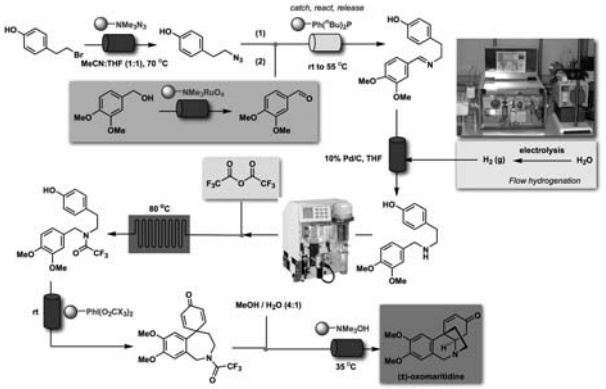
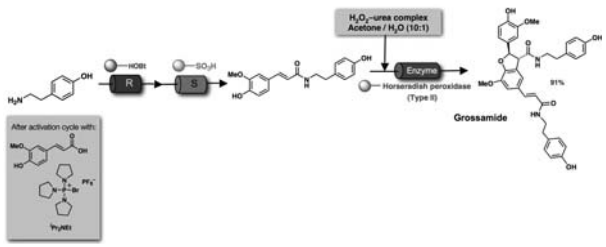
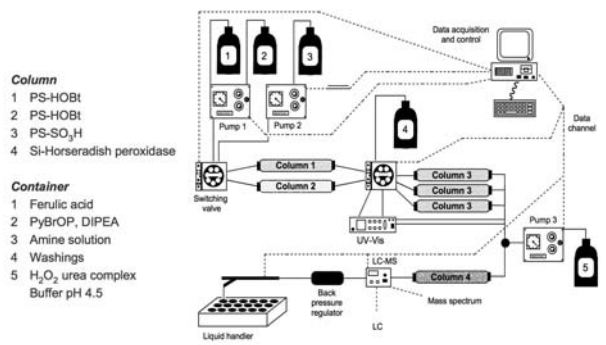
Flow Technology: The Synthesis of Oxomaritidine



Flow Technology: The Synthesis of Grossamide



Schematic diagram of the synthesis and equipment used for the preparation of Grossamide



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L01

INTEGRATION OF FRAGMENT DERIVED STRUCTURE-BASED DESIGN INTO THE DISCOVERY AND DEVELOPMENT OF SELECTIVE KINASE INHIBITORS FOR TREATMENT OF HUMAN CANCERS

Gavin Hirst

Senior Director of Medicinal Chemistry, SGX Pharmaceuticals, Inc., 10505 Roselle Street, San Diego, CA 92121

Rapid discovery and development of potent and selective targeted therapeutic agents depends critically on identification of viable starting points for lead optimization. There is a pressing need for more efficient approaches that complement conventional HTS strategies. The SGX platform utilizes high-throughput X-ray crystallography to guide fragment-based lead identification as a powerful drug discovery tool. This approach uses X-ray crystallography and complementary biophysical techniques to identify low molecular weight fragments

(or scaffolds) that bind to the desired target. This is followed by a structure-guided constructive strategy to enhance affinity and selectivity while preserving ligand efficiency. Integration of this approach into an overall drug discovery process which includes iterative structure-based design that focuses on retention of the intrinsically favorable drug-like properties of the original scaffold hits has yielded high quality development candidates for challenging oncology targets of high therapeutic value.

Structure-based approaches toward the discovery and development of inhibitors of the oncology targets MET, Gleevec®-resistant BCR-ABL, and JAK-2 will be discussed.

L02

FRAGMENT BASED LEAD DISCOVERYNOVEL LEAD FINDING APPROACHES

Professor Roderick E Hubbard

Abstract: Over the past ten years, there has been increasing interest in fragment based methods for drug discovery. At Vernalis, we have developed and applied the approach to generate lead compounds against a number of therapeutic targets[1].

In this presentation, I will describe the discovery and development of clinical candidates that inhibit the function of the molecular chaperone, Hsp90[2]. Fragment and virtual screening methods identified initial hits and the crystal structures of compounds in complex with Hsp90 were used to optimize properties. Compounds from this program are now in Phase I clinical trials.[3]

The experiences will be used to illustrate the advantages and challenges of fragment-based drug discovery. Fragments are just small, weak hits. The main challenges are design of the library, robust identification of which fragments bind and the need for structural informa-tion to decide how and which fragments to progress. The advantages are that a small library can sample a potentially large chemical diversity to generate novel lead compounds and that hits can be identified for new classes of target for which the corporate collection is naive. Various approaches are used to develop the fragment hits into useful leads. These include (a). linking fragments together, (b). growing by directed limited library synthesis or searching for nearest neighbours in the accessible compound databases, or (c). merging where the structures of fragments, existing tool compounds and virtual screening hits provides guidance for how to merge features from different compounds together. Examples of each of these approaches will be discussed.

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L03

SEARCH FOR TAXOID MIMICS USING DYNAMIC COMBINATORIAL CHEMISTRY TARGETING TUBULIN

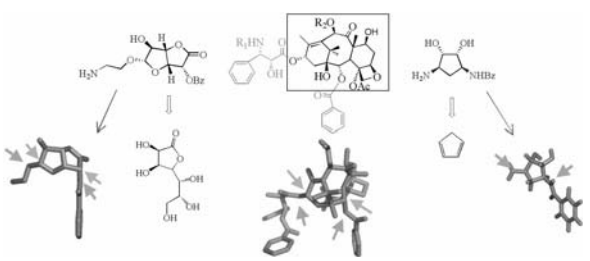
Claire Le Manach, Boris Vauzeilles, Jean-Marie Beau

Université Paris Sud 11, Laboratoire de Synthèse de Biomolécules, UMR CNRS 8132, Institut de Chimie Moléculaire et des Matériaux d'Orsay, Bât. 430, F-91405 Orsay cedex

The tubulin/microtubules system plays a key role during mitosis and disturbing its dynamic equilibrium can prevent cell division and induce apoptosis. Up to now, most of the known antitubulin agents have been characterized by a very complex structure, and therefore are relatively hard to synthesize, especially on a large scale.

We are currently using dynamic combinatorial chemistry along with the reversible formation of imines in water to identify new potential antitubulin agents, possessing simpler structures than the taxoid core. In the presence of tubulin, the distribution of the generated libraries of imines in dynamic equilibrium may be altered, with an amplification of the best binders which can be detected by an adequate analytical method (HPLC).

A model-scaffold possessing a taxoid core was synthesized in order to validate the method of dynamic combinatorial chemistry with tubulin. Then, according to previous SAR studies and using molecular modeling, two types of scaffolds were synthesized, possessing a far simpler structure than taxoids, but allowing a similar orientation of the two side chains which are crucial for activity. These molecules were used in dynamic libraries of imines, with or without tubulin, so that potential amplifications would be identified.



The syntheses and the main results obtained will be presented.

L04

FRAGMENT BASED DRUG DISCOVERY: FROM FRAGMENT TO CLINIC

David Rees

Astex Therapeutics, 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, UK.

Five years ago most scientists did not consider low molecular weight fragments (MW = 120-250) with corresponding binding affinities of only mM to uM to be attractive starting points for drug discovery programs. However, today there is widespread acceptance that these fragments can be progressed into nM lead series and on into clinical trials. Reported examples include candidates that target different protein families such as kinases (CDK, Aurora, Akt, raf), protein-protein interactions (Bcl-X_L), ATPases (HSP-90) and proteases (MMP 2&9).

Fragment based drug discovery uses biophysical screening to identify the initial fragments. Subsequently, in the fragments-to-leads stage a detailed structural understanding of the binding interactions between the fragment and its target protein utilizing X-ray crystallography or NMR is critical. Starting with different fragments allows several lead series to be identified, often by synthesizing only small numbers of compounds.

This presentation has two parts. Firstly, a general overview of the medicinal chemistry techniques associated with fragment based drug discovery and secondly some specific examples from Astex's laboratories of fragments that have been progressed into candidates for clinical trials.

L05

CHEMICAL STRATEGIES FOR SUCCESSFUL CLINICAL DEVELOPMENT

Christopher N. Johnson*,

Neurosciences Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, U.K.

The pharmaceutical industry today faces an immense problem with regard to productivity in that, despite ever-increasing R&D expenditure, new drug approval rates have stayed constant over the last five years. A key factor in this situation is the very high failure rate for drugs entering development, close to 96%, with lack of clinical efficacy cited as the largest single contributory factor.[1] This high rate of attrition means that a high proportion of drug development costs are due to investment in failed projects and, therefore, any strategies which reduce the attrition rate will result in significant improvements in industry productivity. Medicinal chemists have a key role to play in developing such strategies because decisions made by them on which compounds to synthesise and progress have a profound impact at all stages of the drug development process.

This talk will explore a number of medicinal chemistry approaches adopted at GSK for optimising clinical efficacy. Since the 1990s and the advent of ADME screening in early drug discovery, a good understanding has been built of the optimal physicochemical parameters required for good pharmacokinetic (PK) performance, leading to a reduction in the rate of attrition due to poor PK properties. However, to address concerns over efficacy, maximal engagement of the target in the tissue of interest is required, and successful drug design strategies for achieving this aim will be described. However, a key problem remains at the point where a molecule transitions into the clinic: how can one demonstrate target engagement in the target tissue in humans? This problem is particularly acute for compounds acting in the central nervous system. Medicinal chemists have a key contribution to make in this field, for example by collaborating with radiochemists and imaging scientists to develop positron emission tomography (PET) ligands. The talk will conclude with a PET ligand discovery case history, showing the successful application of the technique in the development of a molecule which subsequently demonstrated clinical efficacy.

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*e-mail address: Christopher_N_Johnson @gsk.com

L06

RECENT STRATEGIES AROUND PREDICTING ORAL ABSORPTION

David S. Millan

Pfizer Global Research and Development, Sandwich, UK.

Since Chris Lipinski's seminal publication on 'rule of five' approaches to drug likeness there has been a varied response in the profile of molecules currently being synthesised in leading drug discovery companies. This communication seeks to highlight some of the post-Lipinski approaches various companies have taken to reducing oral bioavailability related compound attrition.

The introduction of topological polar surface area (TPSA) descriptors, number of rotatable bonds, as well as size and shape have also been shown to influence oral absorption. Lipinski's teaching suggests compounds with molecular weight (MW) greater than 500 stand a greater risk of lower intestinal permeability, however it is emerging that it is the consequences of increasing MW, such as concomittant increases in clogP, and TPSA, that are the underlying reason for why MW has been empirically attributed to reduced oral absorption. This communication hopes to provide an overview of these and other post-Lipinski thoughts and learnings.

L07

DISCOVERY AND DEVELOPMENT OF THE NOVEL ANTITHROMBOTIC AGENT RIVAROXABAN, AN ORALLY ACTIVE, DIRECT FACTOR XA INHIBITOR

Susanne Roehrig

Bayer Healthcare AG

Despite recent progress in antithrombotic therapy, there is still an unmet medical need for safe and orally available anticoagulants. The coagulation enzyme Factor Xa (FXa) is a particularly promising target, and recent efforts in this field have focused on the identification of small-molecule inhibitors with good oral bioavailability.

We identified oxazolidinone derivatives as a new class of potent FXa inhibitors. Lead optimization led to the discovery of Rivaroxaban, a highly potent and selective direct FXa inhibitor with excellent *in vivo* antithrombotic activity and high oral bioavailability. The X-ray crystal structure of Rivaroxaban in complex with human FXa clarified the binding mode and the stringent requirements for high affinity; the interaction of the non-basic ligand in the S1 subsite allows for the combination of high oral availability and high potency.

In man, Rivaroxaban demonstrated predictable pharmacokinetic and pharmacodynamic responses with a close correlation of pharmacodynamic effects and plasma concentrations.

The efficacy and safety of Rivaroxaban are currently being investigated in several indications: prevention of venous thromboembolism (VTE) after orthopedic surgery, treatment and secondary prevention of VTE, prevention of stroke in patients with atrial fibrillation and the secondary prevention of acute coronary syndromes.

In a global program of clinical trials involving more than 12,500 patients, comparing Rivaroxaban with the standard of care, injectable enoxaparin, for the prevention of VTE in patients undergoing either total knee or hip replacement surgery, phase III results demonstrate that Rivaroxaban, orally and once daily, showed superior efficacy in preventing venous blood clots while maintaining a similar safety profile.

An anticoagulant such as Rivaroxaban, which can be administrated orally in a convenient once-daily, fixed dose without coagulation monitoring for the prevention of VTE, could be an attractive alternative to currently available therapies.

L08

IDENTIFICATION AND DEVELOPMENT OF PI3K INHIBITORS – PATH TO THE CLINIC

S.-M. Maira, F. Stauffer, J. Brueggen, P. Furet, C. Schnell, C. Fritsch, S. Brachmann, P. Chène, A. De Pover, K. Schoemaker, D. Fabbro, D. Gabriel, M. Simonen, L. Murphy, P. Finan, W. Sellers, and C. Garcia-Echeverria

Novartis Institutes for Biomedical Research

The identification and characterisation of the components of individual signal transduction cascades, and advances in our understanding on how these biological signals are integrated in cancer initiation and progression have provided new strategies for therapeutic intervention in solid tumors and hematological malignancies. In this context, a substantial number of epidemiological and experimental studies support an important role of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/PKB) signaling pathway —also known as the survival or anti-apoptotic pathway— in controlling cell growth, proliferation and survival. Whatever the mechanism, the prevalence of PI3K/PKB signaling abnormalities in human cancers and its potential biological effects (e.g., competitive growth advantage, evasion from apoptosis and therapy resistance) has suggested the potential use of PI3K/PKB pathway modulators as novel targeted therapeutic agents in oncology

Following a structure-based design strategy, we have identified NVP-BEZ235, a dual pan-PI3K/mTOR inhibitor. This imidazo[4,5-c]quinoline derivative exhibits potent antiproliferative activity against a broad panel of tumor cell lines (e.g. IC_{50}= 10 nM, U87MG cells) by specifically blocking the biological function of PI3K signaling components, in particular the phoshorylation and activation of PKB/Akt (e.g. IC_{50} = 10 ± 1 nM, U87MG cells). The antiproliferative activity of NVP-BEZ235 in cellular settings translates well in *in vivo* models of human cancer (e.g, U87MG, PC3M, A549). Thus, the compound displayed disease stasis or tumor regression when administered orally -25 to 50 mg/kg/day-, and enhanced the efficacy of other anticancer agents when used in *in vivo* combination studies (e.g, docetaxel or temozolomide). *Ex vivo* PK/PD analyses of tumor tissues upon acute dose or after termination of *in vivo* efficacy studies showed a time-dependent correlation between compound concentration and inhibition of PKB/Akt phosphorylation. NVP-BEZ235 was well tolerated at the efficacious doses when compared with vehicle treated animals, with no significant difference seen in the body weight. Unlike other modulators of the PI3K/PKB pathway, no elevated blood glucose levels were observed in the animals treated with NVP-BEZ235 after *in vivo* efficacy experiments in mice or rats. Phase I trials with this dual PI3K/mTOR inhibitor in patients with solid tumors are currently ongoing.

L09

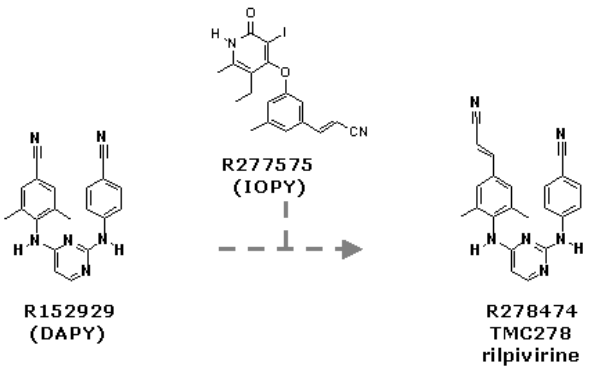
DISCOVERY OF TMC278: A NEXT GENERATION NNRTI DRUG, HIGHLY ACTIVE AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1)

Jérôme Guillemont

Johnson & Johnson Pharmaceutical Research and Development, Medicinal Chemistry Department, Campus de Maigremont BP315, F- 27106 Val de Reuil Cedex, France

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key components of combination anti-HIV therapy. Recently, the US FDA approved the second-generation NNRTI etravirine (TMC125)[(1)], a diarylpyrimidine (DAPY) NNRTI that can successfully treat patients

with NNRTI resistance. We have pursued our efforts in design and synthesis of NNRTI compounds from the DAPY class. The target product profile for a new DAPY candidate was to combine a high activity against wild-type and clinically relevant mutant HIV-1 strains with pharmacokinetic properties allowing once-daily oral treatment of patients at low dose, which is especially important for treatment-naïve patients. Molecular modeling suggested a possibility to improve the interaction between the para substituent on the etravirine left wing and the conserved W229 region within the reverse transcriptase enzyme. A second important suggestion came from the medicinal chemistry knowledge around the DAPY and IOPY[2] classes. This led to a straightforward strategy for the optimization of R152929 towards TMC278 [3,4].



This presentation will illustrate the steps to the discovery of TMC278. We will also describe chemistry, SAR, structural biology studies, biological data and pharmacokinetic properties.

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L10

KINASE SELECTIVITY IN DRUG DISCOVERY-THE CHALLENGES AND OPPORTUNITIES

Dr. Dennis Powell,

Wyeth Research, Pearl River, United States

Selectivity is an important goal for all drug candidates and is a challenge for kinase inhibitors in particular. With more than 500 kinases, and until recently, the limited ability to assess the activity of a drug candidate against the majority of those kinases, drug discovery efforts are hampered by having an incomplete in vitro profile for selectivity. How biochemical selectivity translates into selective cellular function, and eventually, target organ efficacy, is a complex and ongoing question. While potent, highly specific kinase inhibition is often the goal, multi-kinase targeted inhibitors might be preferred in the treatment of human malignancies where numerous signaling pathways are compromised. In addition, resistance to chemotherapy via mutation and upregulation of signaling pathways is a serious concern and a multi-kinase targeted inhibitor may provide significant advantages for both first line as well as second line treatment.

This presentation will discuss some of the challenges in understanding and utilizing kinase selectivity in drug discovery. As examples of this process the discovery of two kinase inhibitors, Bosutinib (SKI-606) and Neratinib (HKI-272), how they were optimized for different selectivity profiles, and how those selectivity profiles were utilized to develop successful clinical candidates will be discussed. Bosutinib, is a multi-kinase ATP competitive inhibitor of Src family kinases as well as Bcr-Abl kinase This profile of kinase selectivity is well suited to the treatment of Imatinib resistant CML, where one route of resistance is through the upregulation of Src family kinases. Neratinib is an irreversible inhibitor of EGFR and HER-2 kinases, forming a covalent bond with a conserved cysteine residue in the ATP binding pocket of these kinases. This cysteine is rare among other protein kinases but it is conserved in three out of four members of the ErbB-family. The ability to irreversibly inhibit the activity of the ErbB family of kinases leads to a significant advantage in the treatment of cancers that have become resistant to the first generation of EGFR ATP competitive inhibitors such as Erlotinib and Gefitinib.

L11

Dr. Louis Lombardo

Bristol-Myers Squibb, United States

Abstract not available at the time of printing.

L12

CDK INHIBITION - SELECTIVITY ISSUES IN PRINCIPLE AND IN PRACTICE

Andrew Thomas

AstraZeneca R&D, Alderley Park, SK10, Macclesfield SK10 4TG, UK

The talk will explore kinase selectivity issues as faced in the AZ CDK2 programme leading to candidate drug AZD5438 and follow-up compound(s). Medicinal chemistry progression within the project and optimisation from early leads to CD will be described. The question of kinase selectivity of project compounds in relation to efficacy, as well as toxicity, will be discussed.

Measuring and interpreting kinase selectivity is often not straightforward. Some principles and practical issues

of measuring kinase selectivity profiles will be covered. The value and appropriateness of different measures of selectivity will be discussed and illustrated, including kinase enzyme selectivity, cellular assays, with protein phosphorylation and phenotypic endpoints, and also in vivo measures.

The difficulty of predicting cross kinase activity of inhibitors on the basis of sequence and likely consequences will also be illustrated. Similarly, issues around predicting and understanding affinity (and selectivity) from kinase inhibitor x-ray crystal structures will be mentioned.

Finally, in concluding, some personal views on the importance of kinase selectivity, or otherwise, will be presented.

L13

PREDICTIVE ADME: EXAMPLES FROM THE REAL WORLD?

Andrew Mark Davis

AstraZeneca R&D, Bakewell Road, Loughborough LE11 5RH, UK

Abstract not available at the time of printing.

L14

VIRTUAL METHODS FOR PREDICTING OFF-TARGET PHARMACOLOGY

Jordi Mestres

Chemogenomics Laboratory, Research Unit on Biomedical Informatics (GRIB), Municipal Institute of Medical Research (IMIM) and University Pompeu Fabra, Doctor Aiguader 88, 08003 Barcelona, Catalonia, Spain, Fax: +34 93 316 0550, jmestres@imim.es

New trends in multitarget drug discovery seem to indicate that the design of a new generation of safer more efficient drugs may partly rely on our ability to anticipate the pharmacological profile of molecules across a panel of targets. In this respect, it is widely recognised that, due to limited time and resources, small molecules are usually not screened systematically through a large panel of protein targets for the sake of acquiring knowledge about their complete pharmacological profile but solely to the few targets of interest for the particular project at work. The consequences are that the drug-target interaction data currently available are largely incomplete and biased toward targets of common therapeutic interest. This talk will focus on current efforts towards developing knowledge-based in silico target profiling methods and their potential impact to designing future drugs with customised pharmacological profiles.

L15

PREDICTING BRAIN TO BLOOD DRUG PARTITIONING: PROGRESS AND LIMITATIONS

Gabriele Cruciani,[a] Massimo Baroni,[b] Goran Westerberg[c]

[a] Chemistry Department, University of Perugia, via Elce di Sotto 10, I06123 Perugia Italy, [b] Molecular Discovery Limited , 215 Marsh Road, Pinner, Middlesex HA5 5NE, England, [c] Siena Biotech S.p.A., via Fiorentina, 153100 Siena - ITALY

The blood-brain barrier (BBB) is the highly regulated interface between the peripheral circulation and the central nervous system (CNS). It consists of an intricate network of tight junctions that separate the CNS from systemic blood circulation.

To be effective as therapeutic agents, centrally acting drugs must cross the BBB. Conversely, to be devoid of unwanted central nervous system effects, peripherally acting drugs must show limited ability to cross the BBB. In both cases, the BBB permeability of drug candidates must be known.

However, the experimental determination of blood-brain partitioning is difficult, time-consuming, expensive and not suitable to screen large collections of chemicals. A broadly applicable method for predicting the BBB permeation of candidates at an early stage of discovery would have a great impact in drug research and development.

However, predicting by in silico methods blood-brain barrier permeation remains a challenge in drug design. Entry into the brain is a complex phenomenon which depends on multiple and inter-related factors. It is known that relatively lipophilic drugs can cross the BBB by passive diffusion as influenced by their H-bonding capacity. Polar molecules normally do not cross the BBB, but sometimes a process of active transport facilitates their penetration. Local hydrophobicity, ionization profile, molecular size, lipophilicity, and flexibility are other important parameters which play a role in BBB permeation. Not only is the number of accepted or donated H-bonds important but also their 3D distribution, due to the anisotropic nature of all biological membranes, or to possible molecular rearrangements due to intramolecular hydrogen bonds. Finally, metabolism at brain level, although not so extensive like in the liver, may modulate the concentration of the compounds into the brain.

The present paper reports the experimental and computational studies conducted at the level of the biological membrane, to demonstrate the value of in silico model obtained so far, and the current limitations we need to address.

L16

PEG CONJUGATION IN DRUG DEVELOPMENT AND DISCOVERY

Francesco M. Veronese

Department of Pharmaceutical Sciences, Padua University, 35100 Padua, Italy

The conjugation of polymers conveys to drugs properties that other way cannot be obtained: In proteins it yields reduction of antigenic responses, increased stability and residence time in blood (1), whereas in the case of small molecular weight anticancer drugs, in addition to a slow release, it allows to take advantage of the so called "EPR effect"(2).

Good results can be obtained only by a proper choice of polymer and of the chemistry of conjugation. Poly(ethylene glycol), PEG, stands as one of the most successful among the polymers: six PEG conjugates of proteins and of one oligonucleotide have already been approved for marketing and few others are in advanced state of clinical experimentation.

The advantage of PEG resides in the absence of toxicity and antigenicity, the availability of a large range of molecular weights at a high degree of purity and in the low polydispersity. A property of PEG is the presence in the chain of two and, in the methoxy form of only one functional group. The methoxy-PEG is more convenient when proteins must be conjugated, since the formation of cross-linked molecules is avoided, but not in the case of non-peptide drug-conjugates for the low drug loading.

From a historical overview one can understand that the development of PEGylation parallels the progression of the chemistry that became more and more specific towards the available functional groups in proteins and drugs: amine, thiol, hydroxyl, amide, carboxyl and disulphide. Chemical methods of coupling are the most employed, but recently enzymatic ones have being developed also. In case of proteins, where several potentially reactive residues are present, special afford was dedicated to obtain site specific conjugation in order to avoid the linking of the polymer chains in, or close to, the active site of enzymes or the recognition sites involved in the binding with receptors. To reach such site specific modifications, genetic engineering was also used in order to introduce amino acids with special reactivity, see for instance cysteine, in wanted sites of protein surface. The linear form of the polymer was also modified and a branched one, called PEG-2 got great success.

However even with the best strategy of PEG conjugation it is difficult to avoid biological activity loss, but generally a convenient compromise between this loss and the improvement of pharmacokinetics and pharmacodynamics can be found. An example is represented by the anti-viral drug PEGASYS that, following modification, maintained only seven per cent of the native protein activity, a decrease that is more then compensated by the great increase of residence time. An alternative solution to the problem of the reduced activity of conjugates is represented by the development of reversible PEGs linkers that release slowly the free native protein in blood, as in the case of the pro-drugs.

Although the most common application of PEGylation is to improve the therapeutic activity of drugs, other uses must be reported, among these the solubilization of enzymes in organic solvents thanks to the anphiphilicity of PEG, a property that is acquired by the protein conjugates.

Small non peptide-drugs were also conjugated to PEG, in this case the diol polymer was mainly used in order to increase the drug loading, but special forms of the polymer, as the fork PEGs that possess several reactive groups at the chain end were also synthesized. For such drug conjugates suitable linkers were proposed that take advantage of releasable mechanisms well known in pharmaceutical chemistry but, although the great amount or research carried out in this direction and the many promised results obtained, so far no PEG-conjugate could reach the market.

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L17

MULTIVALENCY OF POLYMER THERAPEUTICS USED FOR THE INTEGRATION OF ANTI-ANGIOGENIC THERAPY WITH CHEMOTHERAPY

Ronit Satchi-Fainaro, Ph.D.

Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel. Email: ronitsf@post.tau.ac.il

Angiogenesis, new capillary blood vessel growth from pre-existing vasculature, is crucial for tumor progression and metastases. Consequently, the microvascular endothelial cell, recruited by a tumor, has become an important second target in cancer therapy. The successful development of anti-angiogenic therapies for cancer and ocular diseases represents a major medical advance. This validation of the scientific principles elucidated by Folkman and others proves the importance of angiogenesis as a critical "common denominator" pathway in disease. With the successful approval and clinical adoption of anti-angiogenic agents, new questions challenge the scientific and clinical dogmas of the angiogenesis field. Although anti-angiogenic agents offer great therapeutic potential, preclinical and clinical studies suggest that these agents, used as monotherapies, will have a delayed onset of activity and may only induce disease stabilization for advanced malignancy.

Drug delivery technologies including novel polymers promise to create new combination treatments. Multimodality targeted polymer therapeutics that include anti-angiogenic agents offer the potential for improved efficacy and diminished toxicity in the treatment of cancer and other angiogenesis-dependent diseases. However, additional work is still needed to provide a rational basis for the combination of angiogenesis inhibitors with other

modalities so that these agents can be successfully incorporated into existing therapy in a timely and rational manner.

Here we present some novel anti-angiogenic and anti-tumor polymer-drug conjugates that target both the tumor and its microenvironment. These conjugates include combined anti-angiogenic and chemotherapeutic drugs, such as TNP-470 and paclitaxel, respectively. Some also incorporate bisphosphonates as targeting moieties for bone metastases and osteosarcomas or RGD peptidomimetics that target $\alpha V\beta 3$ integrins overexpressed on tumor endothelial cells and several tumor cells. Our results point at our polymer therapeutics as novel bi-specific conjugates targeting both thetumor epithelial and endothelial compartments warranting its use on a wide spectrum of primary tumors and metastatic ones.

L18

5'-PHOSPHATE MIMICS WITHIN ALTRITOL-MODIFIED SIRNAS AND INHIBITION OF MDR1 EXPRESSION

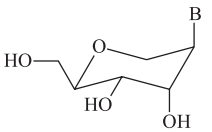
Arthur Van Aerschot[1], Michael Fisher[2], Guy Schepers[1], Mikhail Abramov[1], Jef Rozenski[1], Rudolph L. Juliano[2] and Piet Herdewijn[1]

[1]Laboratory of Medicinal Chemistry, Rega Institute for Medical Res., Minderbroedersstraat 10, B-3000 Leuven, Belgium, [2]Dep. of Pharmacology, School of Medicine, University of North Carolina Chapel Hill NC 27599, USA

We recently reported on siRNA mediated inhibition of MDR1 expression with altritol-modified nucleic acids (ANAs).[1] Overall, their biological activity proved slightly better in comparison with the anhydrohexitol-modified or cyclohexenyl-modified siRNAs,[2] and a large series of modifications at different positions of either the sense or antisense strand or both was studied. It was shown clearly that ANA-modified siRNAs targeting the *MDR*1 gene can exhibit improved efficacy as compared to unmodified controls. This was particularly true of ANA modifications at or near the 3' end of the sense or antisense strands, while modification at the 5' end of the antisense strand resulted in complete loss of activity. Multiple ANA modifications within the sense strand were also well tolerated. Duplexes with ANA modifications at appropriate positions in both strands were generally more effective than duplexes with one modified and one unmodified strand.

The loss of activity associated with ANA modification of the 5'-antisense strand may be due to reduced phosphorylation at this site by cellular kinases. Indeed, a 5'-phosphate introduced chemically on the 5'-altritol nucleoside rescued the activity to a certain extent. Hence, we now have evaluated different phosphate analogues introduced chemically at this 5'-position, which could mimic the phosphate and at the same time should be non-hydrolyzable. Unfortunately, methylphosphonate, thiophosphate, sulphate, sulphamide and phosphoramide modifications did not rescue the activity to the same extent as the straightforward introduction of a phosphate moiety.

In addition, treatment of drug resistant cells with *MDR*1-targeted siRNAs resulted in reduction of P-glycoprotein (Pgp) expression, parallel reduction in MDR1 message levels, increased accumulation of the Pgp substrate rhodamine 123, and reduced resistance to anti-tumor drugs. Interestingly, the duration of action of some of the ANA-modified siRNAs was substantially greater than that of unmodified controls. These observations suggest that altritol modifications may be helpful in developing siRNAs with enhanced pharmacological effectiveness.



altritol nucleic acid (ANA)

siRNA with 2 ANA blocks at 5'-end + phosphate (or mimic)

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L19

EVOLUTION: DESIGN AND IMPLEMENTATION OF A FRAGMENT DISCOVERY PLATFORM

John Barker

EVOTEC, 114 Milton Park, Abingdon, OXON OX14 4SA, UK

Novel starting points for medicinal chemistry programmes can be effectively identified by screening libraries of fragment molecules. Using complementary approaches of virtual screening, fluorescence correlation spectroscopy and NMR screening Evotec has developed a platform for identification of active fragments ahead of determination of their binding modes by X-ray crystallography.

In this presentation the development of the Evotec fragment platform, design of the fragment library and its performance against a number of targets, including the classic target for fragment screening Hsp90, will be discussed in detail. A biochemical screening approach to fragments, using a portfolio of single-molecule Fluorescence Correlation Spectroscopy (FCS+plus) detection techniques to ensure the highest reproducibility and sensitivity, has been enhanced through the use of the orthogonal but highly complementary technique of NMR screening. The addition of both protein and ligand NMR screening techniques to Evotec's biochemical fragment screening approach delivers high information content and

validation of fragment activity ahead of the critical X-ray crystallographic analysis of the binding mode of fragments. The identification of binding modes allows Evotec to employ its expertise in SBDD to rapidly optimise fragment hits.

L20

FRAGMENT-BASED DRUG DISCOVERY: WHAT HAS IT ACHIEVED SO FAR?

Alexander A. Alex and Maria M. Flocco

Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom

Historically, most drugs have been discovered either based on an already existing one or by random screening of compound collections.[1] The onset of combinatorial chemistry and the development of screen miniaturisation and automation in the early 1990s have resulted in much larger compound collections and vastly enhanced high throughput screening capabilities. These developments enabled the screening of collections of hundreds of thousands of compounds, making high throughput screening the predominant approach for hit discovery in large pharmaceutical companies.[1] A different approach, referred to as fragment-based drug discovery, has been described in the literature since the late 1990s, [2] but has only become practical in recent years due to significant advances in technology. Fragment-based drug discovery relies on the identification of low molecular weight compounds that (weakly) bind to a chosen target and attempts to build up drugs from these small molecular pieces and components to achieve the desired biological activity and molecular properties. This approach is largely complementary to HTS as a technique that aids the hit-to-lead or lead discovery processes. Although often synonymous with fragment screening, fragment-based drug discovery is a far wider area covering high-throughput screening, fragment screening and virtual screening efforts. The unifying feature of fragment-based drug discovery is the low molecular weight of the hit rather than the approach it originates from. In this context, the concept of ligand efficiency will also be highlighted [3]. Over the last ten years, fragment-based drug discovery has provided in excess of 50 examples of small molecule hits that have been successfully advanced to leads and therefore resulted in useful substrate for drug discovery programs. We will review efforts in this area and analyse and compare the changes in molecular properties between different approaches in hit to lead, including fragment discovery.[4]

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L21

FRAGMENT-BASED LEAD GENERATION: CHALLENGES AND SUCCESSES. DISCOVERY OF HIGH AFFINITY BETA-SECRETASE INHIBITORS

Jeffrey S. Albert, Edwards, Philip D; Blomberg, Niklas; Breeze, Alexander L.; Brown, Alastair J. H.; Burrows, Jeremy N.;.Folmer, Rutger H. A.; Geschwindner, Stefan; Griffen, Ed J.; Kenny, Peter W.; Nowak, Thorsten; Olsson, Lise-Lotte; Sanganee, Hitesh; Shapiro, Adam B.

CNS Discovery Research, AstraZeneca Pharmaceuticals, 1800 Concord Pike, PO Box 15437, Wilmington, DE 19850-5437; jeffrey.albert@astrazeneca.com

Fragment based lead generation (FBLG) has recently emerged as an alternative to traditional high throughput screening (HTS) to identify chemistry starting points for drug discovery programs. In comparison to HTS screening libraries, the screening sets for FBLG tend to contain orders of magnitude fewer compounds, and the compounds themselves are less structurally complex and have lower molecular weight. We will describe challenges and successes across several projects at AstraZeneca, with particular emphasis on the discovery of high affinity beta-secretase inhibitors for Alzheimer's disease. Using NMR methods, we screened a library of low molecular weight compounds to identify hits that bound to the active site of beta-secretase with affinities (IC50) of 1-5 mM. X-ray crystallography and structure-based design facilitated the rapid evolution of these weak hits into high affinity (IC50 <100 nM) drug leads.

L22

FLUOROMETRY AND FRET IN MEASURING BIOMARKERS AND MONITORING CELL SIGNALLING CASCADE

Ilkka Hemmilä, PhD

PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland

Fluorometric technologies have essential role in analyzing biochemical and cellular events and biomarker search. Adding temporal resolution has given new dimensions to fluorometric analysis and data collection, time, and also has greatly improved sensitivity and specificity of the assays. Time-resolved technologies applied include various imaging modalities, such as lifetime imaging in different time domains, time-resolved fluorometric techniques in immunoassays for biomarker detection, as well as time-resolved way to measure FRET signal.

The present talk will concentrate on millisecond time-gating technologies based on long excited state lifetime lanthanide chelates, and their applications in *in vitro* and cellular cell signaling and drug discovery applications, starting from receptor-ligand interactions, monitoring the GPCR activation by GTP binding, measuring the downstream kinase cascades and monitoring nuclear receptor activation and post-translational control mechanisms.

L23

MASS SENSITIVE LIGANDS USEFUL FOR BIOMARKER DISCOVERY AND VALIDATION

Peter Schulz-Knappe

Chief Scientific Officer, Proteome Sciences plc, UK

Proteomics is considered as key technology for peptide and protein biomarker discovery and development. In the last years, poor reproducibility of the methods used for qualitative and quantitative proteome analysis has severely restricted biomarker discovery as well as validation.

Currently, there is no universal technology for proteomics. The field is segmented into many different technologies and workflows, most likely because there are a myriad of proteins and peptides each possessing very different profiles as to biochemical activity, size, form etc.

Reproducibility of studies has been poor and comparability between labs is often not possible. A comprehensive and sensitive technology is therefore needed in order to improve this situation.

One of the newest and best technologies for biomarker discovery and development is to use mass spectrometry combined with quantitative tags for analysis. Proteome Sciences, has pioneered the field of isobaric mass tags. Recently, Tandem Mass Tags (TMT) have been developed. All tags label amine functions in proteins and peptides on N-termini and Lysine amino acids. The tags have the same overall mass and identical physicochemical properties. After individual labeling of up to six different patient samples, the samples can be mixed and processed together. Labeled proteins behave identically during all steps such as sample handling and chromatography. The idea behind the TMT technology is that the quantitative ratio between individual proteins remains constant after mixing of samples. This conservation of relative proportions is a key for subsequent separation and analysis. Since the mass tags have individual fragmentation patterns the patient proteins can be quantified specifically during mass spectrometry. TMT's only prerequisite is that one must be able to label the sample and that the analyte can be detected with MS. It allows much better control, precision and accuracy to study hundreds to thousands of proteins.

The presentation will illustrate the principle concept of isobaric mass tagging and give several application examples. Proteome Sciences has also made advances in the development of reference standards for proteomics. Clinical laboratories typically use reference materials to calibrate and certify assays. Unfortunately, proteomics lacks such materials to benchmark performance. We decided to address this need and provide TMT-labeled Reference Materials for blood plasma, urine, and other samples. These materials serve as a defined proteome reference. Using this as control sample it is now possible for scientists to have quality control and share their results worldwide. We expect reference materials to help speed up discovery and development of biomarkers. Proteome Sciences has partnered exclusively with Thermo-Fisher to market the universal TMT technology platform for mass spectrometry.

L24

DESIGN OF SPIN-LABELED SULFONAMIDES AS CARBONIC ANHYDRASE INHIBITORS

Cecchi Alessandro[a], Ciani L[b], Winum JY[c], Montero JL[c], Scozzafava A[a], Ristori S[b], Supuran CT[a]

[a] University of Florence, Lab. of Bioinorganic Chemistry, Via della Lastruccia 3, 50019 Sesto Fiorentino,Italy

[b] University of Florence, Department of Chemistry & CSGI, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy; [c] Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS-UM1-UM2, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex, France

Carbonic Anhydrase IX (hCA IX) is a membrane-associated glycoprotein that is observed in many tumor tissues and is strongly overexpressed by hypoxia conditions. Hypoxia is a clinically important tumor parameter and this enzyme can play an important role as a potential *marker* of hypoxic tumor and as a *therapeutic target* too[1]. In the last years, Carbonic Anhydrase IX Inhibitors which possess fluorescent probe were largely used for visualize hypoxic tumor cell lines and for understanding the biological roles of hCA IX in acidification of the external matrix[2,3].

Here, we synthesized a new and original serie of spin-labeled thioureido-sulfonamides as potencial marker of hypoxic tumor cell lines[4]. These compounds possess a 2,2,6,6-Tetramethyl-piperidine-1-oxyl moiety as "tail", which render these inhibitors active in the EPR experiments.

These molecules were tested *in vitro* against the cytosolic hCA I and hCA II, and with the tumor-associated hCA IX. All the compounds behave as strong hCA IX inhibitors, with KI in nanomolar range. Furthermore, the EPR signals of these molecules change in presence of Carbonic Anhydrase, due to the Enzyme-Inhibitor complex formation. In this way, such compounds could interact with the extracellular catalytic domain of hCA IX, and discriminate between the normal and the tumoral cell lines.

The whole results show that these compounds may be used as additional markers of tumor cell line and could also give further information regarding the enzyme-inhibi-

tion complex in solution (i.e. inhibitor mobility, etc.), or developed as diagnostic tools.

Acknowledgements

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L25

BIODIVERSE NATURAL PRODUCTS IN DRUG DISCOVERY

Douglas Kinghorn, Jack L. Beal

Professor and Chair College of Pharmacy, Division of Medical Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210, U.S.A.

For about 150 years, from the beginning of the 19th century, medicinal plants served as organisms of origin of most natural product drugs then used in western medicine. A significant proportion of the official drugs at the time were either crude drugs or extractives, but gradually purified components such as water-soluble alkaloid salts and glycosides were introduced into clinical therapy. From the 1940s to the present, however, microbes have become increasingly significant as sources of anti-infective and antitumor agents, in particular, but also as sources of cholesterol-lowering and immunosuppressant drugs (1,2). In the 21$^{st.}$ century, naturally occurring drugs have continued to be introduced into western medicine, but from a wider variety of organisms than previously (e.g., marine fauna and terrestrial vertebrates, as well as microorganisms and plants).

Small organic molecules from nature ("natural products" or "secondary metabolites") are now generally regarded as occupying complementary "chemical space," when compared to drugs prepared by synthetic and combinatorial chemistry methods alone. Natural products are biosynthesized in the correct chiral form to exhibit activity in biological test systems. The efforts of organic and medicinal chemists can enhance the often scarce supply of natural product lead compounds and improve their efficacy and/or reduce toxicity, among other parameters. Chemical modification of natural products may lead to additional active compounds and provide a more complete understanding of structure-activity relationships. Natural products are commonly utilized as "biochemical tools" to probe cellular targets and thus provide mechanistic insight into many diseases. Complex or unusual natural products may present interesting structure determination challenges, and commercially valuable substances are of interest for "combinatorial biosynthesis" and metabolic engineering investigations.

In this keynote lecture, contemporary factors affecting natural product drug discovery will be reviewed, such as the types of organisms available for study, benefit-sharing and intellectual property issues, and the pressing need for source organism conservation (2). Particular reference will be made to an ongoing multidisciplinary project directed towards the discovery of novel anticancer agents from tropical plants, aquatic cyanobacteria, and filamentous fungi. (Funding of P01 CA125066-01A1 from NCI/NIH, Bethesda, Maryland, U.S.A. is gratefully acknowledged).

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L26

HOW TO USE THE STRUCTURAL DIVERSITY OF NATURAL PRODUCTS FOR DRUG DISCOVERY

Dr. Philipp Krastel

Novartis Institutes for Biomedical Research, CH-4002 Basel, Switzerland)

Natural Products are an important field for the discovery of new drugs. Nature is still a non exhausted source for new pharmacophors. In recent year several launches of new drugs like Ixempra™, Elidel™, Certican™, Cubicin™ or Myfortic™ are based on natural products, showing the high impact of natural product research on drug discovery in different therapeutic areas.

With the emerging need for new drugs against multidrug resistant bacteria natural products have again become an important source for the search for new antibiotics. Compared to the early days of antibiotic research in the last century the techniques used in natural product research have changed dramatically offering a large variety of new methods. This includes the rapid characteriza-

tion of the biological diversity by rDNA sequences including access to new biological sources, the estimation of the chemical diversity by LC-DAD/MS and MS/MS analytics using spectra libraries during de-replication and the rapid structure elucidation of purified compounds.

The integration of new technologies in natural product research opens an efficient way for the build up of highly diverse libraries with structurally unique compounds. These compounds are promising starting points for lead optimization or tools for target or pathway elucidation.

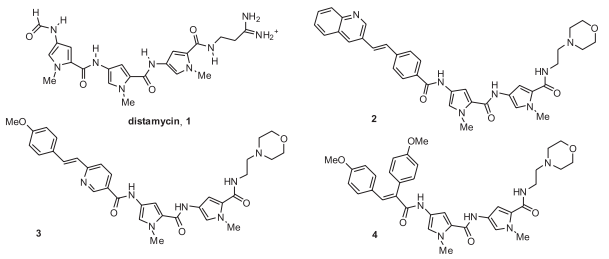
L27

SELECTIVITY IN THE ANTIBACTERIAL ACTIVITY OF MINOR GROOVE BINDERS, DERIVATIVES OF THE NATURAL PRODUCT, DISTAMYCIN

Colin J. Suckling[a], Abedawn I. Khalaf[a], David Breen[a], Elizabeth M. Ellis[b], Iain S. Hunter[b], Samuel Nyabam[b]

WestCHEM, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow, Scotland, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, Scotland

Derivatives of the natural product, distamycin **1**, have been widely studied as potential therapeutic agents. We have found that several analogues (e.g. **2** and **3**) with lipophilic, aromatic head groups such as quinoline, pyridine, and benzene derivatives connected by an ethenyl link, isosteric with the amide found in the natural products, have exceptional selective antibacterial activity with respect to Gram-positive organisms.[1] It is important to understand the reasons for the low toxicity towards mammalian cells if such compounds are to proceed towards development as drugs. To investigate this, we have studied the properties of compounds with tail groups other than morpholino ethyl, which appears to be optimal for antibacterial activity. A new, diversity-oriented synthesis that allows the preparation of many amino-substituted compounds from a common precursor, a thiophosphoamidate, has been developed and used for the synthesis of analogues of **2** and **3** with more basic tertiary amino tail groups (piperidyl and pyrrolidinyl). These derivatives show significant antibacterial activity but also increased toxicity with respect to both L929 and HS27 cell lines emphasising the importance of the pK_a of the tail group. Several alkenyl minor groove binders are intrinsically fluorescent, **4** being the strongest fluorescer of the antibacterial compounds. **4** has been used to probe access to different types of cells using microscopy. It has been shown that **4** penetrates the cells of *Staphylococcus aureus* (Gram-positive) but not *Escherichia coli* (Gram-negative) nor mammalian cell lines. The importance of transport into target cells was emphasised by the observation that **4** penetrated sphaeroplasts from *E. coli* in which the outer membrane and cell wall had been removed. Thus we can conclude that both the physical organic properties of the minor groove binders and the structure of the cell walls and membranes of cells both contribute to the selectivity of these compounds.



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L28

PROTEIN MISFOLDING AND SELF-ASSEMBLY IN NEURODEGENERATIVE DISEASES: FROM MECHANISTIC STUDIES TO THERAPEUTIC STRATEGIES

Hilal A. Lashuel, Ph.D.

Assistant Professor of Neuroscience,Director of the Laboratory of Molecular Neurobiology and Neuroproteomics, Swiss Federal Institute of Technology-Lausanne (EPFL), School of Life Sciences, Brain Mind Institute, Lausanne, Switzerland, Email: hilal.lashuel@epfl.ch, Lab website: http://nmnf.epfl.ch

The process of protein fibrillization, also known as amyloid formation, is implicated in the pathogenesis of most, if not all, age-associated neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease. However, the mechanism(s) by which it triggers neuronal death is unknown. Our group employs a multifaceted approach that aims to bring to bear the power of synthetic chemistry, biophysics, structural biology, proteomics, animal modeling and molecular and cell biology to elucidate the molecular and structural basis of protein aggregation and toxicity in neurodegenerative diseases. More specifically, research efforts in our laboratory cover the following topics: (1) elucidating the biochemical and structural basis of amyloid-associated toxicity in neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's disease; (2) understanding the role of quaternary structure in protein function and disease; (3) developing novel chemical and physical approaches and tools to monitor and control protein misfolding and protein aggregation *in vitro* and *in vivo*; (4) developing novel therapeutic strategies based on modulating protein aggregation and clearance. My talk will focus on highlighting some of our recent findings in these areas and the impact of this work on our understanding of the biochemical and structural basis by which α-synuclein and amyloid-β (Aβ) contribute to neurodegeneration and cell death in Parkinson's and Alzheimer's diseases, respectively. Our recent findings provide novel mechanistic insight into the relationship between protein

aggregation and neurodegeneration and provide the basis for novel targets and therapeutic strategies to treat and/or prevent Parkinson's and Alzheimer's disease.

L29

APPROACHES TO THE TREATMENT OF COGNITIVE DYSFUNCTION AND ALZHEIMER'S DISEASE

Albert J. Robichaud, Ph.D.

Senior Director, Chemical & Screening Sciences, Wyeth Research, CN-8000, Princeton, New Jersey, 08543-8000 USA

Alzheimer's disease is a progressive neurodegenerative disease characterized by gradual and increasing loss of cognitive function and behavioral abnormalities. The formation of β-amyloid plaques and neurofibrillary tangles are recognized as the key pathologies of the disease. Much effort in the past decade has focused on disease modifying approaches and significant progress has been realized. Furthermore, changes in the levels of various key neurotransmitters has been noted in patients with Alzheimer's disease and may represent the earliest biochemical casualty, preceding or signifying the onset of the disease. Over the last 20 years a number of approaches to the palliative treatment of Alzheimer's disease have been scrutinized. The majority of effort has been focused on cognitive dysfunction, as this is the initial and key debilitating symptom of the disease. The identification and commercial development of the acetylcholinesterase inhibitors has, until recently, virtually dominated the field, and although efficacy has been demonstrated, the clinical results suggest alternate approaches are warranted. This presentation will highlight several of our advanced research programs in the areas of disease modifying and palliative therapies aimed at cognitive dysfunction and Alzheimer's disease.

L30

HALOMETHYLKETONES (HMKS): GSK-3 INHIBITORS WITH DISEASE-MODIFYING PROPERTIES FOR ALZHEIMER´S DISEASE THERAPY

S. Conde,[a] D.I. Perez,[a,*] C. Perez,[a] F. Wandosell,[b] C. Gil,[a] and A. Martinez[a]

[a] *Instituto de Quimica Medica-CSIC, Juan de la Cierva 3, 28006 Madrid (Spain),* [b] *Centro de Biología Molecular "Severo Ochoa"-CSIC, Nicolás Cabrera 1, UAM, 28049 Madrid (Spain),* [*] *Present address: Delft University of Technology, Biotechnology, Julianalaan 136, 26 28 BL Delft, The Netherlands*

Alzheimer's disease (AD) is the most common form of dementia affecting more than 15 millions individuals worldwide. Two are the major neuropathological abnormalities presents in the brain of patients with AD: the extracellular senile plaques and the intracellular neurofibrillary tangles.

Glycogen synthase kinase-3 (GSK-3), originally identified as a modulator of glycogen metabolism, has been recently proposed to be involved in the regulation of these two neuropathological hallmarks and there is a strong evidence that GSK-3 plays an important role in AD [1,2]. All these data points clearly identify GSK-3 inhibitors as one of the most promising new approaches for the future treatment of AD [3, 4].

The small heterocyclic halomethylketones (HMKs) represent one of the few ATP non-competitive GSK-3 inhibitors reported to date [5] and have been proposed as new drugs for the effective treatment of neurodegenerative disorders, such as Alzheimer's disease. Kinase selectivity of these new inhibitors, decrease of tau phosphorylation in primary neuronal cultures together with some determined *in silico* and *in vitro* ADME properties using artificial membranes or CODES descriptors will be reported in this communication.

Results show that HMKs compounds are able to cross the BBB and have good oral availability. These data together with the efficacy shown on decrease of tau phosphorylation confirm HMKs derivatives as potential disease-modifying drugs for the future treatment of AD.

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L31

DPP-4 INHIBITORS: GALVUS® / VILDAGLIPTIN AND FOLLOWING GENERATIONS

Daniel Baeschlin

Novartis Institutes for BioMedical Research, Expertise Platform Proteases, Novartis Campus, Basel, Switzerland

Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes mellitus (T2DM). DPP-4 inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain through the conservation of active GLP-1 a peptide hormone, which is deactivated by DPP-4. Galvus (Novartis) and Januvia (Merck) have recently been approved for the treatment of T2DM as the first agents in this new class of T2DM treat-

ment. This novel mode of action has attracted a lot of interest and multiple pharmaceutical companies have DPP-4 inhibitors in clinical development. The presentation will focus on the discovery and characteristics of different DPP-4 inhibitor classes.

L32

PEPTIDIC AND NON-PEPTIDIC GLUCAGON LIKE PEPTIDE 1 RECEPTOR AGONISTS

Jesper Lau

Novo Nordisk A/S, Denmark

Glucagon-like peptide-1 (GLP-1) binds and activates the pancreatic GLP-1 receptor (GLP-1R) and thereby promotes insulin secretion in a glucose-dependent manner. GLP-1 has several additional effects that are important for treatment of type 2 diabetes such as suppression of glucagon release from the α-cells and slowering of gastric emptying as well as down regulation of appetite. Animal studies as well as clinic studies have confirmed all these positive effects of GLP-1 to be used to treat type-2 diabetes.

Unfortunately native GLP-1 has a very short plasma half-life and is not applicable as a drug candidate. This talk will discuss structure activity relationship of GLP-1 analogues and derivatives in order to engineer long acting GLP-1 drug candidates.

The second part of the talk will address structural aspects of the GLP-1 receptor. GLP-1 as well as Exendin-4 bind and activate the GLP-1 receptor (GLP-1R) with similar affinity and potency. GLP-1R belongs to the family B of the seven transmembrane G-protein coupled receptors. The N-terminal extracellular domain (nGLP-1R) is a ligand binding domain with differential affinity for Exendin-4 and GLP-1: low affinity for GLP-1 and high affinity for Exendin-4. The superior affinity of nGLP-1R for Exendin-4 was previously explained by an additional interaction between nGLP-1R and the C-terminal Trp-cage of Exendin-4. We have very recently expressed and crystallised the nGLP-1R/Exendin-4 complex and identified novel important receptor ligand interactions at an atomic level.

The third part of the talk will discuss the discovery of GLP-1 mimetics. Only a few small-molecule agonists to peptide hormone receptors have been described. We have discovered a series of small molecules known as ago-allosteric modulators selective for the human GLP-1 receptor. These compounds act as both allosteric activators of the receptor and independent agonists. Potency of GLP-1 was not changed by the allosteric agonists, but affinity of GLP-1 for the receptor was increased. The most potent compound identified stimulates glucose-dependent insulin release from normal mouse islets but, importantly, not from GLP-1 receptor knockout mice. Also, the compound stimulates insulin release from perfused rat pancreas in a manner additive with GLP-1 itself.

L33

IRREVERSIBLE INHIBITION OF DPP 8/9 BY DIPEPTIDE DERIVED DIARYL PHOSPHONATES

Pieter Van der Veken[a], Anna Soroka[a], Inger Brandt[b], Yuan-Shou Chen[c], Marie-Berthe Maes[b], Anne-Marie Lambeir[b], Xin Chen[c], Achiel Haemers[a], Simon Scharpé[b], Koen Augustyns[a], Ingrid De Meester[b].

[a]Laboratory of Medicinal Chemistry, [b]Laboratory of Medical Biochemistry University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium. and [c]Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhu Nan, Miaoli, Taiwan, ROC350. pieter.vanderveken@ua.ac.be

Recently, inhibition of DPP8/9 has been associated with severe toxicity following *in vivo* studies with *allo*-Ile-isoindoline (**1**), a potent DPP8/9 targeting inhibitor.[1] To verify whether this observed toxicity was caused by inhibition of DPP8/9 or by off-target compound related events and, more general, for the characterisation of the enzyme's physiological role, other structurally or mechanistically distinct inhibitors can be expected to be valuable research tools.[2]

As part of our efforts to develop irreversible diaryl phosphonate inhibitors of DPP8/9, a series of dipeptide derived compounds bearing a diaryl pyrrolidin-2-yl phosphonate at the P1 position was evaluated in order to identify P2 building blocks conferring increased affinity toward the target enzymes. With these products, irreversible inhibition of DPP8/9 was observed. To obtain inhibitors with an improved activity and selectivity profile, a set of selected analogues containing a diaryl isoindolin-1-ylphosphonate at P1 was developed. Within this latter series, the P2-lysine derivative was shown to be a potent irreversible inhibitor of DPP8/9, demonstrating very low affinity for DPP IV and DPP II.[3]

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L34

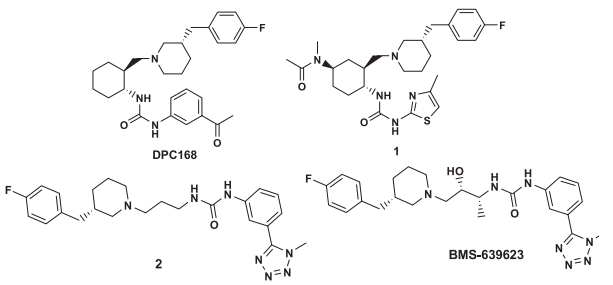
RECENT DEVELOPMENTS IN CCR3 ANTAGONIST

George V. De Lucca

Bristol-Myers Squibb Company, Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543-4000

Selective eosinophil recruitment into inflammatory sites and subsequent activation is a characteristic of allergic diseases, such as asthma, rhinitis, and atopic dermatitis. CC chemokine receptor-3 (CCR3) is the principal mediator of eosinophil chemotaxis and is also known to

be expressed on a variety of inflammatory cells associated with allergic responses. These cells include basophils, mast cells, Th2 lymphocytes, and on resident tissue cells such as airway epithelium. Animal studies suggest that CCR3 is a prominent mediator of allergic responses and that antagonizing the receptor will lead to a reduction in airway inflammation. The potential importance of CCR3 in allergic inflammation has made this receptor a target of drug development. A brief survey of recent reports in the literature of CCR3 antagonists shows that while it has been relatively easy to identify CCR3 antagonists with potent binding affinity (nM), compounds with picomolar potencies in the functional chemotaxis assay were not known until the disclosure of **DPC168**. Conformational rigidity and the proper orientation of the important pharmacophores is the key to the potency seen in these analogs. Analogs with equatorial substituents on the cyclohexyl linker that reinforce this conformation (such as **1)** show equal potency to **DPC168**, while the corresponding axial substituent is almost 100-fold less potent. The use of conformational analysis to design pM inhibitors such as **BMS-639623** starting from nM inhibitors (**2**) suggests that other compounds with pM potencies can be designed.



L35

PI3Kγ INHIBITORS: ASPIRIN OF THE 21ST CENTURY?

Thomas Rückle PhD

Merck Serono S.A., Geneva Research Centre 9, Chemin des Mines, 1202 Geneva, Switzerland. Phone: +41-79-6214063; Fax: +41-22-4149565; email: thomas.ruckle@merckserono.net

Class IB phosphatidylinositol 3-kinase p110γ (PI3Kγ) has gained increasing attention as a promising drug target for the treatment of inflammatory disease.[1,2] Extensive target-validation data are available, which are derived from studies using both pharmacological and genetic tools.[3,4] More recent findings have uncovered further therapeutic applications for PI3Kγ inhibitors, opening up potentially huge opportunities for these drugs.[5] The abstract will focus on our recent findings in EAE, an inducible animal model for Multiple Sclerosis that shares immunologic and histopathologic outcomes with the human disease.

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L36

4-OXO-β-LACTAMS (AZETIDINE-2,4-DIONES) BASED NOVEL POTENT INHIBITORS OF ELASTASE

Jalmira Mulchande[1], Wing Y. Tsang[2], Michael I. Page[2], Jim Iley[3] and Rui Moreira[1]

[1]*i-Med.UL, Faculdade de Farmácia, Universidade de Lisboa, Av. Forças Armadas, 1600-083 Lisboa, Portugal,* [2] *Department of Chemical & Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK,* [3] *Department of Chemistry, The Open University, Milton Keynes, MK7 6AA, UK*

β-Lactams are acylating agents of the active site serine residue of a wide range of serine proteases, including elastase, one of the most destructive proteolytic enzymes [1]. Recently 3-oxo-β-sultams, **1**, were reported as potent inhibitors of porcine pancreatic elastase (PPE), by formation of a covalent enzyme-inhibitor adduct from attack at the carbonyl centre and C-N fission [2], but they are chemically too reactive for studies towards therapeutic application. With this background we designed 4-oxo-β-lactams, **2** as isosteric analogues of **1**, by replacing the sulphonamide by a poorer leaving group such as an amide [3].

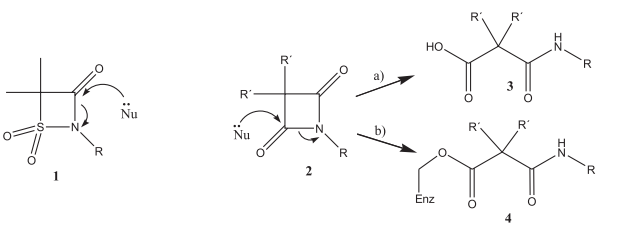

Figure **1** – Hydrolysis of 4-oxo-β-lactams: *a)* by hydroxide ion; *b)* by PPE

We evaluate the effect of different amide leaving groups on a) the kinetics of alkaline hydrolysis and b) PPE inhibition (Fig. **1**). We found that the most reactive derivatives, containing an electron-withdrawing substituent on the aromatic ring (R = C_6H_4-4-CN, C_6H_4-4-Cl), were also the most actives ones against PPE [3]. That evidence supports the use of k_{OH} value for the alkaline hydrolysis as a crude guide to determine the inhibitory potential of an enzyme acylating agent [4]. We conclude that 4-oxo-β-lactams, **2**, are novel, potent time-dependent irreversible inhibitors of PPE, while decreasing dramati-

cally the reactivity towards hydroxide when compared to the isosteric analogues **1**.

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L37

THE POTENTIAL OF ALLOSTERIC MODULATION FOR GPCR DRUG DISCOVERY

Arthur Christopoulos

Drug Discovery Biology Laboratory, Dept. of Pharmacology, Monash University, Melbourne, Australia

G protein-coupled receptors (GPCRs) account for approx. 2% of the human genome and represent the major targets for around 30% of all medicines on the market. Traditionally, optimizing the interaction of lead molecules with the binding site for the endogenous agonist ("orthosteric" site) has been the approach for obtaining selectivity of receptor action. However, it is now evident that many GPCRs possess additional allosteric binding sites, and that ligands can utilize these sites to modulate receptor activity through conformational changes transmitted from the allosteric to the orthosteric site and/or to effector coupling sites[1,2]. Allosteric modulators offer enormous potential for expanding the chemical space associated with GPCR-targeting small molecules. However, in order to capitalize on the promise of GPCR allosteric modulators, a number of ongoing practical challenges also need to be addressed. One such challenge is the need to appreciate and capture as many allosteric behaviors as possible when screening for such ligands. This is because allosteric modulators can have divergent effects on orthosteric ligand affinity versus efficacy[3], while other allosteric ligands can possess intrinsic efficacy in their own right[4,5]. Indeed, there are now clear examples of the following classes of allosteric ligands: a) affinity modulators, b) efficacy modulators, c) allosteric agonists and, most recently, d) bitopic modulators, which contain both orthosteric and allosteric moieties encoded within the same molecule. Another challenge to successfully exploiting GPCR allosteric modulators is the need to develop quantitative models of allosteric drug action to facilitate modulator structure-activity studies. We have recently developed an "operational" modeling approach that allows for the derivation of structure-activity parameters for allosteric modulators from experimental screening data[4]. A third, related, challenge is the determination of structure-function relationships governing allosteric modulator effects on receptor conformation[5,6]. Finally, perhaps one of the most interesting recent observations in this field relates to the ability of some allosteric agonists and modulators to differentially "traffic" receptor stimuli, promising a great potential for further sculpting of cellular signaling, but requiring a broad range of screening assays as a consequence of such pathway-dependent modulation[4].

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L38

THERAPEUTIC OPPORTUNITIES FOR SMALL MOLECULE MODULATORS OF CHEMOKINE RECEPTORS

Thomas J. Schall

ChemoCentryx, 850 Maude Avenue, Mountain View, CA 94043, USA

Abstract not available at the time of printing.

L39

HIGH-THROUGHPUT SCREENING OF POTENTIAL DRUG CANDIDATES FOR GPR-17 RECEPTOR BY FAC-MS: DEVELOPMENT AND APPLICATION OF A NEW GPR17 STATIONARY PHASE

G. Massolini[1], C. Temporini[1], E. Calleri[1], S. Ceruti[2], C. Lambertucci[3], R. Volpini[3], M.P Abbracchio[2], G. Caccialanza[1], G. Cristalli[3]

[1]Department of Pharmaceutical Chemistry, University of Pavia, viale Taramelli 12, Pavia 27100, Italy, [2]Department of Pharmacological Sciences, University of Milan, via Balzaretti 9, Milan 20133, Italy, [3]Department of Chemical Sciences, University of Camerino, Via S.Agostino,1, Camerino 62032 (MC), Italy

The binding assay is a tool for all stages of drug discovery, including the study of disease mechanisms and the screening for the identification of lead compounds. Bioaffinity screening methods have emerged as an alternative to classical screening strategies. Between these methods, frontal affinity chromatography-mass spectrometry (FAC-MS) is turning out to be a viable screening tool that has been successfully applied to a wide range of bio-

logical targets. This method relies on the immobilization of the target biomolecule on liquid chromatographic supports used in frontal chromatography coupled with mass spectrometry detection (FAC–MS) and can be used for the determination of binding constant of individual compounds as well as for measuring and ranking the relative binding affinities of ligands in mixture. The potentiality of this technique has been already demonstrated for membrane-bound ion channels, transmembrane transporters and G protein-coupled receptors. (GPCRs).

Recently, GPR17, a previously orphan receptor belonging to the "purin receptor cluster" has been identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. GPR17s were found to be highly expressed in organs typically undergoing ischemic damage, i.e., brain, heart and kidney. [1]

In this study, cellular membrane fragments obtained from cell lines expressing the target GPR17 were immobilized on the surface of an immobilized artificial membrane (IAM) liquid chromatography stationary phase following a previously reported procedure [2] and the resulting GPR17-IAM stationary phases used to create different columns for the optimisation of the chromatographic system. The columns were then used in FAC-MS studies to characterize ligand-GPR17 interactions.

The binding affinities (Kd) for known ligands for GPR17 were determined and correlated with literature values. The results indicate that GPR17 have been successfully immobilized with retention of its binding activity.

The chromatographic system was then used to screen a wide number of new nucleotide derivatives in order to select high affinity GPR17 ligands as possible lead compounds for acute and chronic neurodegenerative diseases treatment.

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L40

THE DISCOVERY OF MK-0731: THE ROLE OF FLUORINE IN OPTIMIZATION OF KINESIN SPINDLE PROTEIN (KSP) INHIBITORS FOR THE TREATMENT OF CANCER

Paul J. Coleman, Christopher D. Cox, Mark E. Fraley, Robert M. Garbaccio, Michael J. Breslin, David B. Whitman, John D. Schreier, Maricel Torrent, Rob Lobell, Carolyn Buser, Weikang Tao, Keith Rickert, Hans Huber, Nancy Kohl, Thomayant Prueksaritanont, Chunze Li, Donald E. Slaughter, Youwei Yan, Lawrence Kuo, and George D. Hartman

Departments of Medicinal Chemistry, Cancer Research, Molecular Systems, Drug Metabolism and Structural Biology, Merck Research Laboratories, West Point, PA.

Kinesin spindle protein (KSP or Eg5) is a molecular motor essential for proper separation of the spindle poles during mitosis. Disruption of KSP function results in mitotic arrest due to collapse of the bipolar spindle, triggering the apoptotic pathway in tumor cells. KSP inhibitors therefore have potential as antiproliferative agents useful for the treatment of cancer, and may lack mechanism-based side effects common to agents that directly affect microtubule polymerization/depolymerization kinetics. This talk will describe the structure-based optimization of KSP inhibitors beginning with an HTS-derived lead and culminating in the identification of MK-0731, a molecule that recently entered clinical trials for the treatment of taxane-refractory cancer. The presentation will highlight how fluorine substitution was used to control physicochemical properties of leading compounds in order to minimize P-glycoprotein efflux and ion channel activities.

L41

ALLOSTERIC AKT INHIBITORS

George D. Hartman

Departments of Medicinal Chemistry, Cancer Research, Molecular Systems, Drug, Metabolism and Structural Biology, Merck Research Laboratories, West Point, PA.

The PI3K-Akt pathway is dysregulated in the majority of solid tumors as a result of mutations and amplifications in upstream signaling components. Pharmacological inhibition of Akt is a promising strategy for treating tumors resistant to growth factor receptor antagonists due to mutations in PI3K or PTEN. We have developed allosteric, isozyme-specific Akt inhibitors that block the kinase activity of Akt and prevent membrane translocation and activation of Akt in cells. Lead molecules are highly selective inhibitors of Akt1 and Akt2 with equal potency against human and mouse enzymes, possess PK properties suitable for preclinical proof-of-concept studies and their full optimization strategy will be presented.

The efficacy of lead AKT inhibitors was tested in xenografts with the LNCaP tumor cell line, a prostate tumor line with a PTEN deletion and constitutively activated Akt. Treatment of tumor-bearing mice with AKT inhibitor resulted in complete inhibitions of pAkt, induction of apoptosis, and complete inhibition of LNCaP tumor growth. AKTi inhibitor treatment was well tolerated at efficacious doses without weight loss or gross toxicities. Hyperglycemia could be minimized by avoiding large C_{max} to trough variations and this side effect was moderate at doses that resulted in complete tumor growth inhibition. These PD-efficacy studies give insight in defining optimal PK properties of future clinical candidates. Our data suggest that mechanism-based side effects may not limit efficacy in the clinic and support the rationale for clinical development of allosteric Akt inhibitors.

L42

BI 2536, DISCOVERY OF A POTENT AND HIGHLY SPECIFIC INHIBITOR OF POLO-LIKE KINASE 1

Matthias Hoffmann

Department of Chemical Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany

The serine/threonine kinase Polo-like kinase 1 (Plk1) has a pivotal role in regulating mitotic progression. Plk1 contributes to the activation of the cyclin B1/CDK1 complex and is involved in centrosome maturation and bipolar spindle formation at the onset of mitosis. Moreover, Plk1 controls mitotic exit by regulating the anaphase-promoting complex, and has been implicated in the coordination of cytokinesis, the final step of mitosis. Since Plk1 is active in mitosis in all dividing cells, it is likely that Plk1 inhibition will arrest cell proliferation in all human cancers, regardless of their organ derivation or oncogene and tumor suppressor status. Therefore, the inhibition of Plk1 is an attractive approach for the treatment of proliferative disorders such as cancer.

We entered the search for Plk1 inhibitors by an HTS screening campaign of our compound collection. By this screening we identified Dihydropteridinones (DHP) as an attractive scaffold for the inhibition of Plk1. Here we would like to report our activities to elucidate the crucial interaction points of the enzyme–inhibitor complex by classical structure activity relationships and with the aid of molecular modelling. A recently solved X-ray of the wild–type catalytic domain gave further insights into the important interactions of the inhibitor. The result of these efforts was the discovery of BI 2536 which has been selected for development and which is currently under clinical development.

L43

CURRENT ASPECTS AND FUTURE TRENDS IN IMMUNOMODULATION

Murray McKinnon, PhD.

Department of Immunology & Inflammation Drug Discovery, Bristol Myers Squibb Research and Development, Princeton, NJ, USA.

Therapeutic strategies aimed at the treatment of autoimmune diseases such as RA, MS, IBD and Psoriasis have evolved from treating disease symptoms to addressing the underlying pathological mechanisms, through the modulation of the host immune response. While a broad range of small molecule based approaches to immune modulation, from glucocorticoids and methotrexate through mycophenolate and cyclosporine, are used in a variety of indications, they have undesirable side effects that limit their long term clinical utility. Significant efforts have been expended Industry wide in the identification of alternative therapeutic agents with improved efficacy profiles, while minimizing both mechanism and non mechanism based side effects. In the last 5-10 years the major therapeutic advances in the treatment of autoimmune diseases have come from the Biotechechnology Industry. Biologic agents such as the TNF-α blockers (Enbrel®, Remicade®, Humira®), T cell co-stimulation modulators (Orencia®) and B cell depleting strategies (Rituxan®) have been approved in multiple autoimmune indications. The concept of shared immunological mechanisms contributing to diverse autoimmune diseases has further expanded the clinical utility of these agents.

While the clinical success of these biologic agents presents significant challenges to the identification of small molecule agents with a competitive profile, they also provide new oppprtunities. Biologics have helped drive a significant commercial market in autoimmune diseases; they have defined new cell types and novel biological mechanisms/pathways for therapeutic intervention that can be exploited by medicinal chemistry approaches. New therapeutic targets are emerging from preclinical validation studies in animal models of disease, the study of human and animal genetics, and from clinical mechanism of action studies using approved biologic agents. Novel small molecules based programs are emerging in the preclinical literature and compounds are entering clinical evaluation that target the recruitment, proliferation, activation and survival of key cell types, such as the T cell, B cell and monocyte, that underpin disease pathogenesis.

Using Rheumatoid Arthritis as a prototypical autoimmune disease, the presentation will review current approaches to disease treatment, and highlight new biologic and small molecule based therapeutic strategies that are emerging in the scientific and clinical literature. The presentation will also attempt to highlight emerging trends and potential future directions in our understanding and treatment of autoimmune diseases.

L44

ADVANCES IN TARGETING GLUCOCORTICOIDS

Markus Berger, Heike Schäcke, Hartmut Rehwinkel, Khusru Asadullah, Stefan Bäurle, Wolf-Dietrich Döcke, Hartwig Hennekes, Stefan Jaroch, Konrad Krolikiewicz, Manfred Lehmann, Anne Mengel, Roland Neuhaus, Duy Nguyen, Lars Röse, Andrea Rotgeri, Norbert Schmees, Arndt Schottelius, Werner Skuballa, Peter Strehlke;

Bayer Schering Pharma AG, Global Drug Discovery, D-13342 Berlin, Germany; markus.berger@bayerhealthcare.com

Glucocorticoids (GCs) are the most commonly used anti-inflammatory and immunosuppressive drugs. However, their outstanding therapeutic effects are often accompanied by severe and sometimes irreversible side effects, particularly when they are administered systemically. Thus, there is a real need for GCs with a reduced side effect profile which retain the anti-inflammatory and immunosuppressive properties of classical GCs.

The glucocorticoid receptor (GR) is localized in the cytoplasm of cells as part of a multiprotein complex. After

binding of GCs the GR translocates into the nucleus where it modulates gene expression either positively or negatively.

Positive gene regulation is mediated via binding of the activated GR as a homodimer to specific DNA sequences (glucocorticoid response elements, GRE) in promoter and enhancer regions of GC sensitive genes (transactivating activity of GR).

The expression of numerous pro-inflammatory cytokines, chemokines, adhesion molecules and enzymes can be inhibited by the GR via a negative regulation of gene expression. As a ligand-activated monomer the GR binds to other DNA-bound transcription factors such as AP-1 inhibiting their activity (transrepression activity).

Therefore, it is assumed that this mechanism of negative gene regulation by the GR is a major mechanism of anti-inflammatory and immunosuppressive effects of GCs. On the other hand it has been shown that positive gene regulation by the GR often mediates the induction of undesired effects of GCs, especially gluconeogenetic and catabolic effects.

Consequently, our goal has been to identify GR ligands which preferentially induce transrepression with little transactivation activity.

Here we show representatives of a novel class of compounds, selective glucocorticoid receptor agonists (SEGRAs), with a dissociation of transactivation from transrepression activity *in vitro* and *in vivo*. These non-steroidal GR-agonists represent promising new structures with an improved effect/side effect profile.

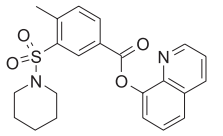
L45

TARGET VALIDATION AND LEAD IDENTIFICATION OF SELECTIVE CB2 RECEPTOR AGONISTS FOR THE TREATMENT OF INFLAMMATION AND PAIN

Doris Riether[1], Renée Zindell[1], Monika Ermann[2], Patricia Amouzegh[2], Angela Berry[1], Todd Bosonac[1], Stephen East[2], James E. Jenkins[2], Beatriz Noya-Marino[2], Innocent Mushi[2], Malcolm Taylor[2], Edward Walker[2], Lifen Wu[1], Mark Gemkow[3], Sabine Löbbe[3], Andreas Ebneth[3], Diane Thome[1], Kathy O'Shea[1], Roger Dinallo[1], Ernest Raymond[1], Kirsten Arndt, Henri Doods, Helmut Romig, Daw-tsun Shih[1], and David Thomson[1]

[1]Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Road, Ridgefield, CT 06877, USA; [2]Evotec (UK) Ltd., 111 Milton Park, Abingdon, Oxfordshire OX14 4RZ, United Kingdom, [3]Evotec AG, Schnackenburgallee 114, 22525 Hamburg, Germany

Selective CB2 receptor agonists are promising potential therapeutic agents for the treatment of inflammation and pain. Cannabinoids act at two known subtypes of the cannabinoid receptor: CB1 and CB2. The observed behavioural effects of cannabinoids are mediated centrally by the CB1 receptor while it is suggested that agonising the CB2 receptor has an anti-inflammatory and anti-hyperalgesic effect. To confirm this we initially performed proof-of-concept studies in both inflammation as well as inflammatory and neuropathic pain models using CB2 selective literature compounds. These data triggered a medicinal chemistry effort targeting the identification of novel CB2 selective compounds. We identified two structurally diverse hits: Commercially available arylsulfonamide **1** which was identified in a high-throughput screen and is a CB1/CB2 dual agonist[1], and benzylamine **2**, a compound with some selectivity for CB2, that originated from the patent literature.



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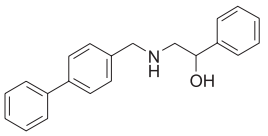

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SAR exploration and optimization toward high quality leads exhibiting a high level of selectivity for CB2 will be discussed. In particular, we will highlight various changes to the benzylamine class of compounds such as cyclising the amino alcohol to form a morpholine group or introducing a linker in the biaryl portion of the molecule, and their effect on CB2 potency and selectivity as well as ADMET properties.

Furthermore, both series were pharmacologically evaluated in acute models of inflammation (zymosan induced paw edema) and demonstrated oral efficacy. One arylsulfonamide was also assessed in a carrageenan induced inflammatory pain model. These experiments led to proof-of-concept for inflammation and pain with our own chemical series. In addition we demonstrated that these CB2 agonists were devoid of typical CB1 side effects such as sedation and catalepsy at doses that show anti-inflammatory or anti-hyperalgesic effects.

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L46

NK3 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF SCHIZOPHRENIA – CHALLENGES AND OPPORTUNITIES

Klaus Simonsen

H. Lundbeck, Ottiliavej 89, DK-2500 Valby, Denmark

Schizophrenia is a chronic and debilitating disorder, consisting of several symptoms (positive and negative symptoms and cognition), with a large unmet need. Despite the discovery of typical and atypical antipsychotics - that mainly treats the positive symptoms - the negative symptoms and cognitive impairments associated with schizophrenia remains largely untreated. Furthermore, the need for antipsychotics with no or fewer side effects, such as weight gain, cardiovascular liabilities

and extrapyrimidal symptoms, is warranted. Consequently, the search for more tolerable antipsychotics - within the classical target avenues as well as the identification of novel targets, with new mode of action - is ongoing. Several novel strategies are being pursued by the industry, e.g. dopamine D_2 antagonists with additional actions on specific serotonin receptor subtypes (e.g. 5-HT_6 receptors), 5-HT_{2C} agonists, phosphodiesterase (PDE10) inhibitors, $mGluR_{2/3}$ agonists and NK3 antagonists.

NK3 (neurokinin receptor 3) is a drugable target expressed in the CNS. Two NK3 receptor antagonists - Osanetant and Talnetant - have recently shown positive results in clinical trials for schizophrenia. Here, we disclose our finding within this novel clinical validated receptor.

A new class of NK3 receptor antagonist, 1-phenyl-2-aminomethyl cyclopropyl-1-carboxamidse, was identified from a HTS screen. Medicinal chemistry follow up resulted in several promising lead series and finally a novel NK3 tool compound was identified. The identification, Structure activity relations ship and preclinical pharmacology of novel NK3 receptor antagonists will be presented. Furthermore, the symptoms associated with schizophrenia and treatment strategies for this debilitating disease will be reviewed.

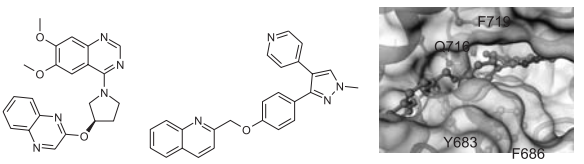
L47

DEVELOPMENT OF MULTIPLE STRUCTURAL LEAD OPTIONS UTILIZING STRUCTURE BASED DRUG DESIGN TO IDENTIFY A PDE10 CLINICAL CANDIDATE TO TREAT SCHIZOPHRENIA

Spiros Liras

PFIZER GLOBAL RESEARCH, Eastern Point Road, GROTON, CT 06340-5146, United States

PDE10A is a dual substrate cyclic nucleotide phosphodiesterase that has expression in the spiny neurons of the striatum. Inhibiting PDE10 in rodents leads to increases in cyclic nucleotides in the striatum and a behavioral anti-psychotic phenotype. Structure based drug design was utilized to identify and develop the SAR of multiple lead options. The design and optimization of the pyrazole series and the quinazoline series into molecules with drug-like properties will be discussed. The structure of the clinical candidate PF-2545920 along with its' preclinical rational and Phase I single dose PK will be disclosed.



L48

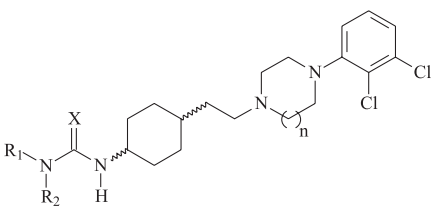
DOPAMINE SYSTEM STABILIZERS

György Domány

Gedeon Richter Plc.

Dopamine system stabilizers are a new class of antipsychotics exerting bidirectional activity on the dopaminergic system. They inhibit overstimulated dopamine receptors but stimulate these receptors when the endogenous dopaminergic tone is low. This balancing feature results in effective blockade of overexcited dopamine D_2 receptors in schizophrenic patients and concurrently prevents the induction of extrapyramidal side effects or secondary negative symptoms by avoiding complete silencing of dopaminergic transmission. The outcome is a stabilized signal near to normal physiological function, resulting in a reduction of psychotic symptoms. The key feature of a compound with system stabilizing character is an appropriate degree of partial agonism at dopamine receptors. Our quest for new antipsychotics has been based on three interrelated hypotheses: 1. dopamine D_2 antagonism/partial agonism is required for antipsychotic activity; 2. dopamine D_3 antagonism may carry favourable effects such as cognitive enhancement and lack of catalepsy; 3. in order to achieve simultaneous *in vivo* manifestation of D_2 and D_3 receptor antagonism the compound should have higher affinity to D_3 than to D_2 receptors.

Starting from known selective dopamine D_3 antagonists after multiple structural modification a series of urea (thiourea) derivatives of general formula **A** were identified as high affinity ligands of both dopamine D_3 and D_2 receptors. Neurochemical studies revealed that several representatives of this series act as dopamine system stabilizers (similarly to e.g. aripiprazole) with various degree of dopamine D_2-like receptor partial agonism.



A

Out of these compounds RGH-188 (cariprazine) possessed very favourable neurochemical profile and demonstrated outstanding activity in several antipsychotic tests in vivo, which, together with its advantageous pharmacokinetic and toxicological profile led to its selection for development. Cariprazine is currently under Phase II clinical investigations.

L49

TARGETTING THE GLYCANS ON THE VIRAL ENVELOPE, A NOVEL THERAPEUTIC CONCEPT

Jan Balzarini

Rega Institute for Medical Research, K.U.Leuven, B-3000 Leuven, Belgium

The envelope of a number of viruses is highly glycosylated (i.e. human immunodeficiency virus (HIV), hepatitis C virus (HCV), coronavirus (CoV), influenza virus). The nature of these glycans is often a mixture of complex-type, high-mannose type and hybrid-type oligosaccharides. In mammalian cells, the nature of the glycans on glycoproteins is predominantly of complex-type. A variety of carbohydrate-binding agents (CBA), including lectins from plant and prokaryotic origin (*Hippeastrum hybrid* agglutinin (HHA) (α(1,3)/α(1,6)-mannose-specific), *Galanthus nivalis* agglutinin (GNA) (α(1,3)-mannose-specific), *Urtica dioica* agglutinin (UDA) (GlcNAc-specific), Cyanovirin N (CV-N) (α(1,2)-mannose-specific)) but also the small-size non-peptidic actinomycete-derived antibiotic Pradimicin A (PRM-A) (α(1,2)-mannose-specific) have been shown to tightly bind to viral envelope glycans. We found that these CBAs efficiently inhibit HIV (and HCV and CoV) entry into its target cells (50%-effective concentration (EC_{50}) ranging between the lower nanomolar range and the lower micromolar range). The CBAs are effective against a wide variety of HIV-1 strains that belong to several clades, as well as against HIV-2 and simian immunodeficiency virus (SIV). They also prevent syncytia formation between persistently HIV-infected cells and uninfected cells, HIV (and HCV) capture by DC-SIGN-expressing cells and macrophage mannose-receptor (MMR)-expressing macrophages, and subsequent transmission of DC-SIGN- and macrophage-captured HIV to uninfected T-lymphocytes. Interestingly, CBAs also efficiently prevent cell-to-cell-based transmission of human T-cell leukemia virus type 1 (HTLV-1) to uninfected peripheral blood mononuclear cells. When HIV-1 is exposed to escalating CBA concentrations in cell culture, mutant virus strains emerge in which glycans were deleted in the HIV-1 gp120 envelope. There is a preference for deletion of high-mannose-type glycans. Several CBAs seem to have a high genetic barrier because several glycan deletions in gp120 are required to exert pronounced phenotypic drug resistance. Interestingly, the deleted glycans allow the exposure of previously hidden immunogenic epitopes on gp120. Whereas some of the CBAs (such as CV-N) display pronounced mitogenic activity and induce a variety of cellular activation markers and chemo/cytokines upon exposure to peripheral blood mononuclear cells (PBMC) (i.e. CV-N), several other CBAs (i.e. PRM-A) do not, or weakly, display such activities. Therefore, we believe that carefully selected CBAs may qualify as potential drug candidates not only for systemic therapy but also for microbicidal purpose. Moreover, the unique resistance profile of CBAs (i.e. glycan deletions in the HIV-1 envelope) may open interesting perspectives, in addition to a direct antiviral effect by blocking virus entry, to also involve the immune system, in the eventual antiviral efficacy of these drugs.

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The work was supported by grants from the European Commission (EMPRO), the F.W.O. (G-485-08) and the Centers of Excellence of the K.U.Leuven (EF-05/15).

L50

LETHAL MUTAGENESIS AS A NEW ANTIVIRAL STRATEGY

Esteban Domingo

Centro de Biología Molecular "Severo Ochoa" (CSIC), Campus de Cantoblanco, Madrid, Spain; and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd)

Quasispecies dynamics of RNA viruses (continuous generation of mutant genomes subjected to competition and selection) represents an important problem for the control of viral disease. In particular, mutant viruses resistant (or with decreased sensitivity) to antiviral agents are readily generated in the course of antiviral treatments. Several approaches have been investigated to try to circumvent the adaptive capacity of evolving viral quasispecies, notably combination therapy and the use of mutagenic agents as antiviral compounds. Here the conceptual basis and experimental results of a new antiviral strategy termed lethal mutagenesis will be summarized. It involves the use of mutagenic agents, specifically nucleotide analogues, to increase the mutation rate of RNA viruses above an error threshold that marks a maximum error rate compatible with maintenance of their genetic information. Studies with foot-and-mouth disease virus (FMDV), lymphocytic choriomeningitis virus (LCMV) and human immunodeficiency virus have documented that: (i) Enhanced mutagenesis (and not merely inhibition) of virus replication can lead to virus extinction; (ii) combinations of a mutagenic agent and non-mutagenic antiviral inhibitors are more efficient than a mutagenic agent alone to produce virus extinction; (iii) low mutagenic activities that lead to the production of interfering mutant viruses (termed defectors) may suffice to produce virus extinction. Molecular mechanisms of the action of mutagenic nucleotides analogues, and prospects for an application of lethal mutagenesis *in vivo* will be discussed.

L51

PROGRESS IN THE DEVELOPMENT OF NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIS) OF HIV; FROM TIBO TO RILPIVIRINE.

IUPAC-Richter Prize in Medicinal Chemistry 2008

Jan Heeres and Paul Lewi.

In 1987 Dr. Paul Janssen initiated a collaboration for screening of a number of compounds on potential HIV activity with Prof. E. De Clerq at the Rega Institute in Leuven. This collaboration led to the discovery of the TIBO and alpha-APA series of compounds with specific HIV-1 activity. Both series of compounds inhibited the essential enzyme Reverse Transcriptase of the HIV-virus by binding in a non-nucleoside binding site.

In 1995 Dr. Paul Janssen founded the the Center for Molecular Design (CMD, part of Janssen Pharmaceutica N.V.) with the intention to work out research programs that did not interfere with other programs within the company.

There were many reasons to continue the HIV project because there was a real medical need to find new drugs which could be utilized in HIV-infected patients all over the world.

Threfore we intended to use state of the art knowledge within the fields of X-ray crystallography, molecular modeling, medicinal chemistry and virology to find potential new drugs for development.

Via the ITU series the DATA and the DAPY series of compounds were discovered.

Within the DAPY series three compounds were discovered, namely Dapivirine, Etravirine and Rilpivirine. All three compounds displayed high potency against the HIV-virus, not only against the wild-type but also against different single and more complex mutants of clinical relevance. The virilogy and clinical studies occurred at Tibotec, founded by Rudi Pauwels in 1994.

Dapivirine has been donated to the International Partnership for Microbicides and is undergoing clinical trials as a microbicide in order to prevent transmission of HIV-infection.

Very recently Etravirine (TMC125) has been approved by the FDA and can now be used in treatment-experienced HIV-patients.

On the other hand Rilpivirine has successfully completed phase II studies in treatment-naive HIV-patients. It is the most potent anti-HIV compound to date both against wild-type and multiple mutants of it.

From the early start of the HIV-project, Janssen has established long-lasting collaborations with Tibotec for anti-HIV screening and with Rutgers University (NJ, USA) for crystallography and X-ray determination of Reverse Transcriptase in complex with NNRTIs.

L52

EMERGING TREATMENTS FOR CHRONIC PAIN: PROSPECTS AND CHALLENGES

Stefan I McDonough

Amgen, Inc. One Kendall Square Bldg 1000, Cambridge, MA 02139, Tel (617) 444-5580, Fax (617) 494-1075, stefanm@amgen.com

Chronic pain is a severe disease and a longstanding unmet medical need. Recently, however, new targets and new technologies have spurred increased efforts by the pharmaceutical and biotechnology industries to invent the next generation of therapeutics. This talk introduces chronic pain as a clinical indication and current theories on the network and systems aspects of pain. Challenges and opportunities in developing drugs for chronic pain will be discussed, including preclinical models, translational biology, and patient stratification. Emerging therapeutic targets and agents will be reviewed, with a detailed focus on the role of subtypes of nicotinic acetylcholine receptors in mediating analgesia.

L53

DISCOVERY OF A SELECTIVE TRPV1 ANTAGONIST, AMG 517: A CLINICAL CANDIDATE FOR THE TREATMENT OF PAIN

Mark H. Norman, PhD

Department of Chemistry Research & Discovery; Amgen Inc, Thousand Oaks, CA

The transient receptor potential vanilloid 1 (TRPV1) is a nonselective cation channel primarily expressed by a subset of nociceptive neurons in the dorsal root and trigeminal ganglia. TRPV1 can be activated by a variety of noxious stimuli, including capsaicin, extracellular acidity, and heat. This activation leads to the influx of ions into the cell causing cell depolarization and leading to the sensation of pain. TRPV1 is up-regulated and sensitized as a result of tissue injury and inflammation and is believed to contribute to the transduction of inflammatory pain signals. In addition, mice lacking the TRPV1 gene, while normal in most respects, exhibit a significantly reduced response to painful thermal stimuli. For these reasons it is believed that TRPV1 antagonsim may provide a novel approach to the treatment of inflammatory pain.

Based on a lead identified from our sample collection by high throughput screening, we have developed a series of potent and orally available 4-oxopyrimidines as TRPV1 antagonists. From this series, we advanced a TRPV1 antagonist, AMG 517, into clinical trials as a new therapy for the treatment of pain. In these studies, AMG 517 was found to significantly increased body core temperature following oral administration. The structure-activity relationships (SAR) studies leading to AMG 517 will be outlined and the preclinical and clinical pharmacokinetic, and pharmacologic will be presented. In addition,

two approaches to eliminate or minimize the on-target hyperthermic effect will also be discussed.

L54

NOVEL α2δ LIGANDS FOR THE TREATMENT OF NEUROPATHIC PAIN

Graham N Maw, PhD

Pain Therapeutics, Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, Kent, CT13 9NJ; graham.maw@pfizer.com

The term neuropathic pain covers a large number of debilitating conditions which are estimated to effect about 1% of the population. Pregabalin has recently been approved for the treatment of two of the most common neuropathic pain conditions, post herpetic neuralgia (PHN) and painful diabetic neuropathy (DPNP). Despite this success a significant proportion of patients do not achieve full pain relief with any of the agents currently on the market, thus there is a high medical need to identify more effective agents. Neurontin and Lyrica are believed to work through interaction with the α2δ subunit of voltage gated calcium channels and evidence for this mechanism of action will be presented.

In our search for proprietary α2δ ligands with potential for improved efficacy in neuropathic pain, a series of novel, alkylated glycine derivatives was identified. Optimisation of these α-amino acids resulted in the discovery of a novel series of proline α2δ ligands with improved potency and efficacy in a broad spectrum of preclinical models of neuropathic pain. SAR in the proline series and the preclinical in vivo profiles of lead compounds will be discussed.

L55

COMPUTER-ASSISTED MOLECULAR DESIGN: VOYAGES TO THE (UN)KNOWN

Gisbert Schneider

Beilstein Endowed Chair for Chem- and Bioinformatics, Goethe-University, Frankfurt a.M., Germany; email: g.schneider@chemie.uni-frankfurt.de

High-throughput screening campaigns are fuelled not only by corporate or “maximally diverse” compound collections, but increasingly accompanied by target- or bioactivity-focused selections of screening compounds. Computer-assisted design methods aid in the compilation of such focused libraries and the *de novo* design of new bioactive agents. We will present the concept of adaptive techniques for fragment-based compound design and chemical space analysis, together with recent case studies. Advantages, limitations and potential future applications will be discussed.

L56

THE QUEST FOR BIOISOSTERISM IN THE “OTHER SIDE” OF CHEMICAL SPACE.

Antonio Macchiarulo and Roberto Pellicciari

Dipartimento di Chimica e Tecnologia del Farmaco. Università di Perugia, 06123 Perugia (Italy); Email: antonio@chimfarm.unipg.it

Nowadays there is a growing awareness that the translation of the increasing number of lead compounds into clinical candidates is still a slow and often inefficient process.[1] In order to facilitate the lead optimization procedure, due consideration must be given to the use of the right bioisosteric replacements. The exploitation of bioisosteric relationships is the ethos of medicinal chemists to improve the properties of a lead molecule such as selectivity and pharmacokinetic characteristics, while retaining the original bioactivity and removing unwanted toxic side effects. Although the quest for bioisosteric groups usually requires an exhaustive process of trial and error, diverse computational methodologies have been developed to navigate the chemical space with the aim of identifying functional groups with an optimal balance of steric, hydrophobic, electronic and hydrogen-bonds properties.[2] These methods are mostly based on the gaining of chemical knowledge from the appraisal of experimental group properties, the analysis of large molecular databases and the development of molecular descriptors that characterize various properties of the functional groups. Very recently, we have introduced explorations into the “other side” of chemical space as a more effective strategy for studying the bioisosteric relationships existing among functional groups.[3] The other side of chemical space constitutes the biological counterpart of the chemical space, being composed by the ensemble of binding sites of protein structures. Charting the “other side” of chemical space provides a biological knowledge that, combining with the chemical knowledge, aids to gain insight into the principles that rules molecular recognition and bioisosteric relationships of functional groups. Applications of navigating the “other side” of chemical space will be presented for some examples of functional groups endowed with the highest contribution to the binding energy.[4]

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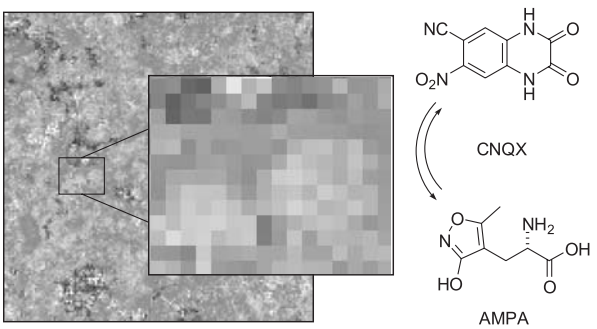
L57

EXPLORATION OF CHEMICAL SPACE FOR DRUG DISCOVERY BY DATABASE GENERATION

Jean-Louis Reymond,

University of Berne, Switzerland

Organic chemisty is the study of molecules made by forming covalent bonds between atoms of carbon, hydrogen, oxygen, nitrogen, halogens, and a few other elements (S, P, Si). The ensemble of all possible molecules forms the so-called chemical universe, or chemical space. Our aim is to explore chemical space in depth by exhaustive generation in silico, going beyond what nature and chemists have synthesized or imagined to date. In one approach, we have generated a database of all molecules up to 11 atoms of C, N, O, F that are possible under simple chemical stability and synthetic feasibility rules, and found that 99.8% of these are unknown and mostly feature new structural types [1]. In another approach, we travel through chemical space between known ligands using a mutational algorithm to produce large databases of intermediates, which are almost exclusively novel, and display a broad range of properties (illustration: map of chemical space between AMPA and CNQX, colored for polar surface area. The map contains 3.2 millions structures ordered by structural similarity). We have used these databases for drug discovery by combining substructure classification methods and docking with organic synthesis and testing of the most promising ligands, and discovered new types of bioactive ligands. Our findings challenge the commonly held notion that all the molecular types that nature may ever need are already available as natural products, and point to a plethora of novel structural types as new targets for synthesis.



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L58

SCAFFOLD HUNTER: CHARTING AND EXPLORING CHEMICAL SPACE

Stefan Wetzel[1], Karsten Klein[2], Steffen Renner[1], Petra Mutzel[2], Herbert Waldmann[1]

[1]Max-Planck-Institute for Molecular Physiology, Otto-Hahn-Straße 11, 44227 Dortmund and Chemical Biology, Technical University of Dortmund, Germany; [2]Chair of Algorithm Engineering, Department of Computer Science, Technical University Dortmund, Germany

"Space", as Douglas Adams famously said "is big. You just won't believe how vastly, hugely, mind-bogglingly big it is."[1] How to navigate in this sheer vastness? And how to explore the opportunities for new small molecules in chemical space?

We developed a hierarchical scaffold classification strategy[2,3] to chart chemical spaces. Our approach is based on Murcko scaffolds and a set of rules which will create a hierarchical scaffold tree by iterative disassembly of the scaffolds. This scaffold hierarchy is then displayed as a radial tree diagram with the corresponding scaffolds forming the nodes.

The Scaffold Hunter program interactively displays these diagrams and greatly thus facilitates navigation in and exploitation of chemical space. It is fairly generic and can also display the results of other hierarchical classifications of molecules, e.g. clustering data, without major modification.

This approach to charting chemical space is very intuitive to chemists since it shows i.e. true chemical structures. In general it allows a quick orientation in the structure space depicted. Features like colour coding of molecular properties further increase the information content without reducing clarity.

Scaffold Hunter enables chemists to directly work with the results of hierarchical classifications in an intuitive and easy way - independent of the underlying algorithm. It is in use for hitlist triaging and compound management but can also serve as a tool to navigate and analyze chemical structure space. Moreover, one can quickly identify "holes" in the structure space analyzed and to link them to bioactivity. Thus promising starting points for library design can be identified and explored.

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L59

CXCR4 ANTAGONISTS: RELEVANCE TO CANCER CHEMOTHERAPY

Nobutaka Fujii and Shinya Oishi

Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan

The CXC chemokine receptor 4 (CXCR4) is a G-protein-coupled cell-surface receptor of a homeostatic chemokine, stromal cell-derived factor 1 (SDF-1)/CXCL12. This ligand-receptor pair regulates a number of physiological processes including migration of progenitors during embryologic development of the cardiovascular, hemopoietic, central nervous systems.[1] Addtionally, this pair is also implicated in multifactorial deseases including HIV infection, rheumatoid arthritis, asthmatic inflammation, pulmonary fibrosis, and cancer metastasis/progression.[2]

Based on the structure-activity relationship study of horseshoe crab-derived antimicrobial peptides, we have identified a series of specific CXCR4 antagonists with different types of scaffolds.[3] These antagonists have served as chemical probes to disclose the physiological and pathological functions of SDF-1 and CXCR4, providing insights into the therapeutic and diagnostic potential of CXCR4 antagonists for these problematic diseases.

Our early finding of the implication of SDF-1-CXCR4 pair to pancreatic cancer metastasis/progression provoked the extensive studies of those on various tumors including breast cancer, chronic lymphocytic B-cell leukaemia (CLL), pre-B acute lymphoblastic leukaemia (ALL), melanoma, bladder cancer, brain cancer, prostate cancer, small cell lung cancer (SCLC).[4] Some of these cancer cells are aggressive and rapidly metastasize with a high propensity for bone marrow involvement expressing high levels of CXCR4. CXCR4 antagonists suppress angiogenesis, cancer metastasis, and drug resistance mediated by bone marrow stromal cell-adhesion.

In the symposium, development of novel functionalized CXCR4 antagonists and the application to visualized cancer cell biology will be presented.[5]

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L60

CCR2 / CCR5 ANTAGONISTS: A NEW APPROACH FOR THE TREATMENT OF AUTO-IMMUNE DISEASES

Wolfgang Miltz*, Pius Loetscher, Philipp Janser, Rene Hersperger, Peter Hiestand, Josef Meingassner#

Novartis Institutes for BioMedical Research, Basel, Switzerland and Vienna, Austria#

Literature data from transgenic and knockout animals underline the importance of chemokine receptor signaling in various diseases and several inhibitors are in development by different companies. For inflammatory diseases, CCR2 and to some extent CCR5 play a crucial role since they are expressed on most inflammatory cells, in particular monocytes.

In this presentation, we would like to show the *in vitro* and *in vivo* results obtained with our dual CCR2/CCR5 antagonists. They potently inhibit the binding of human CCR2/MCP-1 and CCR5/MIP-1α in the nanomolar range and they are cross-reactive with rodent and monkey receptors. In functional assays, calcium-flux and chemotaxis are inhibited, both in transfected and in primary cells. The effects in a mechanistic model of monocyte migration will be discussed and activities in animal models of autoimmune diseases will be presented.

L61

BIOLOGICAL PROFILING OF ANTI-HIV AGENTS USING IN SILICO TECHNIQUES: INSIGHTS INTO CCR5 LIGANDS BINDING THROUGH HOMOLOGY BUILDINGS, 3D-QSAR, DOCKING AND SHAPE MATCHING VIRTUAL SCREENING

A. Carrieri§, A. Fano§, D.W. Ritchie&, J. Teixido¥ and V.I. Perez-Nueno¥.

§ Dipartimento Farmaco-Chimico, Università degli Studi di Bari, Bari, Italy

¥ Grup d'Enginyeria Molecular, Institut Químic de SarriA (IQS), Universitat Ramon Llull, Barcelona, Spain; & Department of Computing Science, King's College, University of Aberdeen, Aberdeen, United Kingdom

Acquired Immune Deficiency Syndrome (AIDS) has become a fatal disease over the whole world with more than 35 million deaths, and many more people affected by the disease.

Novel ligands capable of blocking virus-cell fusion are emerging as promising candidate molecules towards HIV-1 infection because they promise to overcome the major drawbacks of classical highly active antiretroviral (HAART) drugs.

However, due to the paucity of experimentally determined three-dimensional information about the HIV-1 cell surface co-receptors, structure based design continues to be hampered.

Here, we present recent advances gained over the last years to define the molecular requirements and determinants for efficient binding to CCR5, the major biological target of HIV entry blockers, by means of computational techniques based on comparative receptor structure modelling, advanced 3D-QSAR ,and docking.

A fresh pharmacophore hypothesis is proposed together with shape and property based virtual screening of commercially available entry blockers which might be valuable for predicting new HIV entry blocking leads.

L62

MULTIMODALITY IMAGING BY MR, PET, AND CT FOR THE STUDY OF ATHEROSCLEROSIS

Zahi A. Fayad, PhD, FAHA, FACC

Professor of Radiology and Medicine (Cardiology), Mount Sinai School of Medicine, New York, Director, Translational and Molecular Imaging Institute

Atherosclerosis is an inflammatory disease, where the degree of inflammation, not the plaque size, determines risk of rupture and therefore likelihood of a clinical event. Magnetic Resonance Imaging (MRI) can image atherosclerotic plaque with high resolution, and several MRI parameters of disease extent in the carotid arteries and aorta have been shown to correlate with atherosclerotic risk factors. Dynamic-contrast-enhanced MRI (DCE-MRI) is a new technique for the study of plaque composition. In this study, the extent of plaque inflammation determined by FDG uptake was correlated with DCE-MRI. By providing a metabolic image of macrophage activity, F18-Fluorodeoxuglucose (FDG) positron emission tomography (PET) can image atherosclerotic plaque inflammation in patients and in animal models of disease, with a strong correlation between FDG uptake and plaque macrophage content. In addition, autoradiography has confirmed that the FDG signal originates from activated macrophages within the lipid core and fibrous cap of the plaque. This has led to the suggestion that FDG-PET might have a role in identifying 'high risk' plaques and monitoring their response to therapy. Computed tomography (CT) can be used in conjunction with PET to help co-register the PET images and for attenuation corrections. Moreover, CT with its exquisite coronary imaging has the potential to address atherosclerosis in the vessel wall of the coronary arteries. We review in this talk to use of multimodality imaging (MR, PET, and CT) for the study of inflammation of vessel wall may be useful in assessment of plaque vulnerability. We will also discuss the use of these new imaging nanoparticulates not only for imaging but also for drug delivery and treatment of atherosclerosis.

L63

PROFILING SH2 AND TYROSINE PHOSPHATASES TARGET SPECIFICITY

Michele Tinti[§], Anita Palma[§], Stefano Costa[§], Lars Kiemer[§], Martin Miller[||], Emanuela Ferrari[§], Mike Schutkowski[*], Bruce Mayer[**], Rob Hooft[*|], Soren Brunak[||], Luisa Castagnoli[§], Gianni Cesareni[§].

*[§] Department of Biology, University of Rome Tor Vergata. Via della Ricerca Scientifica 1, 00133 Rome, Italy. [||] Center for Biological Sequence Analysis. Technical University of Denmark., DK-2800 Lyngby, Denmark. *Jerini Peptide Technologies, Jerini AG, Invalidenstr. 130 10115 Berlin, Germany.[**]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT 06030, USA. [*|]Merck Serono International S.A., 1211 Geneva, Switzerland.*

Residues cycling between a phosphorylated and a non-phosphorylated form play a key role in the modulation of signal transduction. Over the past few years, we have designed and implemented a strategy to describe the network of interactions linking phosphorylated peptides to their binding domains and to the enzymes that control their phosphorylation levels. We will report the results of the characterization of the substrate specificity of the SH2 domain family and of the family of tyrosine phosphatases. We have cloned and expressed all the 120 human SH2 domains and for 72 of them we have been able to purify soluble GST fusions. Similarly we succeeded in expressing 34 of the 37 tyrosine phosphates in a mutant form (trapping mutants) that still binds to substrate peptides but does not carry the de-phosphorylation reaction through. We have then probed their recognition specificity by incubating the GST fusions with a glass chip containing approximately 6000 phospho-peptides covering most of the human phospho-proteome.

The results have been used to train domain specific Neural Networks and to draw a global "naïve" phosphotyrosine specific interaction network that only takes into account the ability to bind phospho-peptides in an in vitro system.

Next we have used a context score that combines in a Bayesian approach orthogonal information, namely tissue co-expression, sub-cellular localization, target sequence conservation in evolution, vicinity in the protein interaction network etc , to rank interaction according to the probability of being functionally relevant.

L64

SYSTEMS LEVEL RESEARCH INFORMS DRUG TARGET IDENTIFICATION AND THERAPY DESIGN

Harel Weinstein

Department of Physiology and Biophysics, and Institute for Computational Biomedicine, Weill Cornell Medical College, Cornell University, New York, NY, 10065, USA

One of the major shortcomings of target-based drug design is that it does not address causal relationships between pathophysiology of the cell and the effects

of a putative drug on a biomolecular target. Yet such an understanding is imperative in the development of new therapeutics. Construction of useful models of drug effects on disease pathophysiology involves (i) the collection of large and diverse data sets and information on functional connections between cellular components; and (ii) the development and application of computational learning and simulation methods at molecular and systems scales designed to integrate the relevant information and enable prediction of cell behavior. The presentation will illustrate both the challenges presented by the development of a combined experimental and computational approach that includes functional analysis, data-driven simulation and experimental validation, and its power to identify critical cellular components in the activity of drugs and especially combinations exhibiting therapeutic synergy[*see footnote]. The specific focus of the illustration is anticancer therapy based on the experimentally observed and clinically validated synergy between a novel class of experimental anticancer agents, the Farnesyltransferase Inhibitors (FTIs), and the microtubule-stabilizing drug Taxol. Studies of our collaborators have recently shown that the drug combination inhibits cell growth, enhances tubulin acetylation and induces apoptosis in human cancer cell lines. However, the cellular mechanisms underlying the synergistic effects of this promising anticancer drug combination still needs to be understood. To this end, we undertook a collaborative experimental and computational study combining (i) whole-genome transcriptome analysis, (ii) measurements of drug effects on biomarkers (iii) utilization of interaction and functional databases for functional and pathway connections, (iv) computational modeling and prediction based on an *in silico* reverse engineering approach. We report on important insights provided by the results regarding the biology of the combined drug effects. Thus, the dose-dependent effects of treatment with Taxol or the FTI lonafarbin alone were shown to include expression of genes involved in cell death, cell cycle, DNA replication and repair and p53 signaling pathways (LNF had additional effects on genes involved in cellular assembly, organization, and motility). Using the proprietary Reverse Engineering and Forward Simulation Process REFS^{TM} of our collaborators at Gene Network Sciences we discovered genes important for synergy and showed them to belong to signaling networks specific to actin and microtubule cytoskeleton, adherens junctions, and receptor tyrosine kinase signaling, indicating the nature of the synergistic effects. These genes constitute new targets, validated computationally and shown to be directly relevant to the specific therapeutic goal.

[*] The project is carried out collaboratively by the following team: Zeynep H. Gümüs[2,5], Ada Gjyrezi[1,5], Heming Xing[3], Zach Pitluk [3], Ilse Van den Wyngaert [4], William Talloen[4], Hinrich Goehlman[4] ,Iya Khalil[3], Paraskevi Giannakakou[1]

1. Department of Medicine, Division of Hematology and Medical Oncology, Weill Medical College, Cornell University, New York, NY
2. Institute for Computational Biomedicine, Weill Medical College, Cornell University, New York, NY
3. Gene Network Sciences
4. Johnson and Johnson

L65

OVERVIEW ON NEW COMPUTATIONAL TOOLS SUPPORTING DRUG DESIGN

Tudor I. Oprea

University of New Mexico, School of Medecine - Division of Biocomputing, Albuquerque, NM 43183 USA

Abstract not available at the time of printing.

L66

NOVEL OPEN SOURCE TOOLS FOR CHEMICAL-BIOLOGICAL DATA GATHERING

M Karthikeyan[1] and Alex Tropsha[2]

[1]Digital Information Resource Center, Information Division, National Chemical Laboratory, Pune 411008, India; [2] Laboratory for Molecular Modeling, School of Pharmacy and Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599

Harvesting chemical data from public resources is a challenging task. Chemical structures are international language of chemistry, and chemists usually understand pictorial representations. Mathematically speaking chemical structures can be represented as topological graph where the nodes (atoms) connected by edges (bonds). It is important to transform the common name or synonym of chemical into truly computable chemical structures for their re-usability in chemoinformatics based studies. Several attempts has been made to transform raster graphics based chemical structure images into connection table representing atoms and bonds with limited success. Here we present our approach of building chemical-biological databases from various public repositories, and link them effectively with relevant information from literature, web-sources, analytical data etc., We used text-mining strategy for recognition of chemical and biological terms from literature and other web pages and linked them with in-house database containing several millions of molecular structures and biological sequences. Our approach towards building large chemical/biological data and generating new chemical information including defining chemical space from existing data and other information related building java based open-source tools would be presented.

L67

THE SCAFFOLD TREE – A NOVEL TOOLS FOR DATA CLASSIFICATION

Ansgar Schuffenhauer

Novartis Institutes for Biomedical Research

The scaffold tree as hierarchical classification of chemical scaffolds (molecular framework, which is obtained by pruning all terminal side chains) is introduced. The molecular frameworks form the leaf nodes in the hierarchy trees. By an iterative removal of rings, scaffolds forming the higher levels in the hierarchy tree are obtained. Prioritization rules ensure that less characteristic, peripheral rings are removed first. All scaffolds in the hierarchy tree are well-defined chemical entities making the classification chemically intuitive. The classification is deterministic, data-set-independent, and scales linearly with the number of compounds included in the data set. The application of the classification is demonstrated on a data set extracted from the PubChem database, namely, pyruvate kinase inhibitors. The example shown demonstrates that the classification procedure handles robustly synthetic structures and natural products.

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L68

SHIFT: CHEMICAL STRUCTURE HOPPING BY ISOSTERIC FRAGMENT TRANSFORMATIONS

Elisabet Gregori-Puigjané and Jordi Mestres

Chemotargets SL and Chemogenomics Laboratory, Research Unit on Biomedical Informatics, Institut Municipal d'Investigació Mèdica and Universitat Pompeu Fabra, Parc de Recerca Biomèdica, Doctor Aiguader 88, 08003 Barcelona, Catalonia, Spain

The well-known pharmacologist and Nobel laureate Sir James Black is famously quoted to say that "the most fruitful basis for the discovery of a new drug is to start with an old drug". Inspired by this statement, we have developed SHIFT, a new approach to chemical structure hopping by isosteric fragment transformations. SHIFT performs first a systematic fragmentation of a query compound [1]. Once the compound is fragmented, chemical isosters are sought for each fragment and a new molecule is built using this new isoster. In this way, with all combinations of isosteric transformations of its fragments, the chemical space around the original molecule is generated. For both isosteric fragment screening and final molecule re-ranking, SHED descriptors were used [2].

SHIFT has so far been applied to generating structurally novel potentially active molecules from a reference compound, that being a drug, a natural product, an internal novel hit, or a competitor's new chemical entity. The isosteric chemical transformation of this initial bioactive compound provides a set of structurally different, yet pharmacophorically similar, compounds that are expected to retain (most of) the pharmacological profile of the parent molecule. Combining SHIFT with an *in silico* target profiling method [3] can be ultimately used for prioritizing hit series or projecting the pharmacological space relevant to a hit optimisation process.

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L69

Leonard J. Pagliaro

Thermo Fisher Scientific, BioImage Products, Moerkhoej Bygade 28., 2860 Soeborg - Denmark

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L70

RATIONAL DESIGN OF CYTOTOXIC PEPTIDES FROM THE ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN MICROTUBULES

Stefano Pieraccini[a], Giorgio Saladino[a], Pierangelo Francescato[b], Giovanna Speranza[b], Paolo Manitto[b], Daniele Cartelli[c], Graziella Cappelletti[c], Maurizio Sironi[a]

[a]Dipartimento di Chimica Fisica ed Elettrochimica, Università degli Studi di Milano, Via Golgi 19, Milano, Italy [b]Dipartimento di Chimica Organica ed Industriale., Università degli Studi di Milano, Via Venezian 21, Milano Italy; [c]Dipartimento di Biologia, Università degli Studi di Milano, Via Celoria 26, Milano, Italy

Protein-protein interactions are central to many biological processes, from intracellular communication to cytoskeleton assembly, and therefore represent an important class of targets for new therapeutics[1]. In particular we focus on the interactions involved in the stability and self assembly of Microtubules (MTs). MTs are cylindrical polymeric structures formed by the association of tubulin dimers. They are dynamical arrays whose growing and shrinking allows chromosomes separation and organelles migration during the mitotic process in cell proliferation. This fact makes them interesting targets for cytotoxic antitumoral therapies[2]. Presently several MTs targeted drugs which are in use or under clinical trials, are mainly derived from screenings of natural substances and their subsequent modification. Following a different approach, relying on a detailed atomic level energy mapping of the interactions responsible for MTs aggregation, we present here *in silico* design of novel peptides interfering with MTs dynamic and therefore acting as inhibitors of cancer cells proliferation. MTs' protein-protein interactions were characterized in a model system composed of

two tubulin dimers. This tetramer has been studied through molecular dynamics (MD) simulation in explicit water solvent followed by interdimer binding energy evaluations and computational alanine scanning[3] of the residues situated at the interface. The analysis of the results showed that binding energy is not evenly distributed over the protein-protein interface, but is concentrated on some critical aminoacids, that we designed as hot and warm spots depending on the variation of the free binding energy upon mutation. Moreover, these residues showed a tendency to be grouped in small clusters of residues next to one another in the 3D structure and often also in the aminoacidic sequence, forming "hot stretches" of residues of key importance for tubulin aggregation. Based on this observation we selected some groups of contiguous amino acids containing a number of hot and/or warm spots and used their sequences as a starting point for the development of peptides that could bind to tubulin and interfere with its ordered self aggregation to form microtubules. The designed peptides were first simulated with MD in complex with tubulin to evaluate if they conserved binding ability even when no longer inserted in the protein structure. The most promising ones were then synthesised and tested to assess their influence on tubulin polimerization kinetic, microtubule morphology and cancer cell proliferation *in vitro*. The tested peptides exhibited a dose dependent activity against cancer cell proliferation with an IC50 in the micromolar range and noticeably altered MTs morphology and stability both in tumoral cells and in tubulin polimerization assays. These data support the assumption that properly selected tubulin subsequences, corresponding to part of the proteic interface between the tubulin dimers, can interfere with MTs growth and therefore exert a cytotoxic action on cells during their mitotic process. The peptides identified in this study can serve as a basis for the development of novel non peptidic drugs with antimitotic activity that would target different sites on tubulin with respect to traditional chemotherapeutic agents and would be useful in dealing with multidrug resistence.

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L71

PEPTIDOMIMETIC INHIBITORS OF THE PSD-95/NMDA RECEPTOR INTERACTION

Anders Bach

University of Copenhagen, Jagtvej 162, medicnalkemi, 2100 Copenhagen, Denmark

The protein-protein interaction between the NMDA receptor and its intracellular scaffolding protein, PSD-95, is a potential target for the treatment of ischemic brain diseases. Here a peptidomimetic strategy for the development of lead candidates for inhibition of the PSD-95/NMDA receptor interaction is described. Initially, truncation and alanine scan studies were carried out, using an undecapeptide corresponding to the C-terminal of the NMDA receptor as a template. This identified a pentapeptide with wild-type affinity and further examination was performed by systematic substitutions with a range of natural and unnatural amino acids. Peptides were tested for affinity and selectivity in a fluorescence polarization assay and activity of key compounds was confirmed using a pull-down assay. These studies disclosed a modified tetrapeptide, E_{Me}TAV, with improved affinity and a tripeptide, TAV, with micromolar affinity. Both peptides showed wild-type selectivity profile. Molecular modeling studies revealed key interactions between these two compounds and PSD-95.

L72

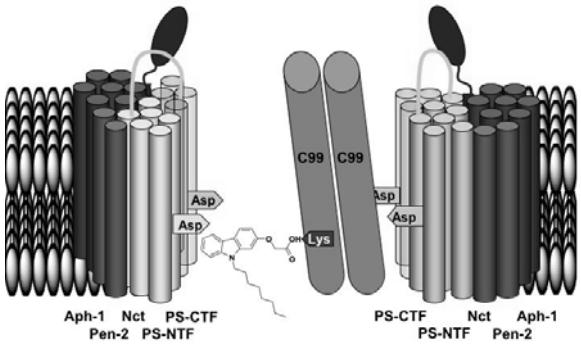
MAPPING THE BINDING CITE OF SUBSTRATE TARGETING GAMMA-SECRETASE MODULATORS. PHASE III CANDIDATES FOR ALZHEIMER´S DISEASE UTILIZE A NOVEL PROTEIN-PROTEIN INTERACTION

B. Schmidt*, R. Narlawar, S. Baumann, N. Höttecke, K. Baumann, T. Kukar, T.E. Golde

a.) Clemens Schöpf Institute for Organic Chemistry, Technische Universität Darmstadt, Petersenstrasse 22, D-64287 Darmstadt, b.) Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville FL 32224.

The inhibition of γ-secretase by active site directed inhibition of the aspartic protease interferes with several vital cell-signalling pathways. Therefore the selective reduction of Amyloid β42 formation by small molecule γ-secretase modulators (GSMs) is a promising therapeutic approach for Alzheimer's disease (AD). The weak modulator Tarenflurbil (Flurizan) is in phase III trials for AD. However, the precise molecular targets of GSMs have not been established. Novel biotinylated photoactivatable GSMs were used to identify the binding site associated with the Aβ pattern mediated by these agents. GSM photoprobes did not label the proteins in the core γ-secretase complex. Instead, GSM photoprobes labelled APP, APP carboxyl terminal fragments (CTFs), and Aβ. Substrate labelling was competed by other GSMs, and labelling of a synthetic APP γ-secretase substrate was more efficient than labelling of a synthetic Notch substrate. Peptide mapping studies localized the region of interaction to residues 28-36 of Aβ, a region critical for Aβ aggregation. Consistent with these data, we demonstrate that numerous compounds known to interact with this region of Aβ or Aβ amyloid act as GSMs, and that mutation of the GSM binding site in APP alters the sensitivity of the substrate to GSMs. Furthermore, GSMs alter the production of cell derived Aβ oligomers. Some GSMs exert two therapeutic actions, alteration in Aβ42 production and inhibition of Aβ

aggregation, which may synergistically reduce Aβ deposition in AD. The data demonstrate the existence and feasibility of "substrate targeting" by small molecule effectors of proteolytic enzymes. If generally applicable, the ability to identify small molecule modulators that target substrate and alter biological processes may significantly broaden the current notion of "druggable" targets. However, there is a caveat for drug development: the binding site is buried in the membrane and thus requires highly lipophilic compounds, which violate Lipinski's rule of 5.



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L73

NETWORK-BASED DRUG DESIGN

Hans V. Westerhoff and friends

Manchester Centre for Integrative Systems Biology, The University of Manchester & Netherlands Institute for Systems Biology, Free University, Amsterdam, EU

Most diseases that plague mankind today cannot be linked to a single factor, genetic or environmental. Epigenetics have been invoked, the cause of the disease has been called elusive, diseases have been called 'multi-factorial'. Therapies and drugs work best in subpopulations of patients and may cause havoc if such complications are not considered. Some block buster drugs seem to involve mechanisms and factors in addition to the ones they were developed for. The diseases are not multi-factorial in the sense that many factors contribute in an additive sense, but in a much more profound way: various factors strongly influence each other's effect. Having four out of five disease factors may enable one to lead a perfectly healthy life. On the other hand, hitting one of the disease factors with a well-targeted drug may not be as effective as anticipated, or have unexpected toxicological implications. What is it that lies at the origin of all these complications? What is it that keeps us from dealing with most disease effectively?

We have called the relevant diseases 'systems biology diseases', emphasizing that network properties dominate molecular properties in their aetiology and should also do so in their therapies.

It is a challenge to deal with Systems Biology diseases in new ways that they have required all along. Most of our existing methodologies, which are highly successful scientifically, are not optimal for approaching network diseases. On the other hand, these approaches should be part of a systems biology approach, as genomics should be.

Here I will discuss our attempt to devise more comprehensive approaches to Systems Biology diseases, notably infectious disease, cancer, and metabolic syndrome. I will discuss the attempt to find drug targets against a parasite, i.e. *Trypanosoma brucei*, and against tumor cells, that do not imply drug toxicity. I will show that a major serendipity in the sleeping sickness case, found through an intensive Systems Biology investigation, may suggest an entirely new set of drug targets.

L74

SYSTEMS BIOLOGY – WHAT DOES IT MEAN FOR PHARMACEUTICAL SCIENCE?

Christian R. Noe

University of Vienna, Faculty of Life Sciences, Department of Medicinal Chemistry

Life sciences are generating a fascinating wealth of biological data, which is rapidly growing. Genomics has been added to the world of proteins and metabolites. Increasingly efficient methods of bioinformatics allow us to handle the huge amount of available information to gain a more comprehensive knowledge on living systems.

Drugs either mimic or counteract metabolites when acting at their biological target. At the same time the elucidation of metabolic pathways and the generation of metabolomic networks are central goals of systems biology. Thus, the rise of systems biology seems to be almost "tailor made" for pharmaceutical science. Medicinal chemists taking up the challenge to broaden their biochemical competence and to widen up their scope of techniques will profit significantly from this development.

The lecture will start from the roots of the presently ongoing paradigmatic change in life science research. Systems biology will be presented first of all as "general pharmaceutical systems biology". This defines an overall commitment in drug research to be always aware of the "systems nature" of the whole field. Some recommenda-

tions will be given to illustrate the advantages of this general approach. The lecture will be concluded by a short outlook towards "specific pharmaceutical systems biology", which is a rapidly growing field. There, "network based drug discovery" is a particularly promising approach.

L75

INTEGRATED APPROACH FOR THE MULTIRECEPTORIAL DESIGN OF ANTIPSYCHOTIC AGENTS

Jana Selent[a], Laura López[a], Anna Bauer-Mehren[a], Christian Fernández[b], María Isabel Loza[b], Ferran Sanz[a] and Manuel Pastor[a]

[a] Computer-Assisted Drug Design Laboratory, Research Unit on Biomedical Informatics (GRIB), IMIM, Universitat Pompeu Fabra, Barcelona, Spain. [b] Faculty of Pharmacy. University of Santiago de Compostela. Santiago de Compostela, Spain.

Some of the most effective antipsychotic drugs in the market are characterized by showing affinity for a large number of related receptors[1], while more selective drugs are less clinically effective. This finding is another piece of evidence suggesting that the classical "target molecule" paradigm must be replaced by another kind of target representing the life complexity. However, there are no clearly established methods to define the exact nature of this new target entity.

In our opinion, a close examination of the structural and pharmacological properties of clinically useful compounds and an appropriate multivariate analysis of this data[2] might help to define a "receptorome", in which a particular binding affinity profile is associated to either therapeutic benefits or side effects. This information will be exploited by appropriate *in silico* drug design methodologies in order to obtain more effective and safe compounds. For this purpose, we generated a series of ligand-receptor complexes for 14 related aminergic receptors[3] on the basis of the new β_2 adrenergic structure[4], which will be used for the structural analysis of the receptorome.

Here we present our progress in an integrated approach[5] aiming to identify this optimum multireceptorial profile in the case of antipsychotic drugs and to translate these findings into novel compounds. The project involves pharmacologist, synthetic chemist and drug design specialists with a clear practical orientation.

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L76

QUANTUM CHEMICAL CALCULATION OF PROTEIN-LIGAND INTERACTION

Isao Nakanishi

Kinki University

Structure-based drug design (SBDD) is widely utilized in the drug design process as a promising methodology in drug discovery. Structural information of the ligand binding site of a target protein provides researchers with clues for designing new compounds. Medicinal chemists usually design new compounds to increase their affinities to a target protein. Therefore, it is very important to analyze the binding energies between ligand and a protein with high accuracy.

A lot of computational methods to predict binding affinity have been proposed such as the linear interaction energy (LIE) theory, the combined molecular mechanics (MM) and the Poisson-Boltzmann/surface area (PB/SA) solvation model (MM-PB/SA), and the free energy perturbation method. However, these methods usually utilize the MM calculation, and thus suffer from the limitations of force fields. Especially, potential parameters are often unavailable for synthetic drug compounds.

The hybrid quantum mechanical (QM) and molecular mechanical (QM/MM) method overcomes some of force field problems by treating drug compounds and possibly some protein atoms by QM. Most of applications employed a semi-empirical QM method that is less rigorous than ab initio approaches. Moreover, the fixed charge description of the MM part can not consider the polarization of protein and drug compound, as well as the charge transfer between them. Both of these effects are properly described by QM methods; however, conventional ab initio approaches require an enormous computation time to compute full-size proteins. Semi-empirical QM calculations using MOZYME program have been applied to protein–ligand binding studies.

The fragment molecular orbital (FMO) method has made possible to calculate the whole protein–ligand complexes with ab initio quality. In the method, system is divided into small fragments and ab initio QM calculations are performed on such fragments and their pairs, to obtain the total energy and other properties of the entire system. Using the FMO method, effects of charge transfer and polarization at the protein-ligand complex formation have been analyzed. In my talk, the results on FK506 binding protein complexes with several ligands are presented. Also, non-canonical interactions found in protein-ligand complexes such as CH-π and CH-O type hydrogen bonds, which are difficult to describe with the current force field method, will be discussed.

L77

ANALYSIS OF METAL ION TOXICITY IN ALZHEIMER'S DISEASE BY X-RAY STRUCTURAL STUDIES OF AMYLOID BETA AND COPPER ION INTERACTIONS

Jose Varghese

CSIRO Molecular and Health Technologies, 343 Royal Pd. Parkville Victoria 3052, Australia

The role of metal ions in Alzheimer's Disease[1] has been studied extensively in recent years. It is thought that Amyloid-Beta (Aβ) the principal component of Amylogenic plaque binds metal ions, in particular copper ions, and under suitable conditions can generate hydrogen peroxide by an oxidation and reduction cycle mediated by a reducing agent like ascorbate or dopamine. This production of H_2O_2 if not controlled by normal cellular regulators can lead to the production of reactive oxygen species that might be the causative agent that leads to neural degeneration in Alzheimer's patents.

We have carried out extensive studies of Aβ complexed with Cu+, Cu++ and Zn by X-ray Absorption Spectroscopy using synchrotron sources of the EXAFS and XANES spectra of these ions to determine the coordination of the ions in the Aβ-metal ion complexes. These experiments have yielded atomic models of the Aβ metal ion complexes that are in agreement with molecular dynamics and quantum mechanical simulations of the complexes and the steps in the oxidative/reductive chemistry of H_2O_2 production[2]. We have also commenced X-ray crystallographic studies of Aβ fusion constructs with the aim of developing a platform for structure based drug design. Such studies will form the basis of a medicinal chemistry program to blockade this reaction pathway and could lead to a treatment to prevent and retard the progression of the disease.

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L78

ACCESS AND BINDING OF LOCAL ANESTHETICS IN THE CLOSED SODIUM CHANNEL

Iva Bruhova, Denis Tikhonov, and Boris S. Zhorov

Department of Biochemistry and Biomedical Sciences, McMaster University,

1200 Main street West, Hamilton, Ontario, L8N 3Z5, Canada

Local anesthetics (LAs) are clinically used to treat severe pain and cardiac arrhythmia. LAs are known to block Na^+ channels in the closed, open, and inactivated states and reach their binding sites via extracellular and intracellular access pathways. Conserved phenylalanine in segment IVS6 is important for both the open and closed-channel block, while conserved tyrosine in the same domain is important for the open, but not the closed-channel block by cocaine [1] and tetracaine [2]. Paradoxically, permanently charged LAs, such as QX-314, block cardiac Na^+ channels ($Na_v1.5$) from both the extracellular and intracellular sides, but block neuronal and muscle Na^+ channels only from the intracellular side [3]. Mutations in segment IVS6 and at the selectivity-filter region affect the extracellular access pathway for LAs [4-6]. Despite many studies, there is no atomic-scale model to explain the diverse experimental data on the LA actions.

We simulated the binding and access of LAs in the homology model of the closed $Na_v1.5$ channel. The S5 and S6 helices were modeled from the X-ray structure of KcsA [7] and the P-loop region was modeled as in ref. [8]. We docked into the channel three structurally different local anesthetics: QX-314, cocaine, and tetracaine. Energetically favorable complexes were searched by generating thousands of starting points followed by Monte Carlo-energy minimizations. We found that the ammonium group of the LAs binds near the focus of the P-loop helices, while the aromatic group extends either along the pore axis (vertical binding mode) or into the III/IV domain interface (horizontal binding mode). Cocaine and QX-314 can adopt both binding modes, however due to size limitations tetracaine can only adopt the horizontal binding mode. The vertical mode was previously predicted for the open channel [9], but only the horizontal mode is in agreement with mutational data on the closed-channel block. To determine the extracellular access pathway(s) of QX-314, we pulled the ligand through the selectivity filter, the closed activation gate, and the III/IV domain interface. Only the III/IV interface, which leads into the horizontal binding mode, does not impose steric hindrance. A Na^+ ion bound in the selectivity-filter DEKA locus destabilizes the vertical mode and thus favors the horizontal mode. LA ingress and binding into the inner pore requires the resident Na^+ ion to escape the central cavity via the selectivity filter, while LA egress should be coupled with the reoccupation of Na^+ into the cavity. This hypothesis on the coupled movement of Na^+ and LA in the closed channel explains seemingly contradictory data on how the outer-pore mutations as well as why tetrodotoxin, but not μ-conotoxin prevents LA access to the closed channel. Supported by CIHR grant to BSZ and CIHR scholarship to IB.

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L79

ENGINEERING OF PURINE RECEPTORS AND THEIR LIGANDS

Kenneth A. Jacobson

National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

Extracellular adenosine, purine nucleotides (such as ATP and ADP), and pyrimidine nucleotides (such as UTP, UDP, and UDP-glucose) act as signaling molecules by activating G protein-coupled adenosine and P2Y nucleotide receptors. These ubiquitous receptors modulate the function of diverse mammalian cells under both normal and pathophysiological conditions. We have designed ligands for these receptors using classical and structure-guided (e.g. molecular modeling and docking) approaches. Selective agonists for the A_3 adenosine receptor (AR) are sought as anti-inflammatory, cardioprotective, cerebroprotective, and anticancer agents. We designed and synthesized novel A_3ARs agonists (1) containing a conformationally constrained ribose substitution, i.e. a North (N)-methanocarba (bicyclo[3.1.0]hexane) ring system, which maintains a conformation that is highly preferred by the A_3AR. Dual acting A_1/A_3 agonists have been designed for cardioprotection. A_3AR antagonists are efficacious in reducing intraocular pressure in glaucoma models. The problems of species dependence of affinity as A_3AR antagonists (i.e., many of the nonnucleoside antagonists are effective in humans but not rats) have been overcome with nucleoside antagonists that are truncated or otherwise impaired in flexibility or H-bonding ability at the 5′-uronamide group. Platelets express a proaggregatory $P2Y_1$ receptor and an antiaggregatory A_{2A}AR. We recently reported the first nanocarriers with covalently conjugated functionalized congeners of AR ligands, which activate the A_{2A}AR to display antithrombotic activity (2). The multivalent conjugation to polyamidoamine (PAMAM) dendrimers alters the pharmacological properties and provides targeting options. The bisphosphate nucleotide MRS2500 is a potent $P2Y_1$ receptor antagonist (Ki = 0.79 nM) and the first one demonstrated to be suitable for use *in vivo* (3). This antagonist contains a (N)-methanocarba ring system in place of the ribose moiety, which maintains a preferred conformation for both $P2Y_1$ agonists and antagonists. Activation of the $P2Y_6$ receptor in microglial cells induces phagocytosis (4). Thus, this receptor might be a target for neurodegenerative diseases. The conformational requirements of the ribose moiety of UDP in binding to the $P2Y_6$ receptor are very different from those of the $P2Y_1$ receptor. UDP analogues locked in the South (S) envelope conformation, predicted by receptor docking to be $P2Y_6$-preferred, were synthesized and found to be more potent and selective than the native ribose-containing nucleotide at this subtype (5). Another potential means of using the protective effects of AR activation was achieved through receptor engineering. We have introduced the approach of neoceptors (6), intended for eventual delivery by tissue-targeted vectors for gene therapy, in which the putative agonist binding site is redesigned to accept only agonist molecules altered in a complementary fashion. We are exploring this approach conceptually with tailor-made agonist ligands (de novo designed neoligands that are selective for the neoceptor and not the native receptor) in combination with receptor mutagenesis. Complete orthogonality in a neoceptor/neoligand pair has been achieved for the H272E mutant A_3AR.

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L80

NEW MODULATORS OF A_3 AND A_{2B} ADENOSINE RECEPTORS

Pier Giovanni Baraldi,[a] Delia Preti,[a] Mojgan Aghazadeh Tabrizi,[a] Giulia Saponaro,[a] Stefania Baraldi,[a] Romeo Romagnoli,[a] Pier Andrea Borea [b]

[a]Dipartimento di Scienze Farmaceutiche, [b]Dipartimento di Medicina Clinica e Sperimentale-sezione di Farmacologia, Università di Ferrara, 44100 Ferrara Italy

Compounds presenting an additional fused ring on the xanthine nucleus have been reported to exhibit antagonistic activity with various levels of affinity and selectivity towards the four adenosine receptors subtypes A_1, A_{2A}, A_{2B} and A_3 (ARs). We recently reported the synthesis and biological evaluation of new 1-benzyl-3-propyl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-diones and 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-diones, among which we identified potent and selective A_3 adenosine receptors antagonists.[1] In particular compound 1-benzyl-7-methyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione shows a K_i (hA_3) value from binding assay of 0.8 nM and high level of selectivity over A_1, A_{2A} and A_{2B} ARs (K_i ratios > 1,250).

We have recently discovered a number of 8-heterocyclesubstituted xanthine derivatives as potent and selective A2B AR antagonists.[2] A new series of 1,3-dipropyl-8-(1-phenylacetamide-1H-pyrazol-3-yl)-xanthine derivatives has been identified as potent A_{2B} adenosine receptor antagonists.[3] The products have been evaluated for their binding affinities for the human A_{2B}, A_1, A_{2A}, and A_3 adenosine receptors. Compound *N*-(4-chloro-phenyl)-

2-[3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-5-methyl-pyrazol-1-yl] showed a high affinity for the human A_{2B} adenosine receptor K_i = 7 nM and good selectivity (A_1, A_{2A}, A_3/A_{2B} > 140). Synthesis and SAR of this novel class of compounds is presented herein.

The lack of molecules endowed with selective and potent agonistic activity toward the hA_{2B} adenosine receptors has limited the studies on this pharmacological target and consequently the evaluation of its therapeutic potential. We reported the design and the synthesis of the first potent (EC_{50} in the nanomolar range) and selective hA_{2B} adenosine receptor agonists consisting of 1-deoxy-1-[6-[((hetero)aryl-carbonyl)-hydrazino]-9*H*-purin-9-yl]-N-ethyl-β-D-ribofuranuronamide derivatives.[4] The concurrent effect of 6-substitution of the purine nucleus with a ((hetero)aryl-carbonyl)-hydrazino function and a 2-chloro-substitution has been investigated in such NECA derivatives. Moreover, a new series of N^6-[hetero)aryl/(cyclo)-alkyl-carbamoyl-methoxy-phenyl]-(2-chloro)-5'-*N*-ethylcar boxamido-adenosines has been synthesised and tested in binding assays at hA_1, hA_{2A}, and hA_3 adenosine receptors, and in a functional assay at the hA_{2B} subtype.[5] The examined compounds displayed high potency in activating A_{2B} receptors with good selectivity versus A_{2A} subtypes. The introduction of an unsubstituted 4-[(phenylcarbamoyl)-methoxy]-phenyl chain at the N^6 position of 5'-*N*-ethylcarboxamido-adenosine led us to the recognition of a potent full agonist displaying the highest efficacy of the series (EC_{50} hA_{2B} = 7.3 nM). These compounds represent the first report about adenosine-related structures capable of activating hA_{2B} subtype in the low nanomolar range.

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L81

ALLOSTERIC MODULATION OF ADENOSINE RECEPTORS

Ad P. IJzerman

Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, PO Box 9502, 2300RA Leiden, The Netherlands

Allosteric modulation of G protein-coupled receptors (GPCRs) has evolved from an almost academic concept that was thought to hold for just a few GPCRs into a very active field of drug discovery for virtually all GPCRs. This is only logical, since it offers new ways of intervening with GPCR ligand binding and function. Classically the mechanism of action for many drugs is either to mimic or to inhibit the action of endogenous signaling molecules, leading to the traditional classification of agonists as well as antagonists/inverse agonists, respectively. Through competition at the binding site for the endogenous neurotransmitter or hormone the desired effect is exerted. In contrast, allosteric modulators are thought to act at sites distant from the (primary or orthosteric) ligand binding crevice, potentially leading to a number of benefits. One of these is that an allosteric drug does not necessarily have an action *per se*. It can rather modulate the action of the naturally occurring hormone or neurotransmitter when the latter is released. In this way the temporal and spatial aspects of the natural signaling mechanism may be preserved.

It now emerges that allosteric modulation is dependent on the nature of the orthosteric ligand and on the system (species, tissue, signalling route) the effect is assessed in, such that opportunities for subtle receptor interaction are increased even more. This apparent complexity can be well explained by receptor theory and pharmacological modeling.

In this presentation I will take the adenosine A_1 and A_3 receptors as templates to illustrate the above aspects. Firstly, our synthetic efforts to develop selective allosteric modulators for the two receptor subtypes will be discussed. Their biological evaluation enabled us to derive structure-activity relationships for allosteric modulation. Secondly, some of these materials were used to probe the receptors, and study the proteins' behaviour in more detail with respect to orthosteric ligand binding, species differences, and signaling route.

L82

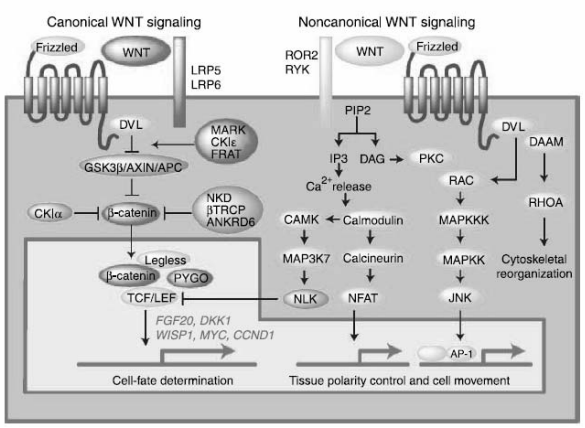
ALTERNATIVES TO KINASE INHIBITORS FOR CANCER THERAPIES

Giovanni Gaviraghi

Sienabiotech S.p.A., via Fiorentina, 1, 53100 Siena Italy

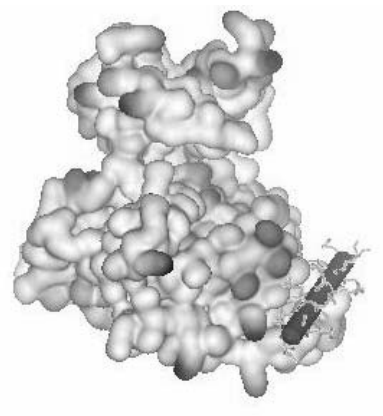
Kinases are central elements (nodes) in many cellular processes including tumour propagation and of proven value as targets for selective therapeutic agents particularly for oncology applications. There are over 500 protein kinases which form a well defined protein class based on consideration of ATP-binding site homology defined by a 28 amino acid fingerprint. As such kinases where one of the first protein classes to be investigated at a systematic level[1] and constitute a rich area for discovery of novel small ligand-based therapies largely based around ATP binding site inhibitors[2]. Obtaining adequate selectivity at a therapeutic level can be difficult given the homology within the ATP binding site and resistance to current kinase inhibitors can limit overall effectiveness.

This talk will concentrate on 2 alternatives to ATP binding site inhibitors – pathway or phenotype screening where function is the key to novel therapeutic agents and kinase signalling inhibition via kinase-effect or interaction inhibitors protein-protein interactions.



Pathway or phenotype screening offers particular advantages where the complexity of pathobiology of disease such as glioblastoma render effective target identification difficult and thus choosing the right target would be assisted by first selecting compounds with a relevant mechanism of action in a disease context. The example of the Wnt signalling pathway and it's relevance to brain disease will be used to highlight this approach.

Protein-protein interactions are less well explored but may provide alternatives to unlocking the potential of kinases as key components in signalling pathways via a more direct block of signalling. Some PPi interfaces are very difficult to approach with small molecules, but with the right assays some "systems" or "target classes" such as "α-helix groove binding sites" might be amenable to small molecule inhibitors. Novel approaches to the design of small molecules for such protein-protein interactions for brain tumours such as glioblastoma will also be highlighted.



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L83

ENDOTHELIN CONVERTING ENZYME INHIBITORS AS ANTICANCER AGENTS FOR HUMAN GLIOBLASTOMA. A COMPARISON WITH ENDOTHELIN RECEPTOR ANTAGONISTS

Lucienne Juillerat-Jeanneret

University Institute of Pathology, CHUV and UNIL, Lausanne, Switzerland

Glioblastoma are very aggressive and deadly human cancers, which almost invariably develop multiresistance and poor response to conventional chemotherapeutic agents. Therefore alternative therapeutic protocols must be found and developed. The endothelin system may represent such a treatment opportunity, either as monotherapy but more likely in drug combination therapy. The endothelin system is composed of a precursor peptide, the pro-endothelins-1,2,3, which are activated to the active endothelin (ET-1,2,3) peptides by the protease endothelin converting enzyme-1 (ECE-1), of which four isoforms have been described with different cellular localization. The ETs act, either via autocrine or paracrine loops, on two cellular receptors, the ET_A and ET_B, inducing multiple intracellular responses, including cell survival signals. Therefore blocking this ET-dependent survival signals in cancer either using ET_A or/and ET_B receptor antagonists or ECE-1 inhibitors, may allow the control of tumor growth. In cancer, alternative variants of ECE-1 and of the ET_A and ET_B receptors may also exist, with properties differing of their normal counterparts, including their cellular localization and requiring antagonists and inhibitors with adapted chemical properties. Thus families of synthetic antagonists for ET_A/ET_B receptors or inhibitors of ECE-1 have been synthesized, and were evaluated on human glioblastoma and brain-derived angiogenic endothelial cells with known expression of ET receptors and ECE-1. Drug combination with conventional chemotherapeutics has also been attempted. These synthetic approaches and the results of the biological experiments will be presented and discussed. In particular, the consequences on the physicochemical properties of the antagonists and inhibitors of the cellular localization of the receptors and enzymes of the endothelin axis in cancer will be emphazized.

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L84

IDENTIFICATION OF A SMALL MOLECULE AKT KINASE INHIBITOR, SK690693

Dirk A. Heerding

Over-expression of AKT has an anti-apoptotic effect in many cell types and expression of dominant negative AKT blocks the ability of a variety of growth factors to promote survival. In addition, PTEN, a critical negative regulator of AKT, is lost in many cancers, including breast and prostate carcinomas, glioblastomas, and several cancer syndromes including Bannayan-Zonana syndrome, Cowden disease, and Lhermitte-Duclos disease. Therefore inhibitors of AKT kinase activity might be useful as monotherapy for the treatment of tumors with activated AKT.

This presentation will describe our lead optimization studies culminating in the discovery of GSK690693. GSK690693 is a novel ATP competitive, pan-AKT kinase inhibitor with IC_{50}s of 2, 13 and 9 nM against AKT-1, 2 and 3, respectively.

Treatment of tumor cells with GSK690693 causes dose dependent reductions in the phosphorylation state of multiple proteins downstream of AKT, including GSK3β, PRAS40 and Forkhead (FOXO1/FOXO3a). GSK690693 inhibits proliferation and induces apoptosis in a subset of tumor cells with potency that is consistent with intracellular inhibition of AKT kinase activity.

A single intraperitoneal (IP) administration of GSK690693 inhibits GSK3β phosphorylation in SCID mice bearing BT474 breast tumor xenografts in a dose and time-dependent manner. GSK690693 treatment (once daily for 21 days) produces significant tumor growth delay in mice bearing established human SK-OV-3 ovarian, LNCaP prostate, and BT474 and HCC-1954 breast carcinoma xenografts. Immunohistochemical analysis of tumor xenografts after repeat dosing with GSK690693 demonstrates reductions in phosphorylated AKT substrates. The pharmacodynamic and antitumor effects of GSK690693 supports its evaluation as an anticancer agent.

GSK690693 is currently in clinical development as an IV agent for use in patients with solid tumors or hematological malignancies.