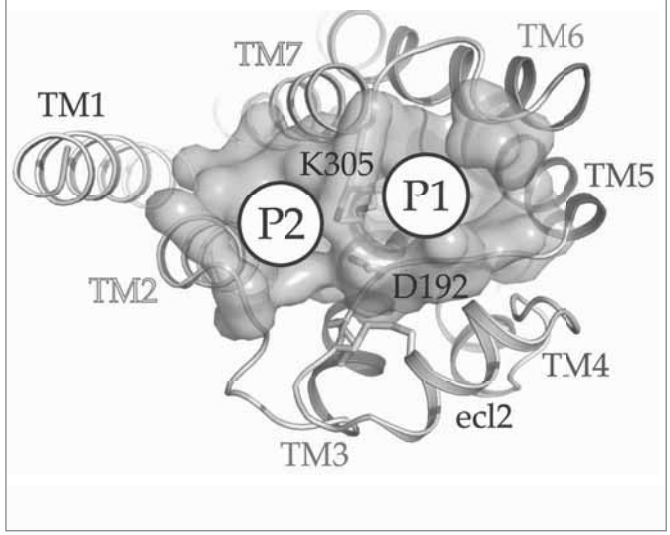
1. Thiry, A.; Dogne, J. M.; Supuran, C. T.; Masereel, B. *Curr. Pharm. Des.* **2008**, *14*, 661-71.
2. Gitto, R.; Ferro, S.; Agnello, S.; De Luca, L.; De Sarro, G.; Russo, E.; Vullo, D.; Supuran, C.T.; Chimirri, A. *Bioorg Med Chem.* **2009** *17*, 3659-64.

IMPACT OF LIGAND ENTRY PATHWAYS ON SELECTIVITY IN BETA ADRENERGIC RECEPTORS

Ángel González (1), Leonardo Pardo (2), Xavier Deupi (2)

(1) Centre for Bioinformatics CBUC, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
(2) Laboratory of Computational Medicine, Biostatistics Unit, School of Medicine, Universitat Autònoma de Barcelona, Bellaterra, Catalunya, Spain

The beta 1 (β1AR) and beta 2 (β2AR) adrenergic receptors belong to the amine family of Class A GPCRs. Despite their similarity in sequence, a range of ligands bind these receptors with different affinities. The crystal structures of β1AR and β2AR have revealed that their ligand-binding pockets are virtually identical. Thus, ligand selectivity must be encoded in a different region of the receptors. While the β1AR has a relatively exposed entry to the binding pocket, the β2AR has an ionic interaction between Asp192, in the second extracellular loop, and K305(7.32), in the extracellular side of TM7, that partially occludes the mouth of the binding site (see figure). As a result, β2AR has a preferred ligand entry pathway near TM3/TM5/TM6/TM7 (P1) and a secondary pathway near TM2/ TM3/TM7 (P2). Using steered molecular dynamics to simulate the process of ligand entry, we have detected a putative secondary binding site along the main entry pathway, formed by His296(6.58) and Asp300/Asn301 in the third extracellular loop. Sequence analysis shows that these residues are exclusive of the beta 2 subfamily. These differences can alter the process of ligand binding, and may be related to differences in the pharmacological profile between families 1 and 2 of the beta adrenoreceptor family.



POSTER COMMUNICATIONS – PC.158

DEVELOPMENT OF MONOACYLGLYCEROL LIPASE (MGL) INHIBITORS

Silvia Ortega-Gutiérrez (1), José A. Cisneros (1), Janling Hu (2), Christopher J. Fowler (2), and María L. López-Rodríguez (1)

(1) Dpto de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense, E-28040 Madrid, Spain and
(2) Dpt of Pharmacology and Clinical Neuroscience, Umeå University, Sweden

Recently, the enzyme monoacylglycerol lipase (MGL) has been proposed as the responsible of degradation in brain of 2-arachidonoylglycerol (2-AG)[1], one of the main endocannabinoids which is involved in a broad number of physiopathological processes[2].

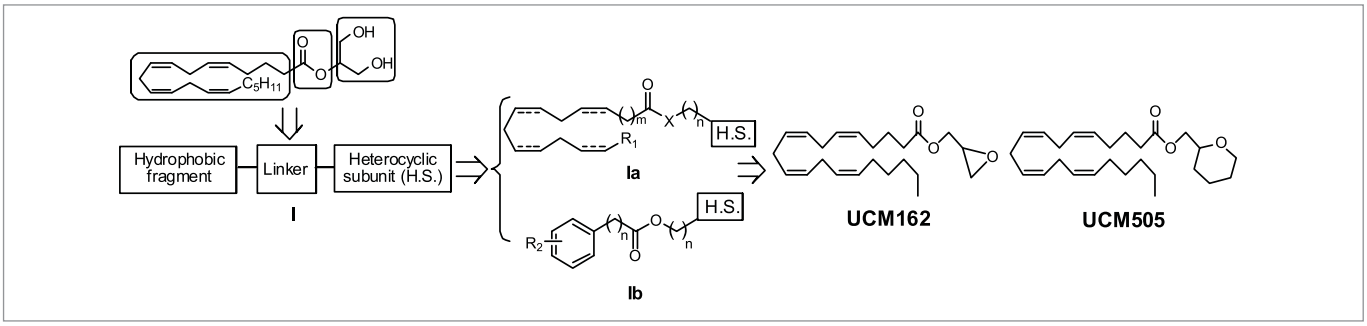
The development of potent and selective MGL inhibitors will allow to study the roles played by 2-AG. However, data available regarding the structural features involved in the recognition of substrates by the enzyme are very scarce.

Considering the lack of the 3D structure and lead compounds for MGL, we have designed and synthesized a series of compounds **I** based on the structure of 2-AG that have allowed us to begin to delineate the structural requirements involved in the recognition of substrates[3]. The most promising compounds emanating from this study were UCM162 and UCM505. These compounds inhibit cytosolic MGL completely with IC_{50} values of 4.5 and 5.6 μM, respectively, being less potent fatty acid amide hydrolase (FAAH) inhibitors (IC_{50}=12 and 51 μM, respectively). Moreover, the pyrane derivative has allowed the identification of a novel MGL enzymatic activity in microglial cells[4].

These results prompted us to extend the structural space with a new series of compounds where the arachidonic acid chain is mimicked by an aromatic moiety. The most potent compound in the series inhibits hMGL with an IC_{50} value of 0.8 μM being selective towards FAAH (39% inhibition at 100 μM).

This work has been supported by MICINN (Predoctoral fellowship to J.A.C., Ramón y Cajal grant to S.O.G. and SAF-2007/67008) and CAM (S-SAL-249-2006).

1. (a) Dinh, T.P. *et al. Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10819. (b) Blankman, J.L. *et al. Chem. Biol.* **2007**, *14*, 1347. (c) Long, J.Z. *et al. Nat. Chem. Biol.* **2009**, *5*, 37.
2. Viso A. *et al. Curr. Top. Med. Chem.* **2008**, *8*, 231.
3. Cisneros J.A. *et al. J. Med. Chem.* **2007**, *50*, 5012.
4. Muccioli G.G. *et al. J. Neurosci.* **2007**, *27*, 2883.



DISCOVERY OF NOVEL BICYCLIC PYRIDINE AND PYRAZINE HETEROCYCLIC SYSTEMS WITH ANTI-HIV-1 ACTIVITY

Sonia de Castro (1), Olga Familiar (1), Jan Balzarini (2), María-José Camarasa (1), and Sonsoles Velázquez (1)

(1) Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, 28006 Madrid, Spain; (2) Rega Institute for Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

The reactivity of the 4-amino-5*H*-1,2-oxathiole-2,2-dioxide (β-amino-γ-sultone) heterocyclic system has been scarcely studied. In a continuation of our studies on the reactivity of the β-amino-γ-sultone system towards electrophiles and amines[1], we now explore the synthetic usefulness of this ambident nucleophile for the synthesis of unusual fused nitrogen-containing heterocycles with a γ-sultone moiety. Even though the poor nucleophilicity of the amino group, which is considered to have an "amide like" character, the β-amino-γ-sultone reacts with a variety of bis-electrophiles. As a result, previously unknown pyridine-and pyrazine-based bicyclic heterocycles were obtained and evaluated against a broad panel of viruses in cell culture.

The synthetic approaches for the synthesis of these novel heterocycles and their biological evaluation will be described. Interestingly, some substituted pyrido-fused compounds showed a significant activity against HIV-1 replication, being inactive against a variety of other DNA and RNA viruses. The new "hit" compounds deserve further studies and are a good starting point to explore what it seems to be a novel class of HIV-1 nonnucleoside inhibitors.

1. S. de Castro, A. Lozano, M. T. Peromingo, M. J. Camarasa and S. Velázquez. *Chemistry-A European Journal*, **2008,** *14,* 9620-9632.

POSTER COMMUNICATIONS – PC.160

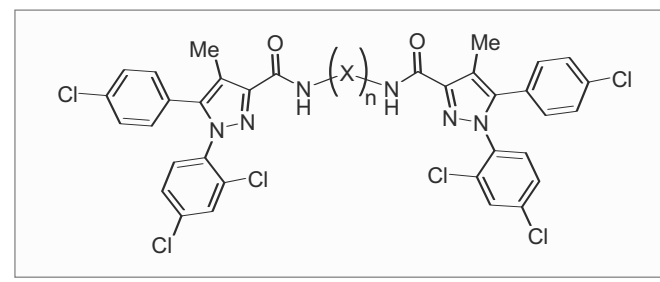
BIS-PYRAZOLE-3-CARBOXAMIDES AS CANNABINOIDS. *IN VITRO* AND *IN VIVO* CHARACTERIZATION

Cristina Fernández-Fernández (1), Juan Decara (2), Francisco Javier Bermúdez (2), Eva Sánchez (3), Patricia Rodríguez (4), María Gómez (4), Pilar Goya (1), Fernando Rodríguez de Fonseca (2), M. Isabel Martín (3), Javier Fernández-Ruiz (4), and Nadine Jagerovic (1)

(1) Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain, (2) Fundación IMABIS, Hospital Carlos Haya, Avda. Carlos Haya 82, 29010, Málaga, Spain, (3) Unidad de Farmacología, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Avda. Atenas, s/n, E-28922 Alcorcón, Madrid, Spain, (4) Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain

Pyrazole-3-carboxamides as ligands for cannabinoid receptors have attracted much attention this last decade to the point that one of its representatives, rimonabant, was the first of the CB1 blockers to be launched. However, this anti-obesity agent had to be withdrawn due to important psychiatric effects. In spite of this, diaryl pirazoles continue to be interesting heterocycles in cannabinoid chemistry, and within this context, and in connection with our ongoing studies in obesity we now report a novel series of bivalent compounds[1].

In radioligand binding studies, the new compounds have shown affinity to cannabinoid CB_1 and/or CB_2 receptors and were identified as new lead structures. Since both receptors are attractive drug target due to their functional role in several physiological and pathological processes, evaluation of the new ligands have been carried out in isolated tissues and *in vivo* studies. In food intake studies, one selected compound turned out to have excellent *in vivo* efficacy in an animal model.



These studies were supported by SAF2006-13391-C03-02, CANNAB-CM (S-SAL-0261-2006) and RTA (RETICS RD06/001/0014)

1. Patent application number ES P200930120.

BENZIMIDAZOLE DERIVATIVES AS NOVEL SEROTONIN 5-HT$_6$ RECEPTOR ANTAGONISTS. A COMPUTATIONAL AND MUTAGENESIS STUDY OF LIGAND-RECEPTOR INTERACTION

Mar Martín-Fontecha (1), Bellinda Benhamú (1), Tania de la Fuente (1), Jessica Sallander (2), Mercedes Campillo (2), Henar Vázquez-Villa (2), Lucie Pellisier (3), Sylvie Claeysen (3), Aline Dumuis (3), Leonardo Pardo (2), and María L. López-Rodríguez (1)

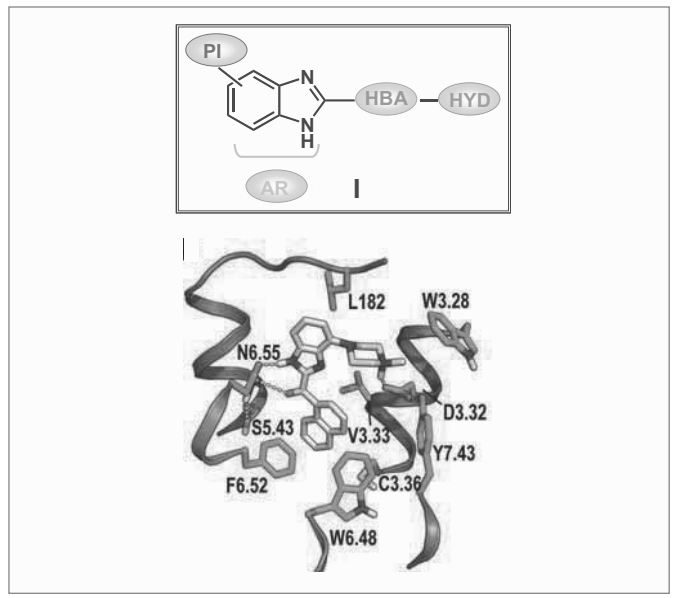
(1) Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain.
(2) Laboratori de Medicina Computacional, Unitat de Bioestadística, Universitat Autònoma de Barcelona, E-08913 Bellaterra, Barcelona, Spain.
(3) Département de Neurobiologie, Institut de Génomique Fonctionnelle, CNRS, Université de Montpellier, F-34094 Montpellier, France

The G protein-coupled 5-HT$_6$ receptor (GPCR, 5-HT$_6$R) is one of the mostrecently added subtypes to the serotonin receptor family. Since it is almost exclusively localized in the brain, this receptor has generated considerable interest as a promising target for CNS-mediated diseases such as Alzheimer or obesity[1]. In the past few years several 5-HT$_6$R agents have been identified, mainly by HTS of pharmaceutical companies, but few compounds have entered clinical trials. Therefore, significant efforts are currently focused on a rational drug discovery in this research area.

In a project aimed at the development of new 5-HT$_6$R ligands, we have generatedthe first known 3-D pharmacophore for 5-HT$_6$R antagonists recognition[2]. Based on this model, a series of new benzimidazole derivatives of general structure **I** has been designed *de novo,* synthesized and evaluated for their binding affinity at the h5-HT$_6$R. Benzimidazole-4-substituted derivatives (**Ia**) show high 5-HT$_6$R affinity whereas 5-substituted analogues (**Ib**) are inactive. Indeed, the structural elements in compounds **Ia** optimally fit the pharmacophore features of the 3-D model, validating our previous proposal. The ligands have been characterized asantagonists in COS7 cells transfected with the human receptor, representing a new family of potent 5-HT$_6$R antagonists.

In order to get a better understanding of the mode of binding of these new 5-HT$_6$R ligands, we have performed site-directed mutagenesis and computational modeling studies with antagonist UCM-258, identified in this work. Five point mutations were generated and the results have shown that W6.48, F6.52, and N6.55 are key residues for the binding of the benzimidazole derivatives **I** to the 5-HT$_6$R.

These combined experimental and computational studies have permitted to propose the molecular mechanism for the inactivation of the receptor, which is a key subject in medicinal chemistry. Thus, we will report valuable information that should help in the optimization of 5-HT$_6$R ligands, which may contribute to the rational design of new compounds with predetermined pharmacological properties.



Financial support was provided by grants from the Ministerio de Educación y Ciencia (SAF-2007/67008) and the Comunidad Autónoma de Madrid (SAL-0249/2006).

1. Holenz, J.; Pauwels, P. J.; Diaz, J. L.; Mercè, R.; Codony,X.; Buschmann, H. *Drug Discov. Today***2006**, *11*, 283-299.
2. López-Rodríguez, M. L.; Benhamú, B.; de la Fuente, T.; Sanz, A.; Pardo, L.; Campillo, M. *J. Med. Chem.***2005**, *48*, 4216-4219.

POSTER COMMUNICATIONS – PC.162

THE INFLUENCE OF BIOMARKERS RESEARCH IN MEDICINAL CHEMISTRY

Josep Prous, Jr. (1), Miriam Bayés (1), Peter Revill (1), Tim Miller (2), Dave Paterson (2), Colin Williams (2) and Jemima Tonks (2)

(1) Thomson Reuters Healthcare & Science, Provenza 388, 08025 Barcelona, Spain, (2) Thomson Reuters Healthcare & Science, 77 Hatton Garden, London, EC1N 8JS, UK

At present, researchers in the biomedical field are demanding for an increasing amount of information about the specific forms of diseases and how they might be treated or cured with new therapies custom-designed according to individual clinical status.

Historically, medicinal chemistry has benefited from other scientific and technological disciplines in order to evolve. Examples include QSAR and computer-aided drug design (IT), combinatorial chemistry and high-throughput screening (robotics), among others.

With this in mind, biomarker information may provide the opportunity to generate new drug discovery hypotheses in a more selective and faster way.

As an example, the commercial success of agents such as Imatinib and Trastuzumab has alerted the pharmaceutical industry as to the potential value of "biomarker-guided" drugs. In addition, new evidence continues to emerge showing how the early institution of biomarke-based assays can more reliably predict both efficacy and toxicity in the preclinical and early clinical phases of drug development. This can allow for both improved success rates in the selection of compounds to move forward and cost savings associated with earlier cessation of the evaluation of novel compounds destined to fail.

In particular, the creation of a repository of disease-specific biomarkers that includes their biological basis and their target identification and validation would be a significant asset for all those involved in the discovery of better and safer drugs.

A review on how medicinal chemistry can benefit from the advances in biomarkers research will be presented.