Lectures

Inaugural Lecture

The Future of Medicines Research and The Pharmaceutical Industry in Europe

T. Jones

CBE. FRSC. C. Chem. PhD. DSc. Hon. FRCP Allergan Inc . BAC bv and ReNeuron Group plc., UK

The post genome revolution has opened up an entirely new approach to understanding disease and discovering new therapeutic and prophylactic interventions. Medicinal Chemistry is the core discipline to make this happen and ,in parallel advances in physical sciences, particularly analytical chemistry are playing an important part .Biotechnological advances ,although based on biological sciences rely heavily on medicinal chemistry and ,going forward, the distinction between these fields is likely to narrow.

Meanwhile the pressures on the pharmaceutical industry in Europe from a variety of sources e.g. political, regulatory, societal, financial make this a tough environment where there is a danger that companies will seek alternative locations for their R&D ;most notably (in addition to the USA) India and China. Creating a stable, sustainable science and economic base for the industry to grow in Europe and increase it's productivity will demands a commitment from governments , academia and industry alike.

Prize Lectures

I – Nauta Award on Pharmacochemistry

Unifying themes in the design of ligands as tools for the investigation of opioid receptors

P. Portoghese

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, MN, USA

Molecular tools are essential for the investigation of pharmacologic receptors and development of new drugs. For example, selective agonists and antagonists are employed routinely for the pharmacologic characterization of new ligands. In this Award Address I will focus on research from my laboratory that encompasses several themes to illustrate different approaches we have used for the design of ligands that are widely employed in the investigation of opioid receptors. These include the design of a) a novel class of affinity labels, b) ligands based upon the message-address concept, and c) ligands that target dimeric receptors. Some potential clinical applications of such ligands will be discussed.

II – GSK Award on Chemical Biology

Biology Oriented Synthesis for Chemical Biology Research

Herbert Waldmann

Max-Planck-Institut für molekulare Physiologie, Department of Chemical Biology, Otto-Hahn-Str. 11, D-44227 Dortmund, Germany, and Universität Dortmund, Fachbereich 3, Chemische Biologie. Herbert.waldmann@mpi-dortmund.mpg.de

Relevance to nature is one of the most important criteria to be met by compound classes for chemical biology and medicinal chemistry research. The underlying frameworks of natural products (NPs) provide evolutionary selected chemical structures encoding the properties required for binding to proteins, and their structural scaffolds represent the biologically relevant and prevalidated fractions of chemical space explored by nature so far.

Biology oriented synthesis (BIOS) builds on these arguments. It employs core structures delineated from NPs as scaffolds of compound collections and creates focussed diversity around a biologically prevalidated starting point in vast structural space. BIOS, therefore, builds on the diversity created by nature in evolution and aims at its local extension in areas of proven biological relevance. Consequently BIOS offers a conceptual alternative to other guiding strategies for library design which for instance are based on mechanistic considerations, sequence or structure homology or on the creation of chemical diversity.

In the lecture the trains of thought leading to the BIOS concept will be detailed, including the development of a Structural Clustering of Natural Products (SCONP) in a tree-like arrangement and its combined use with Protein Structure Similarity Clustering (PSSC) as hypothesis generators for the development of NP-derived and –inspired collections, the chemical feasibility of their synthesis on the solid phase and in solution and the investigation of these compound collections in selected biochemical and biological assays.

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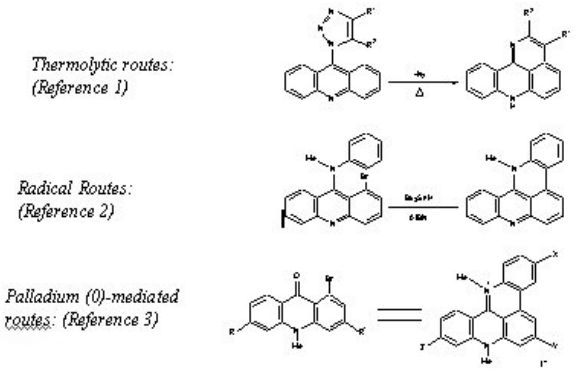
III – IUPAC-Richter Prize in Medicinal Chemistry

Azapentacycles as DNA telomere signalling-targeted agents

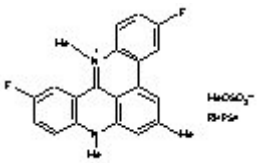
M. Stevens

Cancer Research UK Experimental Cancer Chemotherapy Research Group, School of Pharmacy, University of Nottingham, NG7 2RD, UK

The tetra- and pentacyclic acridine scaffold can be decorated with substituents which confer unique biological properties on the products. A range of synthetic methodologies has been applied to provide access to molecules which selectively bind to the $(TTAGGG)_n$ base sequences in telomeric DNA and show pronounced antitumour effects.



The 8,13-dimethylquinoacridinium salt RHPS4 has been selected as a clinical candidate: this agent stabilises G-quadruplex isoforms of DNA,[4] compromises the integrity of telomeres, and leads to profound biological sequelae.[5] Two separate syntheses of RHPS4 will be described and its pharmaceutical and biological properties revealed.



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IV – Prous Award for New Technologies in Drug Discovery

Vascular Tumor Targeting

D. Neri

Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

One avenue towards the development of more selective anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific for tumor-associated markers.

The targeted delivery of therapeutic agents to newly-formed blood vessels (vascular targeting) opens a broad palette of biomedical opportunities. Angiogenesis, i.e., the proliferation of new blood vessels from pre-existing ones, is an important process not only in cancer, but also in relevant diseases such as certain blinding ocular disorders and rheumatoid arthritis. The ability to selectively target and occlude neovasculature promises to be useful for the diagnosis and treatment of angiogenesis-related diseases.

In collaboration with Philogen SpA and with Luciano Zardi (Genova), my laboratory has developed human monoclonal antibodies, capable of selective targeting of neo-vascular structures in solid tumors and in a number of angiogenesis-related diseases. Three derivatives of these antibodies (i.e., two immunocytokines and a radiolabeled antibody) are currently being investigated in clinical trials.

The identification of novel vascular markers of pathology and the development of novel methodologies for the isolation of high-affinity small organic binding molecules are possibly the most pressing technological challenges for future developments in the field of vascular targeting. In the first research area, we have developed a novel chemical proteomic methodology based on the in vivo perfusion of tumor-bearing animals with reactive ester derivatives of biotin, followed by purification of the biotinylated vascular proteins in normal organs and tumors and by a comparative mass spectrometric analysis, for the identification of novel accessible markers of pathology. In the second research area, my laboratory has developed novel DNA-encoded chemical library technologies (e.g., encoded self-assembling chemical libraries), which allow the construction and screening of chemical libraries of unprecendented size.

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V – UCB Award for Excellence in Medicinal Chemistry

Nexavar® – A novel Inhibitor of Signal Transduction

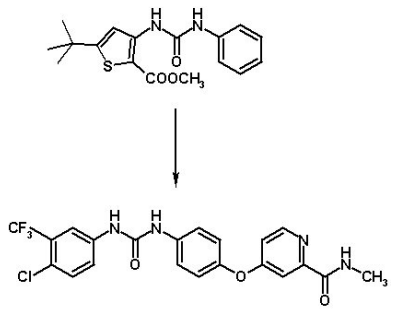
B. Riedl

Bayer HealthCare AG PH-R&D-EURC-PRR-CEF Elberfeld, 0456, Germany

The discovery of Nexavar® (sorafenib tosylate) is the result of a collaboration between Bayer HealthCare and Onyx Pharmaceuticals. Sorafenib belongs to a novel class

of kinase inhibitor exhibiting a dual mode of action. This compound inhibits Raf kinase, a key mediator of the MAP kinase pathway, thereby blocking tumor cell proliferation. In addition, sorafenib inhibits a series of receptor tyrosine kinases involved in angiogenesis and stromal activation, notably VEGFR and PDGFR. Therefore, the kinase profile of sorafenib results in inhibition of both tumor growth and tumor angiogenesis in xenograft models.

The medicinal chemistry program started from a lead structure identified via high-throughput screening. The confirmed hit, a thienyl-phenyl-urea (IC_{50} = 17 μM), was optimized using classical medicinal chemistry as well as combinatorial chemistry techniques.



Nexavar® was first approved in the US in late 2005 for the treatment of advanced renal cell carcinoma. Additional Phase III clinical trials are currently ongoing, evaluating its potential in the treatment of melanoma, hepatocellular carcinoma, and non-small cell lung carcinoma, either as a single agent or in combination with cytotoxic therapies.

Plenary Lecture I

Chemical Genetic Analysis of Protein and Lipid Kinases in Disease and Normal Physiology

K. Shokat

Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 600 16th Street, Box 2280, San Francisco, CA 94143-2280, USA

Our goal is to develop chemical means to perturb each protein and lipid kinase in the human genome. We have explored two avenues to acheive this goal. Using chemical genetics we have developed a systematic means to engineer each protein kinase to be inhibitable by a bio-orthogonal small molecule inhibitor that does not inhibit any wild-type protein kinases. Using this approach of combining chemistry and genetics we can analyze pathways with unparalleled specificity and rapidity revealing phenotypic effects often missed by traditional genetic studies. Using a more traditional pharmacological approach for the study of the much smaller lipid kinase family we synthesized a structurally diverse set of drug-like molecules based on their potential to serve as potent inhibitors of one or more enzymes involved in PIP3 generation. Our goal was to produce different chemotypes with distinct target selectivities as probes of lipid kinase signaling. We have applied this matrix of inhibitors to study both normal physiological pathways such as the insulin pathway and also disease pathways such as cancer.

Plenary Lecture II

Protein-protein interactions and the control of GPCR-mediated signal transduction

G. Milligan

Molecular Pharmacology Group, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

It has recently become clear that many, if not all, G protein-coupled receptors (GPCRs) are able to form dimers or higher-order oligomers. Using the α_{1b}-adrenoceptor as a model we have established that symmetrical interactions between elements of both transmembrane domain I and transmembrane domain IV allow this receptor to link into chains of monomers [1] with a pattern reminiscent of the in situ organisation of rhodopsin in murine rod outer segment discs. Mutations in transmembrane domains I and IV modulate this structural organisation, interfere with core glycosylation, prevent cell surface delivery and eliminate signal transduction.

Many GPCRs are co-expressed by individual cells. When co-expressed the orexin-1 receptor and cannabinoid CB-1 receptor interact and this interaction regulates their cellular distribution, pharmacology and function. Such 'hetero-dimers' may thus provide novel and selective targets for drug design [2]

A wide range of GPCR-interacting proteins have been identified via approaches that include yeast 2 hybrid screens and proteomic technologies. A number of these interact with 'helix 8' of members of the rhodopsin-like, family A GPCRs. Using the melanin concentrating hormone receptor-1 as an example we have shown that both periplakin [3] and neurochondrin interact. As 'helix 8' plays an important role in interactions with G proteins and hence signal transduction, we have shown that both periplakin and neurochondrin interfere with agonist-mediated signalling and as such modulate the function of the melanin concentrating hormone receptor-1.

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Plenary Lecture III

How can or should the Medicinal Chemist use the progress in the field of Systems Biology in novel approaches

J. Van Der Greef

TNO Systems Biology, The Netherlands; Center for Medical Systems Biology, Leiden University, The Netherlands BG Medicine Inc, USA

The continued commercial success of the pharmaceutical industry as a whole depends on reversing the trends in a number of leading indicators of its productivity such as the recent year-over-year decreases in new product launches, increases in product development time, withdrawals of blockbuster drugs, and a non-linear increase over time in research and development expenditures.

Systems biology delivers tools for improving drug discovery and development especially in the form of systems biomarkers to improve the insights in disease processes, phenotypes, translational aspects and drug response patterns, but the major step forward is not only comprised of a technological one, but encompasses a conceptual shift towards systems thinking. From a *systems-based view* the key topic is understanding the *interconnectivity* within systems and the study of the *organizing principles*, realizing that new properties emerge at different levels of complexity.

More importantly for the medicinal chemist new tools emerge by applying translational and *reverse-translational approaches*. Historically, very little useful information relating to efficacy or safety issues, in the form that drug discoverers/designers can use, is passed back to the preclinical phase from clinical trials. A molecular systems approach to clinical efficacy and safety of a first-in-class drug can surmount this challenge, dramatically facilitating the process of second generation drug discovery.

The overview will comprise a general introduction into systems biology and its impact on the pharmaceutical discovery and development chain with emphasis on 2nd generation drug design using correlation network approaches.

L1

The role of ABCG2 multidrug transporter in cancer drug resistance and drug metabolism

B. Sarkadi*, C. Özvegy*, Á. Telbisz*, G. Várady*, K. Német*, A. Váradi**

*National Medical Center, and **Institute of Enzymology, Hung.Acad. Sci., Budapest, Hungary

The ABCG2 multidrug transporter is an “ABC half-transporter”, its functional form is a homodimer. This protein is a key player in xenobiotic transport and cancer multidrug resistance, as ABCG2 can actively transport several hydrophobic drugs, as well as various anionic compounds, e.g. methotrexate. We have expressed this human protein both in Sf9 insect cells and in various mammalian cells lines, in order to study its function, drug interactions, and mechanism of action. We have also expressed various substrate mutants and polymorphic variants of ABCG2 to examine their functional features. In this presentation we review the basic function of ABCG2 in physiology and drug metabolism, especially the possible role of this protein in new cancer treatment modalities, such as the use of tyrosine kinase inhibitors. We present new data regarding the interaction of ABCG2 with a cell-surface reacting monoclonal antibody and the modulation of the ABCG2 transport activity by drugs and membrane lipids. We also show recent data regarding the mutant and polymorphic variants of ABCG2, the latter being present in large human populations with different ethnic backgrounds. Since the polymorphic ABCG2 proteins may differently affect cancer treatment, general drug absorption and toxicity, represent risk factors in fetal toxicity, or alter the differentiation of stem cells, their characterization is a major challenge in this field.

L2

Future Antipsychotics: Magic bullets or add-on treatments?

K. BøGesø

Medicinal Chemistry Research, H. Lundbeck A/S, Denmark

Schizophrenia is a psychiatric disorder that affects approximately 1% of the world’s population. The disease comprises a range of symptoms such as positive, negative, cognitive, and depressive symptoms. Current typical and atypical drugs mainly affect positive symptoms, but even on these symptoms the drugs show a partial response in patients and the discontinuation rate is high.

The typical and atypical drugs act on a multitude of receptors. They all block (fully or partially) dopamine D2 receptors, but in addition they bind to serotonergic, adrenergic and muscarinic receptors. Although atypical antipsychotics have a low propensity to induce extrapyramidal side effects, they induce other side effects such as sedation, weight increase, QT prolongation, orthostatic hypotension, etc.

A number of new targets are currently being investigated either as targets for potential add-on treatments, especially to treat cognitive deficits (NMDA enhancers, D1 agonists, α_2 antagonists, NA uptake inhibitors, etc), or as new antipsychotic targets (NK3 antagonists, PDE10 inhibitors, etc).

The aim of the talk will be to outline the current situation with regards to strategies for pharmacotherapy of schizophrenia, followed by a number of case stories relevant to Lundbeck, exemplifying different approaches.

L3

Smart polymeric micelles as nanocarriers for gene and drug delivery

K. Kataoka

Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo, Tokyo, Japan. Division of Clinical Biotechnology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan. Center for NanoBio Integration, The University of Tokyo, Tokyo, Japan

Polymeric micelles, the self-assemblies of block copolymers, are a promising nanocarrier system for drug and gene delivery. They have several advantages, such as controlled drug release, tissue-penetrating ability and reduced toxicity. Also, nano-engineering of the block copolymers might allow the preparation of polymeric micelles with integrated smart functions, such as targetability as well as stimuli-sensitivity. pH-sensitive polymeric micelles, in which doxorubicin (Dox) is attached to the side chain of the core-forming segment of the block copolymers via an acid-labile hydrazone bond, were developed in our group to show a drug release selectively under the lysosomal/endosomal pH conditions (5.0~5.5). A biodistribution study revealed the micelle to show the longevity in blood circulation due to a minimal leakage of free drug, resulting in the highly selective accumulation in solid tumors. Eventually, the micellar-Dox achieved a significantly higher antitumor activity in C-26-bearing mice over a broader range of injection doses than free Dox without any serious side effects. Besides the hydrophobic interaction, an electrostatic interaction was found to be available for the formation of the so-called polyion complex (PIC) micelles. This system is particularly useful for the delivery of charged compounds, including genes and siRNA. We developed a novel PEG-polycation block copolymer carrying the ethylenediamine moiety at the side chain (PEG-PAsp(DET)). Due to the regulated location of primary and secondary amino groups in a side chain, this block copolymer possessed both the sufficient DNA complexation ability and buffering capacity for the efficient endosomal escape of the polyplexes based on the proton sponge effect. These properties of PEG-PAsp(DET) enabled the transfection to various primary cells. Notably, the PEG-PAsp(DET) polyplex micelles incorporating pDNA encoded with osteogenic factors were found to successfully transfect recipient cells in mouse calvaria bone defects to induce bone regeneration, demonstrating their utility in the field of tissue regeneration.

L4

Synaptic pain amplifier in spinal cord in vivo

J. Sandkühler

Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, A-1090 Wien, Austria

At the level of the spinal cord pain-related information may be amplified for prolonged periods of time. A synaptic model of enhanced pain sensitivity, the long-term potentiation (LTP) of synaptic strength at the spinal origin of pain pathways has been challenged recently: 1.) LTP has always been induced by electrical conditioning high frequency (~100 Hz) stimulation of peripheral nerves at C-fibre strength. This does not, however, resemble the real discharges in C-fibres during inflammation or trauma which are within the low frequency range (less than 10 imp. s^{-1}). 2.) Low frequency pre-synaptic activity normally fails to trigger LTP but rather induces long-term depression at all synapses studied so far.

We now provide evidence for a synaptic pain amplifier in spinal dorsal horn which is switched-on by natural low level afferent barrage following subcutaneous injections of capsaicin or formalin. Natural discharge patterns in nociceptive C-fibres trigger substantial and sustained rise in calcium ion concentration (to 143 ± 9% of control, n = 15) in spinal dorsal horn lamina I neurons *in vivo*. This triggers calcium-dependent signal transduction pathways which potentiate synaptic strength (to 173 ± 9, n = 5) in pain pathways *in vivo*.

Upon acute withdrawl opioids such as the ultra-short acting remifentanil may induce a state of hyperalgesia. In intact anaesthetized rats i.v. remifentanil infusions depressed synaptic strength. When the infusions were stopped, responses became potentiated (to 134 ± 11%, n=15) throughout the rest of the recording period. When remifentanil was applied after LTP had been induced by electrical nerve stimulation (to 207 ± 13% of control), then a depotentiation (to 163 ± 14% of control, n = 10) was induced upon withdrawl from the opioid. In conclusion, depending upon the functional state of the spinal cord, acute withdrawl from a μ-opioid receptor agonist may either amplify pain related information, or depotentiate synaptic strength. The former may be a cellular mechanism of opioid-induced hyperalgesia, the latter a novel opioid action: the lasting reversal of pain amplification (i.e. long-term anti-hyperalgesia).

Supported by grants from the Austrian Science Fund (FWF)

L5

Posttranslational modifications in lantibiotic biosynthesis

W. Van Der Donk

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave, Urbana, USA

Lantibiotics are peptide-derived antimicrobial agents that are ribosomally synthesized and post-translationally modified by a multienzyme complex. Nisin has been used for decades in the food industry against food-borne pathogens without development of significant resistance. The compound has also attracted much attention due to its novel mechanism of action including specific binding to the bacterial cell wall precursor lipid II, followed by membrane permeabilization. We have recently succeeded in the reconstitution of the biosynthesis of nisin[1] as well as

lacticin 481.[2] Furthermore, we have determined the X-ray structure of the nisin cyclase, which surprisingly reveals structural homology to farnesyl transferase. The implications of this finding will be discussed as will re-engineering efforts of nisin and lacticin and the mechanism of their biosynthesis.

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L6

Fragment Based Drug Discovery – From Crystal to Clinic

D. Rees and colleagues,

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

Fragment-based lead discovery is becoming established within pharmaceutical and biotechnology companies as a complementary approach to traditional HTS screening for discovering new chemical leads in drug discovery programmes.[1-3] The starting fragments are low molecular weight ligands (MW=120-250) whose binding interactions with a target protein are structurally understood (eg by X-ray crystallography or NMR) and typically with IC50s in the mM range. This structural knowledge allows the fragments to be progressed into chemical lead series with IC50s in the nM range by synthesizing around 20-80 compounds.

Methodology developed at Astex for fragment-based lead discovery utilizes high throughput X-ray crystallography, NMR and other biophysical techniques to screen fragments against various protein targets including kinases, proteases etc. [4-6] The development of multiple oncology lead series using this approach will be outlined for targets such as cyclin-dependent kinase (CDK), aurora and PKB/Akt. Using this strategy a CDK inhibitor, AT7519, has been progressed from first synthesis to dosing in cancer patients in 18 months.

Attractions of the fragment-based technique include the requirement to screen and synthesise only a few compounds and the high success rate of generating multiple chemical series with lead-like properties.

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L7

Selective sodium channel modulators: new avenues for drug discovery

A. Termin, D. Wilson, J. E. Gonzalez

Vertex Pharmaceuticals, 11010 Torreyana Road, San Diego, CA 92121, USA

Ion channels have been shown to play a significant role in neuro-degenerative diseases. They can be classified broadly into ligand gated ion channels and voltage gated ion channels. Within the voltage-gated ion channel family, potassium, calcium, and sodium channels have been implicated in the pathology of neurodegenerative diseases.

Voltage gated calcium and potassium channels have slower kinetics than sodium channels (Na_v) and can be studied with a variety of techniques. Voltage gated sodium channels with their fast action kinetics are difficult to assay which presents a significant hurdle for medicinal chemistry.

The Na_V gene family consists of nine subtypes with different expression patterns in the brain, muscle, and the peripheral nervous system (PNS). Several Na_V subtypes are believed to play key roles in sensory neurons and are currently targets for novel analgesic agents.

In addition, Na_V blockers are also used as antiarrhythmic, neuroprotectant, and anticonvulsant agents in man. These agents were often discovered and optimized in animal pharmacological models, and only later were their mechanisms of action on Na_Vs elucidated.

Using Electrical Stimulation Voltage/Ion Probe Reader (E-VIPR) screening technology coupled with rapid parallel medicinal chemistry, we have discovered subtype selective sodium channel blockers which can be used to probe the role of specific voltage-gated sodium channels in neurodegenerative diseases.

L8

New vistas for the treatment of DNA virus and retrovirus infections

E. De Clercq

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

Current therapeutic strategies for the treatment of DNA virus infections consist of only a limited number of compounds, i.e. those used for the treatment of herpes simplex virus (HSV) infections (acyclovir, valaciclovir, famciclovir), varicella-zoster virus (VZV) infections (acyclovir, valaciclovir, famciclovir and brivudin), cytomegalovirus (CMV) infections (ganciclovir, valganci-

clovir, foscarnet and cidofovir) and hepatitis B virus (HBV) infections (interferon-α, lamivudine, adefovir dipivoxil and entecavir). For the treatment of human immunodeficiency virus (HIV) infections, more than twenty antiviral drugs are currently available, encompassing NRTIs (nucleoside reverse transcriptase inhibitors: azidothymidine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine), NNRTIs (non-nucleoside reverse transcriptase inhibitors: nevirapine, delavirdine, efavirenz), PIs (protease inhibitors: saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir), the NtRTI (nucleotide reverse transcriptase inhibitor) TDF (tenofovir disoproxil fumarate) and the FI (fusion inhibitor) enfuvirtide. The acyclic nucleoside phosphonates represent a new dimension in the therapy of virus infections, essentially because they possess a uniquely broad spectrum, long-lasting activity and resilient resistance profile. Together, cidofovir, adefovir dipivoxil and tenofovir disoproxil fumarate span virtually all DNA virus and retrovirus infections. In addition to the aforementioned indications, cidofovir could be used against human papilloma virus (HPV), adenovirus, herpesvirus (i.e. Epstein-Barr virus, human herpesvirus type 6, 7 and 8, in addition to HSV, VZV and CMV) and poxvirus infections (i.e. variola, vaccinia, cowpox, monkeypox, molluscum contagiosum, orf), and TDF, in addition to its anti-HIV activity, could be used successfully in the treatment of HBV infections. Moreover, cidofovir still remains the only antiviral drug which has been reserved for the treatment of poxvirus infections [including smallpox (variola), should this virus strike inadvertently]. The crucial chemical entity underlying the unique antiviral activity profile of cidofovir, adefovir and tenofovir is the phosphonate group, which is also present in a new generation of acyclic nucleoside phosphonates, i.e. the 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines HPMPO-DAPy, PMEO-DAPy and PMPO-DAPy, which like their predecessors, cidofovir, adefovir and tenofovir, offer substantial promise for the treatment of a broad range of retro-, hepadna-, adeno-, herpes-, pox- and papillomavirus infections.

L9

Virtual Screening - The Road to Success

H. Kubinyi

University of Heidelberg,Germany

Several computer-aided techniques for automated database searches and docking developed over the past ten to fifteen years. Chemical database filters, pharmacophore searches, and docking and scoring provide a powerful and flexible set of computational techniques for virtual screening. In the most effective application, cascades of different steps reduce very rapidly the number of potential ligands of a certain target from hundred thousands or even millions of structures to a manageable size, e.g., by first applying simple filters (molecular weight, polar surface area, number of rotatable bonds, presence or absence of certain structural features, lead-likeness rules, drug-likeness neural nets, Lipinski bioavailability rules), followed by pharmacophore generation and topological or 3D pharmacophore searches. If a 3D structure of the biological target is available from protein crystallography or NMR studies, or can be modeled by homology, the last step is flexible docking, followed by scoring and a careful visual inspection of the obtained results. In this manner, virtual screening became a routine technique in the search for new leads, as illustrated by many successful applications.

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L10

Recent Advances in Orphan Receptor-Based Drug Discovery

W. Childers

Wyeth Research CN-8000 Princeton, NJ 08543-8000, USA

Orphan receptors have enticed researchers for nearly two decades as potential new targets for drug discovery. As biologists and pharmacologists have worked to attach identities to these gene-derived proteins, medicinal chemists have followed closely in their own efforts to provide tools with which to assess the pharmacology and to investigate the therapeutic potential of these novel targets. A number of nuclear receptors have been “de-orphanized” and modulators of these receptors have already entered clinical trials. Success with orphan G-protein coupled receptors has lagged behind that obtained with other proteins, but recent progress has stimulated interest. This presentation will highlight some of the recent successes in orphan receptor-based drug design, with emphasis on orphan G-protein coupled receptors.

L11

Everything a Medicinal Chemist should know about Antibodies

M. Eaton

UCB - Celltech, 216 Bath Road, Slough, Berks, SL1 4EN, UK

Antibodies evolved to be one of the body's front line defences against disease but have also become a major drug class in the last decade. Pivotal to their use as drugs has been the development of humanisation technologies that have avoided issues around immunogenicity that bedevilled their earlier use. As whole antibodies or fragments such drugs utilise their unique targeting abilities plus their inherent effector roles. Recent developments have led to the development of techniques to rapidly produce antibodies with picomolar affinities for their target. Antibodies are also extremely good internalising agents capable of taking nanometre-sized payloads into cells. However they are no longer the only targeting entity being used; such diverse molecules as aptamers and peptides are also finding a role. At this interface the distinction between Novel Chemical Entities and Biologicals becomes less clear.

Increasingly "antibodies" have been functionalised with various chemical entities - polymers, cytotoxins and isotopes to target various diseases. Whilst the concept of the magic bullet is centuries old we are now entering an era where an unparalleled number of such biopharmaceuticals are in the clinic. Later speakers will no doubt add detail to this statement.

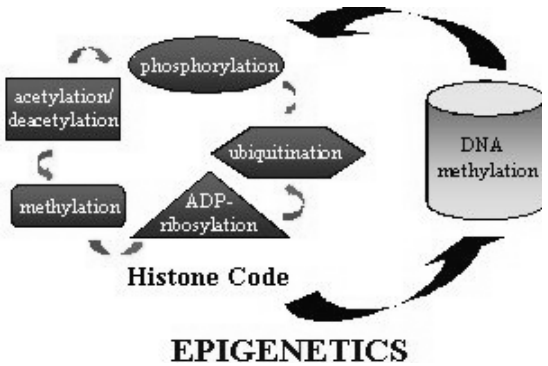
L12

Chromatin modifiers as useful tools for epigenetic therapeutics

A. Mai

Dipartimento Studi Farmaceutici, Università degli Studi di Roma "La Sapienza", 00185 Rome, Italy

Histone deacetylase (HDAC) and histone acetyltransferase (HAT) are key enzymes involved in determining the histone acetylation, which play an important role in epigenetic regulation of gene expression [1]. HAT-promoted acetylation is related with nucleosomal relaxation and gene transcription, HDAC-promoted deacetylation with a tightness of nucleosomal integrity and gene silencing. HDAC inhibitors can reactivate gene expression and result potent inducers of growth arrest, differentiation, or apoptotic cell death in a variety of transformed cells in culture and in tumor bearing animals. In the last ten years, a number of HDACi have been reported as useful tools to study the function of chromatin acetylation/deacetylation and gene expression. Some of them are in clinical trials as anticancer agents. In recent years, we reported various series of hydroxamates as HDACi, from pyrrole derivatives to uracil-based hydroxamates, both unselective and class II HDAC-selective. About sirtuins (class III HDACs), only a few inhibitors have been reported in literature. One of these, sirtinol, pushed us to design and synthesize a series of sirtinol analogues that were tested against yeast Sir2, human SIRT1 and SIRT2, and *in vivo* with a yeast phenotypic screening. Two analogues, namely *meta*- and *para*-sirtinol were 2 to 10 fold more potent than sirtinol against human SIRT1 and SIRT2 enzymes. HATs misregulation is invariably associated to human pathologies such as tumors. We identified MC1626 as a novel, cell permeable Gcn5p inhibitor able to inhibit yeast cell growth, the Gcn5-dependent transcription, and HAT acetylation in vivo. Such compound represents a useful starting point for the further development of new molecules that can be applied to study the expression profile of genes regulated by histone H3 acetylation. About histone methyltransferases (HMTs,) we postulated the hypothesis of the o,o-dibromophenol moiety as pharmacophore group for the design of inhibitors, and a number of such compounds have been synthesized and tested.



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L13

Targeting heterodimeric G protein-coupled receptors as an added dimension in drug design. Opioid receptors as proof of concept

P. Portoghese

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA

The potential biochemical diversity arising from heterodimerization of G protein-coupled receptors (GPCRs) offers the possibility of developing drugs with reduced side effects or different pharmacologic profiles. Opioid receptors fall into this category. To explore this possibility we have investigated the interaction of bivalent ligands with spinal opioid receptors in mice and in cultured cells coexpressed with delta and kappa opioid receptors. A bivalent ligand that contains pharmacologically selective delta and kappa opioid antagonist pharmacophores (KDN-21) was found to selectively target heterodimeric

delta-kappa opioid receptors in cultured cells and in mouse spinal cord and have selectivity characteristics of the delta$_1$ and kappa$_2$ putative subtypes. A significant finding from this study was the apparent localization of delta-kappa heterodimers in the spinal cord but not the brain. This has led to the development of 6'-GNTI, the first spinally selective analgesic that derives its selectivity through targeting of delta-kappa heterodimers. The bivalent ligand approach was also employed to investigate the possibility of mu-delta heterodimers as the functional signaling unit that mediates tolerance and dependence of mu opioid agonists, given mounting evidence for involvement of delta opioid receptors in these adverse effects. Bivalent ligands that contain mu agonist and delta antagonist pharmacophores (MDAN series) that contain specific length spacers were highly potent analgesics and devoid of tolerance and dependence. The advantage of targeting heterodimeric GPCRs as a drug design strategy will be discussed.

L14

Using Drug Metabolism and Pharmacokinetic Data to Generate High Quality Leads

A. Baxter

AstraZeneca Charnwood, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, UK

At AstraZeneca Charnwood Drug Metabolism and PharmacoKinetic (DMPK) data is generated early in the lead generation process. DMPK profiling of hits includes the generation of *in vitro* metabolic stability, solubility and lipophilicity. Metabolite identification is also undertaken and this helps to identify the key areas of weakness in hit series. All this data is only useful if it is understood by the medicinal chemists and used to design new targets with improved properties. Just as important is realising when DMPK properties cannot be easily improved and abandoning series based on this data. One DMPK end-point for lead generation is a set of compounds that have moderate *in vivo* clearance, are orally bioavailable and have understandable clearance mechanisms. Scaling of *in vitro* with *in vivo* data is an important part of this analysis. Various examples will be discussed to illustrate these points.

L15

Enterohepatic Orphan Nuclear Receptors as Targets for Drug Discovery

T. Willson

High Throughput Chemistry, GlaxoSmithKline, 5 Moore Drive Research Triangle Park, NC 27709, USA

Many Orphan Nuclear Receptors (ONRs) are abundantly expressed in the liver and intestine. We have used structure-guided design and high throughput chemistry to generate tool compounds for several of these receptors, namely FXR [1], LXR [2], CAR [3], and LRH1 [4]. These tools have proven useful in dissecting the role of ONRs in regulation of lipid, bile acid and xenobiotic metabolism. The cocrystal structures have also provided insights into the mechanism of small molecule activation of the receptors. I will review the potential of ONRs as targets for discovery of new drugs for metabolic and liver diseases.

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L16

In combo hit and lead profiling: The hybrid wet and web ADME screening strategy

H. van de Waterbeemd

AstraZeneca, DMPK, Mereside, Alderley Park, Macclesfield, SK10 4TG, UK

Modern approaches in chemistry and biology involving the synthesis and high-throughput testing of large libraries of compounds, have led to an increased number of hits against potential targets. It is now widely recognised that early evaluation of ADME (or DMPK) properties and toxicity (T) liabilities is key to the selection of a successful clinical candidate and reducing the risk of attrition during later stage development [1,2].

Much effort went into developing medium to high-throughput screens for *in vitro* measurement of physical chemistry and DMPK properties [3].

ADMET modelling and prediction can be based on data or structure modelling [4]. The latter includes molecular structures of e.g. cytochrome P450 isoenzymes or other enzymes involved in human metabolism, as well as various transporter proteins. Typical molecular modelling techniques also used in activity/affinity modelling are applied. Also based on structure are the predictive tools

for metabolites and site of metabolism. Data modelling is based on building predictive models for any property of relevance to absorption, distribution, metabolism and elimination (ADME). A wide range of techniques known from over 40 years of experiences with quantitative structure-activity relationships (QSAR) are being applied to ADME/Tox modelling. Another group of modelling tools are based on physiological concepts and are used in prediction and simulation of oral absorption, and a range of pharmacokinetic properties in humans.

Extensive *in vitro* (wet) screening appears very expensive. *In silico* (web) screening is more cost efficient and often as predictive. A hybrid approach combining both *in vitro* and *in silico* methods is an appealing compromise. Such *in combo* approach to drug discovery will also heavily rely on a good computational infrastructure.

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L17

Polymorphs, Salts and Solvates: How To Avoid Disaster

T. Laird

Scientific Update, Maycroft Place, Mayfield, East Sussex TN20 6EW, United Kingdom

During these days of fast-tracking the development of new drug substances, the emphasis in early development is usually on the synthetic route and the impurities in the drug substance and on making the first kilogram. This talk will emphasise that it is important to consider the salt form of the new drug and to screen at an early stage for polymorphs and solvates, particularly hydrates, which can affect the solubility and bioavailability of the new substance. The decision on which form to progress in development is crucial and can affect the subsequent development, scale up and large scale manufacture, as well as the formulation and stability of the drug substance or product. Issues such as hygroscopicity, which can be relatively unimportant in the initial stages, may have profound effects later in the development cycle.

The increasing molecular complexity of new drugs and particularly the introduction of aryl-aryl (or heteroaryl) bonds (eg via Suzuki coupling) often makes crystallisation more difficult and may lead to the material being initially amorphous. It is often advisable to try, early in development, to obtain a crystalline form since this enables the drug to be purified easily and poorer quality batches to be upgraded.

The molecular complexity may enable the molecule in the solid state to exist in a number of conformations of closely similar energy, resulting in a large number of polymorphs. Several new drugs have more than 20 different forms, though it is often said that the number of forms depends on how long and how rigorously you screen.

The multiplicity of solid forms also can be an issue for IP. Characterisation of new forms relies on, not only traditional methods such as X-ray crystallography and Solid State NMR, but on newer techniques which will be briefly mentioned. Case studies and some entertaining horror stories from published papers or, more often, from unpublished work presented at Scientific Update conferences will be provided. Company names may be withheld to protect the guilty.

L18

Membrane protein function in a structural context: Computational modeling integrates ligand-dependent activation with protein interactions in cell signaling mechanisms

H. Weinstein

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

Advances in understanding dynamic mechanisms in

A)-signaling by G protein-coupled receptors (GPCRs), and

B)-the function of neurotransmitter transporters (NTs - especially SERT and DAT) will be presented in the context of novel approaches to drug design.

The findings from our multi-authored collaborative approach combining computation and experiment revealed ligand-specific modes for activation of GPCRs, distinct ligand-dependent receptor states, and determinants for ligand binding specificity in the NTs. Two topics in ligand-dependent signaling mechanisms related to the design of ligands that modify GPCR or and NT activity, will be discussed in mechanistic detail:

Topic (1): Dynamics of GPCR oligomerization interfaces - Function-related rearrangements at the interface of GPCR homodimers were mapped with an approach in which molecular modeling and bioinformatics served to identify the putative interface, and crosslinking of substituted cysteines along the entire length of the TM segment tested the predictions. Results revealed the changes in the interface upon transition from inactive-to-active forms of the GPCR, identifying conformational change as a critical part of the receptor activation mechanism, and thus new opportunities for selective ligand design.

Topic (2): Interactions of GPCRs and NTs with scaffolding proteins - Specifically:

- ligand-dependent regulation of the interaction of GPCRs with PDZ domains,
- the basis for PDZ biding specificity in the context of the interaction of the PICK-1 PDZ domain with the dopamine transporter (DAT),
- and computational simulations of PDZ-ligand complexes.

We identify mechanisms that integrate the membrane proteins in the signaling cascade through activation-dependent interaction with PDZ domains, and the selectivity of PDZ domain recognition, as putative novel targets for drug design.

L19

Tyrosine Kinases: Tracking and hitting a moving target

M. Eck

Dana-Farber Cancer Institute and Harvard Medical School, 44 Binney Street Boston, MA 02115, USA

Although tyrosine kinases use a well-conserved active site and catalytic mechanism to carry out the phosphotransfer reaction, they have evolved distinct mechanisms of regulation to allow their integration into various biological pathways. Regulation typically involves “deconstruction” of the active site by deformation of one or more moving parts in the active site, in particular the C-helix and the kinase activation loop. These inactivating deformations are produced by the binding interactions of other proteins or of adjacent domains or loops within the kinase. Not surprisingly, these deformations can greatly affect the shape of the ATP binding cleft and are therefore a critically important consideration in the design and development of small molecule tyrosine kinase inhibitors. The nature of these conformational changes will be reviewed, and as will their effect on inhibitor binding in the context of Abl and other tyrosine kinase targets. A second type of “movement” in tyrosine kinase domains is mutational – treatment with small molecule TKIs is complicated by the emergence of drug resistant mutants in both hematologic and solid tumors. Additionally, in non-small cell lung cancer, diverse mutations in the EGFR kinase have been observed to underlie tumorigenesis. These diverse mutations can be expected to alter the structure of the active site in a manner that affects inhibitor binding. Our recent work with the mutant EGFR in complex with both reversible and irreversible TKIs will be used to illustrate the importance of this issue and the likely need for mutant-specific inhibitors of the EGFR kinase in lung cancer.

L20

New Strategies in Prodrug Design: Effective prodrug strategies to improve oral bioavailability

B. Clement

Department of Pharmaceutical Chemistry, Pharmaceutical Institute, Christian-Albrechts-University Kiel, Gutenbergstr. 76, 24118 Kiel, Germany

Prodrugs are an established concept to overcome barriers to a drug´s usefulness. In general about 10% of all marketed medicines can be classified as prodrugs, an estimate based on a conservative prodrug definition that does not include soft drugs and limited prodrugs. The latter are defined as active agents whose metabolite(s) also contribute(s) to the observed therapeutic activity. Approximately 50% of all marketed prodrugs are activated by hydrolysis and about 30% by a biosynthetic reaction. Noteworthy blockbuster prodrugs are for example omeprazole, simvastatin, lovastatin, enalapril and acyclovir [1].

The early phases of lead discovery and optimization rely extensively on the design and synthesis of analogues (the „analoguing“ strategy). However, situations exist where the SAR for the drug target is incompatible with pharmacokinetic objectives due to irreconcilable structural prerequisites. In such cases, a prodrug strategy can offer a viable alternative and should be consulted early. The present lecture intends to provide in the first part a broad overview of successful applications of prodrug strategies in drug discovery concentrating mainly to improve oral bioavailability. Marketed prodrugs are used as examples [1].

In the second part of the lectures amidoxime (*N*-hydroxyamidine) prodrugs will be presented in greater detail. Amidines and guanidines are functional groups, which are very often incorporated into active molecules because the cations formed after protonation are necessary for interactions with the negatively charged carboxylates of target molecules. Very often, benzamidines are imitating the guanidine functional group of arginine. So amidines can be found in various drug candidates.

Amidines like guanidines are protonated at the double bonded nitrogen atom and thus form a highly mesomerically stabilized cation. They are the strongest known organic bases, protonated under physiological conditions, very hydrophilic, usually not absorbed from the gastrointestinal tract, and thus not orally available. The *N*-hydroxylated derivatives (amidoximes, *N*-hydroxyguanidines) are less basic because of the introduction of the oxygen atom. They are not protonated under physiological conditions and lead to sufficient oral absorption and therefore to improved bioavailability.

Because of these properties and the intensive reduction of benzamidoximes, the prodrug principle benzamidoximes instead of benzamidines was developed in my laboratory [for a review see 2]. The principle has been applied to a lot of drug candidates in particular for the optimisation of antithrombotics [for a review see 3].

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L21

PTP1B Inhibitors: Progress and Pitfalls

S. Taylor

Dept. of Chemistry, University of Waterloo, Waterloo, Ontario, Canada

Type II diabetes and obesity are characterized by insulin and leptin resistance. Studies suggest that insulin and leptin resistance may be due to defects in the insulin and leptin signaling pathways**.** Over the last decade a considerable body of evidence has been amassed that indicates that PTP1B is involved in the down regulation of insulin and leptin signaling. Consequently, compounds that inhibit PTP1B have potential as therapeutics for treating type II diabetes and obesity. Although many potent inhibitors of PTP1B have been developed by both academia and industry, their development into effective orally bioavailable drugs for treating diabetes and/or obesity has yet to be realized. The challenges in developing PTP1B inhibitors into therapeutics will be discussed. These challenges will be illustrated by an overview of our own efforts as well as others to develop potent and selective PTP1B inhibitors.

L22

Understanding hits and decoys in molecular docking

R. Brenk, N. Huang, S. Boyce, J. Irwin, B. Shoichet

Dept. of Pharmaceutical Chemistry, University of California San Francisco, USA

Molecular docking is widely used to screen compound collections for novel leads. Because of approximations in docking scoring functions and under-sampling of configurations, many docking predictions prove false; even more perniciously, they are almost impossible to understand because of the entangled approximations. We are taking two strategies to investigate this problem. In the first, we have turned to simple, small buried cavities where the interactions are dominated by one particular term. Thus, the L99A cavity in T4 lysozyme is dominated by non-polar complementarity, the L99A/M102Q cavity in T4 lysozyme has a single hydrogen bond acceptor, and the W191G cavity in cytochrome C peroxidase is dominated by a single ionic interaction. The simplicity of these sites makes mis-predicted ligands and geometries as informative as correct predictions. We are using experimental testing of predicted binding, geometry, and protein motion, using crystallography in a cycle of theory development and experimental testing. In a second strategy, we are developing a large database of good "decoy" molecules as a background for evaluating enrichments when docking to a large number of druggable binding sites. We find that the quality of the decoy molecules in the database is a critical factor when judging docking results.

L23

COX-2 and beyond; modulation of enzymes and receptors in the arachidonic acid cascade

G. Fitzgerald

Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia PA 19104, USA

Evidence from 5 placebo controlled trials of 3 structurally distinct compounds – rofecoxib, celecoxib and valdecoxib - has established that selective inhibitors of prostaglandin G/H synthase (COX) – 2 confer a small, but absolute risk of cardiovascular disease. Mechanistically, this is compatible with suppression of COX-2 derived prostacyclin (PGI_2), which acts as a constraint on endogenous modulators of thrombogenesis, hypertension and vascular remodeling, atherogenesis and cardiac function. Emergence of such a hazard is conditioned by drug exposure – dose, duration of action and of dosing - selectivity for inhibition of COX-2, the underlying risk of cardiovascular disease and concomitant therapies. Substantial interindividual differences in response to COX-2 inhibitors exist in humans with roughly 30% of the variance attributable to genetics. Strategies might be pursued to identify individual determinants of both efficacy and risk with these compounds. While both COX-1 and COX-2 exist as dimmers, we have recently shown that the two enzymes might heterodimerize which may also have implications for drug therapy. Deletion of microsomal PGE synthase (mPGES) – 1 is effective in models of pain and inflammation. However, unlike inhibition or disruption of COX-2, deletion of mPGES-1 does not result in a predisposition to thrombosis or hypertension. Indeed, atherogenesis is retarded in LDL receptor knock out mice in which mPGES1 is deleted. These cardiovascular effects may reflect – at least in part-substrate rediversion to PGI_2 . Strategies such as this and targeted blockade of discrete prostanoid receptors may permit expression of therapeutic efficacy while limiting adverse outcomes observed with inhibitors directed at targets high in the biosynthetic cascade.

L24

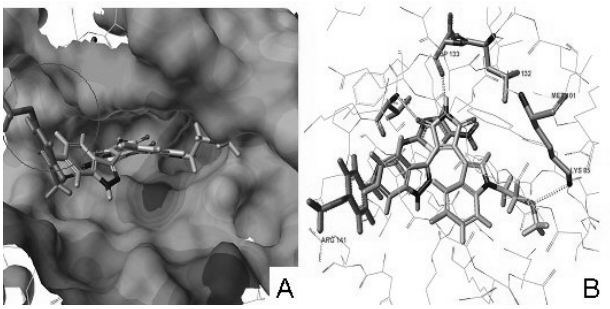
Discovering New Therapies for Cancer, Infectious Diseases, and CNS Disorders - Taking Hints from Nature

A. Kozikowski

Drug Discovery Program Univ. of Illinois at Chicago Dept. of Medicinal Chemistry and Pharmacognosy, Chicago, IL, USA

In this lecture I shall review the group's research activities aimed at the design, synthesis, and biology of novel ligands that target certain enzyme, receptor, or transporter systems relevant to both normal brain activity as well as to certain disease states such as Alzheimer's disease, Parkinson's disease, bipolar disorder, depression, and drug abuse. These collaborative research efforts

combine the tools of molecular modeling, chemical synthesis, biochemistry, pharmacology, and behavioral studies. In the majority of our chemical undertakings, natural products have served as the lead candidates in our quest to identify new pharmacological research tools as well as possible CNS therapeutics. In particular, my lecture shall focus on the natural products huperzine, cytisine, teleocidin, cocaine, and staurosporine, all of which have been modified in order to transform them to novel CNS active agents.



L25

Biomimetic modulation of kinases as an approach to inhibition by small molecules

D. Flynn

Deciphera Pharmaceuticals LLC, 4950 Research Park Way, Lawrence, KS 66047, USA

Currently, the pharma industry and academia focus most of their efforts on developing small molecule inhibitors of kinases by targeting the ATP cofactor pocket. After more than fifteen years of global R&D dedicated to mining the ATP pocket, only a few such inhibitors have made it to the marketplace. In searching for a general approach to kinase modulation that not does utilize the ATP pocket, we have targeted the endogenous switch control pockets that exist in kinases. These pockets are utilized by kinases to regulate overall shape and catalytic activity *in vivo*. This lecture will highlight the attributes of targeting kinase switch control pockets by demonstrating its application to bcr-abl kinase and B-raf kinase. The bcr-abl program has afforded potent inhibitors of this oncogenic kinase that inhibit the unphosphorylated form, phosphorylated forms, and the T315I mutant clinical isolate form that is resistant to gleevec and dasatinib. This general approach has afforded small molecules with unprecedented selectivity over off-target kinases, due in large measure to the heterogeneous populations of switch control pockets in the kinome as compared to the relatively conserved ATP pocket.

L26

Ionotropic Neurotransmitter Receptors as Targets for Rational Drug Design

M.L. Mayer

Laboratory of cellular and Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 210892 USA

Glutamate receptor ion channels mediate fast synaptic transmission in the mammalian central nervous system. Numerous subtypes have evolved with different ligand selectivity, kinetics, ion permeability, expression patterns and regulation by second messengers. The three major families, named AMPA, kainate and NMDA receptors were initially identified on the basis of their ligand binding properties. Subsequently the cDNAs for mammalian 18 genes were cloned, and the domain organization of the receptors determined by biochemical techniques. The key role these receptors play in normal function and disease has not yet been exploited by medicinal chemists to develop novel ligands with therapeutic properties. In part this stems from two factors: 1st because the receptors are so widely expressed in the CNS, where they mediate a vast range of synaptic activity, antagonists which shut down their activity have profound side effects; 2nd the available ligands have until very recently lacked subtype selectivity, and thus have too broad an effect to the useful either as drugs or experimental tools. This situation is now changing as a result of efforts in structural biology. During the past 5 years the ligand binding domains for GluR2, GluR5, GluR6, NR1, NR2A and NR3A have been crystallized with a range of agonists and antagonists, and currently the PDB contains > 60 structures, a large number at resolutions of better than 2 Angstrom. Several features emerge from these crystallographic studies. Glutamate binds in a cleft located between two domains, and in the case of agonists triggers a large conformational change. Competitive antagonists act via a foot in the door mechanism and either trap the receptor in its resting state, or produce too small a conformational change to trigger ion channel gating. In the glutamate, or in the case of NR1 and NR3 subtypes glycine, bound states for the majority of structures the domains close sufficiently to trap the ligand in a cavity, from which it cannot escape until the cleft opens. The volume of the cavity differs substantially between receptor subtypes and contains a variable number of trapped water molecules. Displacement of these by synthetic ligands provides medicinal chemists with the opportunity to design novel ligands. Although the availability of the crystal structure for the GluR2 AMPA receptor subunit in 1998 immediately suggested that with homology modeling its should be possible to begin rationale drug design for individual receptor subtypes a difficulty arose when additional crystal structures were solved. These revealed that the conformational change induced by both agonists and partial agonists, as well as competitive antagonists, that is the extent of domain closure, differed between receptor subtypes, as well as between ligands. However, as the range of crystal structures continues to grow the increasing number of templates available for modeling and drug design suggests that the time is now ripe to try and develop more selective ligands, especially for receptor sub-

types, such as the NR2 and NR3 families, and GluR6 for which selective agonist and antagonists are sorely lacking. Also of note, the crystal structures of GluR2 revealed for the 1st time the binding site for allosteric modulators and explained their mechanism of action. Currently such ligands are only widely available for AMPA receptors, but the available crystal structures now provide the necessary templates to design ligands for other receptor families. Finally, from an experimental perspective, the availability of crystal structures provides the opportunity for rational protein engineering, and the fruits of this are just staring to emerge.

L27

Chemical Methods for Tracing Protein Kinase Cascades

K. Shokat

Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 600 16th Street, Box 2280, San Francisco, CA 94143-2280, USA

Our laboratory focuses on the development of novel chemically based tools to decipher signal transduction pathways on a genome-wide scale. We have developed a method for producing small molecules that are specific for any protein kinase of interest in a signaling cascade by combining protein design with chemical synthesis. These highly specific inhibitors of individual kinases have revealed a number of new principles of signal transduction that have complemented genetic and biochemical studies of cell signaling. Examples where new pathways and new functions can be revealed by small molecule inhibitors of protein kinases will be highlighted. A second area of interest in our laboratory is the tracing of direct kinase substrates. We have designed and synthesized unnatural ATP analogs which are substrates of our engineered kinases but are poorly accepted as substrates of wild-type kinases. This specific nucleotide substrate of any kinase of interest allows for the radiolabelling of the direct substrates of a wide variety of protein kinases including both serine/threonine and tyrosine kinases. New methods for the isolation and identification of low abundance substrates of kinases from cells will be discussed. Once a phosphoprotein substrate of a kinase is identified, the specific phosphorylation site is often difficult to identify using traditional tryptic peptide phosphorylation site mapping. Using a novel strategy based on the design of tailor made proteases which specifically cleave proteins after sites of phosphorylation, we have developed a rapid means to map protein phosphorylation patterns. Finally, a potential link between the unnatural ligands of engineered kinases and a set of plant hormones, the cytokinins, will be discussed in the context of a custom designed database created for the genome wide analysis of protein kinase catalytic domains.

L28

Targeting trypanosomatids

K. Augustyns

University of Antwerp, Department of Medicinal Chemistry, Universiteitsplein 1, B-2610 Antwerpen, Belgium

Parasitic diseases continue to take an enormous toll on human health, particularly in tropical regions. The major burden is caused by helminths and protozoa. Among the latter the family of trypanosomatids is responsible for important diseases such as leishmaniasis (*Leishmania* spp.), African trypanosomiasis (*Trypanosoma brucei gambiense*, *T.b. rhodesiense*) and Chagas' disease (*Trypanosoma cruzi*). The drugs used to treat these diseases are far from ideal, due to problems with resistance, toxicity, cost and length of treatment. Therefore, there is an urgent need to discover new effective and save drugs for the treatment of these diseases.

Several drug discovery and development projects will be reviewed. Points to consider in selecting parasite molecular targets will be emphasized (selectivity, validation, biochemical properties, druggability, assays). The recent completion of the genomes of *L. major, T. brucei* and *T. cruzi* is an important development in this field. Hit and lead identification and lead optimization for several of these targets will be discussed.

The absence of *de novo* purine biosynthesis in trypanosomatids and their complete dependence on purine salvage pathways reveal several interesting target enzymes. One of these enzymes is nucleoside hydrolase, responsible for the hydrolysis of the glycosidic bond of purine nucleosides. Other interesting potential targets can be found in the metabolism of trypanothione, polyamines, glucose, lipids, sterol, proteins and folate. The importance of recent developments in these fields will be reviewed.

L29

Finding New Drug Targets in the 21st Century

M. Lindsay

Biopharmaceutics Research Group, Airways Disease, National Heart and Lung Institute, Imperial College, Dovehouse Street, London SW3 6LY, UK

The past 30 years have witnessed a steady decline in the number of novel drug targets. This presentation will concentrate upon the initial process of target identification and argue that current problems have resulted from a decrease in clinical research, an overemphasis on the discovery of new targets through an understanding of the molecular causes of disease and the adoption of cell and animal models that are poor predictors of human disease. To resolve this situation, the presentation will argue that an intervention at the physiological level, using drugs to target the extracellular signalling pathways, will facilitate

identification of novel drug targets in the 21st century. The various strategies that can be used to identify these novels targets will be discussed, as well as the recent emergence of biopharmaceutics including recombinant proteins, monoclonal antibodies and the utilisation of the RNA interference pathway.

L30

Inhibiting MDR pumps from gram-negative bacteria: lessons and perspectives

O. Lomovskaya

Mpex Pharmaceuticals. , Inc San Diego, CA, USA

Multidrug resistance (MDR) efflux pumps play a prominent role in intrinsic and acquired antibiotic resistance in gram-negative bacteria. It makes efflux pumps attractive targets for inhibition. It is expected that the resultant efflux pump inhibitor (EPI)/antibiotic combination drug should exhibit increased potency, enhanced spectrum of activity and reduced propensity for acquired resistance. To date, several classes of broad-spectrum and narrow-spectrum EPIs have been extensively characterized. While these efforts indicated a significant potential for developing small molecule inhibitors against efflux pumps, they did not result in a clinically useful compound. Stemming from the continued clinical pressure for novel approaches to combat drug resistant bacterial infections, second-generation programs have been initiated based on a number of recent developments in the field, including structural elucidation of all three individual components of MDR efflux pumps and ligand-based insights into the mechanism-of-action of drug transporters. These new approaches show early promise to significantly improve the clinical usefulness of currently available and future antibiotics against otherwise recalcitrant gram-negative infections.

L31

In silico methods for prediction of P-glycoprotein substrates

B. Zdrazil, S. Schindler, G. Ecker

Emerging Focus Pharmacoinformatics, Department of Medicinal Chemistry, University of Vienna, A-1090 Wien, Austria

The ABC (ATP binding cassette) family of polyspecific membrane transport proteins includes the best-known modulators of the activity of anticancer drugs: ABCB1 (P-gp, MDR1), ABCC1 (MRP1) and ABCG2 (BCRP, MXR). Additionally, ABCB1 is responsible for bad absorption properties of drugs in the gastrointestine as well as improper permeation of the blood-brain barrier. Although considerable efforts have been undertaken to establish *in silico* tools for predicting drug-protein interactions of such multispecific targets, especially in the field of ABC pumps general applicable models are still rare.

With the increasing knowledge on the physiological role of P-glycoprotein for bioavailability and brain uptake of drugs the focus of interest changed from design of inhibitors to prediction of substrate properties. This primarily involves the areas of anticancer agents and cns-active compounds. Due to the broad substrate specificity and fuzzy SAR-pattern, only a few models for P-gp substrate prediction have been published so far. They rely on support vector machines, decision tree analysis or simple filter rules. We used the VSA-descriptors developed by Labute to establish predictive models for a set of 431 structurally and functionally diverse P-gp substrates and non-substrates. The data set included 263 compounds taken from the literature and 168 derived from the NCI60 screen. VSA-descriptors are calculated on basis of the 2D-topology and represent the contribution of logP, molar refractivity and partial charge to the van der Waals surface in a binned form. Binary QSAR as implemented in MOE (Chemical Computing Group) gave models with a cross-validated accuracy of up to 0.81 Due to the high speed of VSA-descriptor calculation, this might be a versatile model for high throughput *in silico* filtering of large compound libraries.

L32

Mechanism of action of allosteric modulators of mGlu receptors

J. P. Pin, C. Goudet, J. Kniazeff, L. Prezeau

Department of Molecular Pharmacology, Institut of Functional Genomics CNRS, INSERM, University of Montpellier 1&2 34094 Montpellier, France

MGluRs are key regulators of synaptic transmission. Multiple theraputique applications are expected for compounds regulating mGluR activity. Unlike most other GPCRs, mGluRs possess a large extracellular domain where agonists bind, and are constitutive dimers linked by a disulphide bridge. The complex structure of these receptors offers multiple possibilities to regulate their activity. High-throughput screening strategies allowed the identification of highly selective compounds acting at a site different from the agonist binding site. Such compounds include non-competitive antagonists that have inverse agonist properties, and positive allosteric modulators that can increase both the efficacy and potency of agonists. These compounds bind in the heptahelical domain of mGluRs. The activity of such compounds was examined on receptors truncated of their large extracellular domain. Non-competitive antagonists were found to retain their inverse agonist property, but the positive allosteric regulators were converted into full agonists. This demonstrates that, like rhodopsin-like GPCRs, the HD of mGluRs can be inhibited and activated by synthetic ligands. Two non-competitive antagonists per dimer are required to block receptor activity, whereas a single positive modulator is sufficient. These data support the idea that in such dimeric receptors, a single HD is turned on at

a time. A model for the activation process and its regulation by allosteric regulators will be presented.

L33

A Superior Antipsychotic: Rational Design or Irrational Dream?

A. Payne

GlaxoSmithKline, UK

A detailed analysis of the *in vitro* properties of current antipsychotic drugs reveals some very promiscuous receptor interaction profiles. Many of these properties are thought to be unnecessary for an antipsychotic action, and contribute to a range of side-effects. Furthermore, it is conceivable that some of these receptor interactions could mask or detract from the desired antipsychotic action, which in many cases has been found to be sub-optimal. A review of the medicinal chemistry literature indicates that all of the current atypical antipsychotic agents have evolved from a D_2 receptor *in vitro* and *in vivo* screening approach, and have not been discovered by targeted design based on a specific and selective receptor profile. Indeed, the core structural motif of all existing antipsychotic drugs can easily be recognised as originating from a promiscuous chemical series, and not from *de novo* design. Schizophrenia is probably the only therapeutic area in modern drug discovery which, perhaps because of its unique complexity, has not yet benefited from rational targeted drug design. In response to this challenge, we have identified five key receptors for specific and selective antagonism (D_2, D_3, $5HT_{2A}$, $5HT_{2C}$, $5HT_6$) for the treatment of schizophrenia. SAR data will be provided around one lead series to illustrate the challenges in targeting affinities at multiple receptors. From this study we have identified an advanced lead compound, which possesses the desired receptor fingerprint along with a promising *in vivo* profile, as a prototype for a new generation of antipsychotic agents.

L34

The Development of PR-104: a Hypoxia-Activated Prodrug for Cancer Therapy

W. Denny, G. Atwell, A. Patterson, W. Wilson, S. Yang

Auckland Cancer Society Research Centre, University of Auckland, New Zealand

The low oxygen levels (hypoxia) caused by the poor blood supply in solid tumours is a physiological difference that distinguishes them from normal tissue. Such hypoxic tumour cells are resistant to conventional therapy, and occur to a significant extent in most human solid tumours. This has given rise to the concept of hypoxia-activated prodrugs, where relatively non-toxic prodrugs are designed to be selectively activated in hypoxic tumour cells, by oxygen-inhibited bioreduction. The resulting cytotoxic active drug then undergoes limited redistribution within the tumour (the “bystander effect”), exploiting the small proportion of hypoxic cells for enhanced cell killing across the tumour. The requirement for both the non-toxic prodrug and the cytotoxic active form of the drug to efficiently diffuse in tissue places special restraints on the design of such compounds. Techniques were developed to quantitatively measure these diffusion properties, which were used to guide drug development in the dinitrobenzamide mustard class of prodrugs. A subset of these compounds were recently identified as having high activity against hypoxic cells in human tumour xenografts, and the analogue PR-104 has just entered clinical trial. The development of this class of novel anticancer drugs, and their biological properties, will be discussed.

L35

Cancer nanotechnology: nanoparticles of biodegradable polymers for new-concept chemotherapy

S. Feng

Department of Chemical & Biomolecular Engineering and Division of Bioengineering,National University of Singapore

Cancer is a leading cause of deaths and has become the #1 killer in many countries including Singapore. Nevertheless, no substantial progress can be observed in the past 50 years in fighting against cancer. The cancer death rate in US was 1.939‰ of the total population in 1950 and still 1.940‰ in 2001. Cancer nanotechnology will radically change the very foundations of cancer diagnosis, treatment and prevention. The current regimen of chemotherapy is far from being satisfactory. Its efficacy is limited and patients have to suffer from severe side effects. This presentation will demonstrate through a full spectrum of proof-of-concept research how nanoparticle technology could provide an ideal solution and with further development, promote a new concept of chemotherapy, which may include sustained, controlled and targeted chemotherapy; personalized chemotherapy; chemotherapy across various physiological drug barriers; and eventually, chemotherapy at home. Paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles were prepared by a modified solvent extraction/evaporation technique with vitamin E TPGS as emulsifier. Drug-loaded nanoparticles were characterized by various state-of-the-art techniques, including laser light scattering for size and size distribution, scanning electron spectroscopy (SEM) and atomic force microscopy (AFM) for surface morphology, X-ray photoelectron spectroscopy (XPS) and Fourier transformation infrared spectroscopy (FTIR) for surface chemistry, and zeta-potential for surface charge. Drug encapsulation efficiency and in vitro drug release profile were measured by high performance liquid chromatography (HPLC). The cellular uptake of fluorescent nanoparticles was imaged by confocal laser scanning microscopy (CLSM) with Caco-2 cells employed

as an in vitro gastrointestinal drug barrier model for oral chemotherapy. The results were found strongly dependent on particle size and particle surface coating. In vitro HT-29 cell viability experiment demonstrated that the paclitaxel formulated in the nanoparticles was 5.64 times more effective than that for Taxol® after 24 hours of treatment, which should be even better with the sustainable release feature considered. In vivo pharmacokinetics measurements confirmed the advantages of the nanoparticle formulation versus Taxol®. The plasma concentration of the nanoparticle formulation had an area-under-the-curve (AUC) value comparable with that of Taxol®, but never exceeded the maximum tolerance level and hence should have much lesser side effects than Taxol®, for which 28.8% AUC was associated with side effects. The nanoparticle formulation had a sustainable therapeutic time of 168 hours (7 days) in comparison with 22 hours for Taxol® and achieved four times greater drug tolerance than Taxol®.

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L36

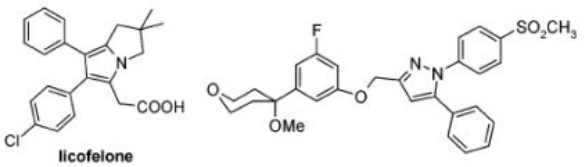
Dual inhibition of 5-LOX and COXs as an alternative to classical NSAIDs - Structural insights into human 5-LOX inhibition

C. Charlier

Laboratory of Structural Biological Chemistry, Faculty of Sciences, University of Namur (F. U. N. D. P.), Rue de Bruxelles 61, 5000 Namur, Belgium

Non steroidal anti-inflammatory drugs (NSAIDs) represent a choice treatment in inflammatory diseases such as rheumatoid arthritis. However, their use is often limited by the adverse effects they cause, particularly gastric injury and gastric ulceration, renal failure and asthma. Therefore, several new strategies have been considered, and notably the simultaneous inhibition of COXs and 5-LOX. [1] This approach constitutes an interesting alternative to classical NSAIDs to provide safer anti-inflammatory agents. Indeed, by inhibiting both major arachidonic acid pathways, these dual compounds may lead to an enhanced anti-inflammatory activity accompanied by reduced gastric and allergic side effects. Licofelone is the first molecule of this new class in the most advanced stage of development and preliminary data seem promising. Furthermore, as 5-LOX and COX-2 have been shown to be involved in cellular proliferation processes, the design of dual inhibitors also opens up new perspectives in the treatment of several types of cancer. [2]

Whereas the COX pathway has already been extensively studied, little structural or mechanistic information is available regarding human 5-LOX. Therefore, we focussed on this enzyme and characterized its 3D structure as well as its interaction with non redox inhibitors, in order to help the design of dual 5-LOX/COXs inhibitors. [3]



Structure of dual 5-LOX/COX inhibitors

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L37

Cannabinoid and glutamate receptors in pain modulation

E. Borsani, R. Lanzi, R. Rezzani, R. Bianchi, L. F. Rodella

Unit of Human Anatomy, Department of Biomedical Sciences and Biotechnologies, University of Brescia, Brescia, Italy

Although pain perception is thought to be controlled mainly by neurotransmitter systems that operate within the central nervous system, anti-nociceptive mechanisms also occur in peripheral tissues. The potent analgesic effects of cannabis-like drugs and the presence of cannabinoid receptors in pain-processing areas of the brain and spinal cord indicate that endogenous cannabinoids such as anandamide may contribute to the modulation of pain transmission. Mammalian tissues contain at least two types of cannabinoid receptors, cannabinoid CB_1 receptor and cannabinoid CB_2 receptor, both coupled to G proteins. Cannabinoid CB_1 receptors are expressed mainly by neurones of the central and peripheral nervous system whereas CB_2 receptors occur centrally and peripherally in some non-neuronal tissues, particularly in immune cells and it has been shown their involvement both in acute and in chronic pain.

On the other hand the glutamate is the transmitter of the vast majority of the fast excitatory synapses in the mammalian central nervous system with two kinds of receptors: ionotropic and metabotropic receptors expressed in relevant areas of the brain, spinal cord and periphery involved in pain sensation and transmission. In the last years the role of metabotropic glutamate recep-

tors (mGluRs) has been focused; they are preferentially involved in transmitting noxious sensory information in the thalamus and may be involved in induction of long-term enhancement of responses to noxious stimuli in the spinal cord. Selective antagonists for the mGluRs subtypes that interact with transmission of noxious sensory information could be useful in the treatment of acute and chronic pain.

The state of the art of cannabinoid and glutamate receptors in pain control and particularly the potential role of their synergic modulation will be discussed.

L38

The quest for novel mechanism of antibacterial agents

D. Payne

Department Microbiology, MMPD CEDD, GlaxoSmithKline, South, Collegeville Rd, Collegeville, PA19426, USA

The need for novel mechanism antibacterials has now become a global healthcare concern. In the mid 1990s many large pharmaceutical and biotechnology companies set up genomic-based antibacterial programmes believing that genomics would fuel a flow of targets that could be screened to rapidly identify new classes of antibiotics. Genomic exploitation was spectacularly successful in delivering hundreds of novel antibacterial strategies such that a review of the literature between 1995-2004 reveals >35 different companies ran >120 screens on >60 different antibacterial targets. However, the number of novel acting antibiotics currently in the industry pipeline is concerningly small. A number of factors have contributed to this situation, firstly, the challenging commercial and regulatory environment for new antibacterials has led to many companies withdrawing from the area and secondly there are some substantial and unique scientific challenges that have compromised the success of the 'genome to antibiotic paradigm'. This presentation will review the genomics program we put in place between 1995 -2001 and how it successfully identified and validated >160 novel antibacterial targets and led to 70 high through put screens (HTS) on antibacterial strategies. However, the number of screens that resulted in hits and leads was surprising small. Furthermore, even when leads were identified, optimizing such molecules to antibacterial development candidates with all the necessary microbiology and drug developability properties is a high hurdle and very resource intensive. Consequently, the changes we have put in place to tackle novel antibacterial discovery will be illustrated along with how these approaches are helping to grow a portfolio of novel mechanism antibiotics.

L39

The Discovery of Novel Antibacterial Agents; the Challenges and the Opportunities

J. Primeau

AstraZeneca R&D Boston, USA

A key strategy, used to discover and develop antibacterial agents that will address the growing challenge of bacterial resistance, has focused on the design of ligands for new or under-exploited bacterial targets. The application of technologies such as HTS, X-ray crystallography and NMR screening has provided the opportunity of new lead scaffolds for these targets. As these new leads are optimized into potential drug agents, a key challenge of managing off-target activity must be addressed. Using case studies, this presentation will describe how structure-based design tools have been used to improve on-target potency and blunt off-target "side-effect" activity.

L40

TINS: New Opportunities for Fragment Based Drug Discovery

G. Siegal

Leiden Institute of Chemistry, Leiden University and ZoBio BV, The Netherlands

Fragment based drug discovery (FBDD) is gaining increased attention because it generates "lead like" compounds and has been successfully applied to challenging targets such as protein-protein interactions. However, FBDD is generally only applicable to proteins that are available in large quantities (100's of mg) that are soluble or can be solubilised.

We have developed a method we call TINS, for target immobilized NMR screening, that is applicable to targets that are not possible to obtain in large quantities and/or are insoluble such as integral membrane proteins. In TINS, the target is immobilized on a solid support. The mixture of compounds to be tested for binding (up to 10 at a time) is pumped over the support and binding is detected by 1D ^{1}H NMR spectroscopy of the ligands. Binding to the immobilized target results in a simple reduction in peak amplitude, which is conveniently detected by comparison with a control sample. We have shown that more than 2,000 compounds can be applied to a single sample of the target with no effect on ligand binding, thereby opening the way to screening an entire fragment library using a single sample of the target.

A dual-cell sample holder and an 8 mm, triple gradient probe have been developed to enable TINS in flow-injection mode. Using this hardware and an optimized fragment library, screening can be carried out in an automated manner using roughly 5 mg of the target and a reference protein. The TINS screening system has been successfully applied to an increasing number of targets. The hardware yields reliable and readily interpretable results. Here we will report our latest results using the instrument for early stage drug discovery, including initial attempts to apply TINS to membrane proteins, one of the more challenging pharmaceutical targets.

L41

Simultaneous screening and chemical characterization of bioactive compounds using LC-MS based technologies

H. Irth

Vrije Universiteit Amsterdam, Department of Analytical Chemistry and Applied Spectroscopy, De Boelelaan 1083, 1081 HV Amsterdam, (The Netherlands)

LC-MS is a widely used tool in the chemical analysis of target compound relevant to environmental chemistry. The use of LC-MS in high-throughput screening (HTS), i.e., the biological characterization of analytes, is far less common: modern HTS platforms rely mainly on fluorescence detection as readout for biochemical assays. The use of different technology platforms for the chemical and biochemical characterization of bioactive compounds requires sophisticated logistics and is, therefore, costly and time consuming.

The present lecture gives an overview on our attempts to develop analytical screening technologies, where LC-MS is used to measure simultaneously the chemical and biochemical characteristics of bioactive compounds. MS-based biochemical assays are equivalent to fluorescence HTS assays by using appropriate reporter molecules and enzyme substrates for the development of receptor-ligand binding and enzyme inhibition assays. Electrospray MS is used to detect concentration changes of these reporter molecules upon interaction with receptors or enzymes and consequently allows the detection of (unknown) active ligands. Next to the biochemical readout, molecular mass information on the active compound is generated simultaneously and allows the rapid identification of the chemical species involved

The presentation will focus on both ligand binding and substrate conversion assays using nuclear receptors, proteases and acetylcholinesterase as biomolecular targets. Special attention will be paid to requirements for assay development and validation of assays.

L42

Therapeutic approaches for treatment Huntington's and Parkinson's diseases

A. G. Kazantsev*, S. Altmann*, R. Bodner**, T. Outeiro*, M. Maxwell*, M. Coufal**, B. Hyman*, D. Housman**, A. B. Young*

*MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital and Harvard Medical School **Center for Cancer Research, Massachusetts Institute of Technology, Charlestown, MA 02129-4404, USA

One of avenue towards drug discovery for Huntington's Disease, non-curable neurodegenerative human disorder, is a search for small chemical molecules capable of reversing, blocking or delaying pathological processes.

Research on neurodegenerative disorders over the years has revealed an increasing complexity of disease genetics, biochemistry, and molecular pathology, Despite significant progress in basic research on disease biology, identification of the most significant cellular pathways leading to neuronal dysfunction and degeneration have remained elusive. Complex disease phenotypes at the molecular and cellular levels make a choosing drug targets quite challenging. Basic research has demonstrated that disease pathogenesis seems to involve the recruitment of multiple biochemical pathways.

That each of these pathways may be amenable to modulation by small molecules. Since there isn't sufficient knowledge to prioritize the different targets for neurodegenerative disorders, the most rational approach is a broad one in which multiple pathways are tested, the resulting 'hits' optimized and validated and their relative values determined by their therapeutic effects in genetic mouse models. In this way the most promising leads can be moved forward towards drug development unhindered by any scientific biases about which mechanisms are most important. Progress on different disease mechanisms, leading to identification novel therapeutic leads, will be discussed.

L43

Current Aspects and Future Trends in Neurodegenerative Diseases

G. Gaviraghi, G. Robertson, G. Terstappen

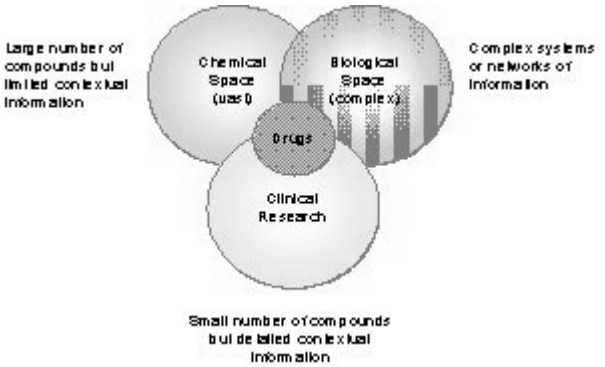
Sienabiotech S.p.A., via Fiorentina, 1, 53100 Siena Italy

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amylotrophic lateral sclerosis (ALS) feature common cellular and mechanisms including protein aggregation and inclusion body formation where brain function appears to be impaired by cell damage caused by the interaction of the aggregated proteins with cellular components. [1,2]

Current therapies are focussed on symptomatic treatment of the effects of protein aggregation, future therapies will need to be neuroprotective (disease modifying) to address the formation of protein aggregates or inclusion bodies and maintain normal brain function. Overall a combined approach may be the most effective.

Disease	Pathology	Toxic Proteins
Alzheimer's Disease	Neuritic Plaques Neurofibilary tangles	Aβ Tau
Parkinson's Disease	Lewy bodies & neurites	α-Synuclein
Huntington's Disease	Intracellular inclusions & cytoplasmic aggregates	Huntingtin with polyGlu repeats
Amylotrophic lateral sclerosis	Bunina bodies & axonal spheroids	SOD1

The current trend towards phenotype screening and chemistry driven approaches to unravelling the complexity of these pathopathways to promote the integration of biology, chemistry and clinical research will be presented. Examples that highlight this integration, bringing together medicinal chemistry, SAR, and mechanism of action studies will be presented for the highlighted diseases.



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L44

Antiviral imidazo[4,5-c]pyridines

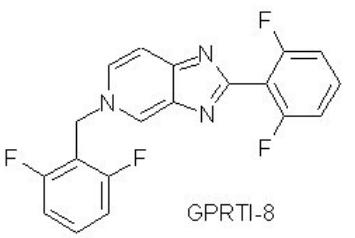
G. Puerstinger*, J. Paeshuyse**, E. De Clercq**, J. Neyts**

*Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria **Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

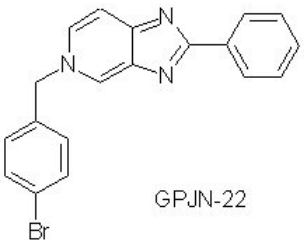
In the course of a screening program dedicated to the discovery of inhibitors of BVDV (bovine viral diarrhea virus), used as a surrogate for HCV (hepatitis c virus), the antiviral activity of GPRTI-8 was discovered.

Modifications of the substitution pattern led to the development of analogues with high selectivity against BVDV and HCV (GPJN-22 and GPJN-135, respectively).

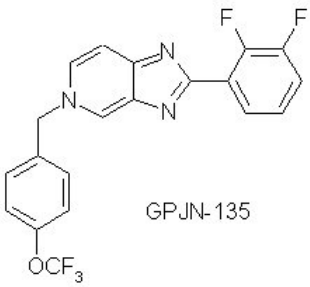
The synthesis and structure-activity relationships within this class of compounds will be reported.



GPRTI-8



GPJN-22



GPJN-135

L45

R278474 (TMC278, rilpivirine). The result of 14 years of interdisciplinary anti-HIV research

J. Heeres, P. J. Lewi

Johnson&Johnson Pharmaceutical Research ans Development, Janssen Pharmaceutica NV, 2340 Beerse, Belgium

This lecture is a tribute to the late Dr. Paul Janssen, who favored interdisciplinary research. He saw his role as a conductor and his collaborators as members of an orchestra.

In his last scientific paper Dr. Janssen listed the various requirements that should be satisfied in order to obtain a successful anti-HIV drug.

These constraints deal with the activity, bioavailability, safety, formulation, cost of manufacturing and patentability of the compounds and he very often reminded his collaborators of these requirements.

TMC278, Dr. Janssen's word champion among NNRTIs meets these criteria to a very large extend. This overview recapitulates the main steps of a long journey that started from TIBO and alpha-APA and that finally lead to the DAPYs, with ITU and DATA analogues as intermediate stations.

It took 14 years to get to this point, and almost 4 years have passed since TMC278 was synthesized by Jerome Guillemont in Val de Reuil (France).

TMC278 was originally labelled as R278474 and received the generic name rilpivirine.

Currently two members of the family are in full development for oral treatment of HIV-infections, namely TMC125 and TMC-278.

Another compound TMC120 or dapivirine has been transferred by Johnson and Johnson to the International Partnership for Microbicides with the aim of preventing the transmission of HIV.

L46

Deciphering biological effects by combining comparative modelling and structure-based approaches

W. Sippl

Institute of Pharmaceutical Chemistry, Martin-Luther-University of Halle-Wittenberg, 06120 Halle/Saale, Germany

Protein homology models have been used in conjunction with structure-based approaches to successfully identify novel inhibitors over the last few years. It is widely accepted that for example docking to homology models is more challenging and less successful than docking to X-ray structures of proteins. To successfully apply structure-based methods to homology models, in addition to accurate docking programs, high quality protein models are needed.

The present talk will highlight the results obtained for two protein targets where we used a combination of comparative protein modelling and structure-based methods in order to find novel leads. For both targets (the nuclear hormone receptor CAR[1,2] and the histone modifying enzyme PRMT1) we were able to identify new ligands based on a structure-based virtual screening. Besides the successful identification of PRMT inhibitors and CAR agonists the validated homology models were used to explain the structural basis for yet not understood biological effects. Our findings suggest that high quality homology models can be used as structural basis for lead finding of yet not crystallized protein targets and are able to provide important information concerning their biological effects.

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L47

Understanding metabolism in human cytochromes from the chemist's perpective

G. Cruciani*, R. Vianello**, I. Zamora***

*Laboratory for Chemometrics and Cheminformatics, Chemistry Department, University of Perugia, Via Elce di Sotto 12, I-06123, Perugia – Italy. **Molecular Discovery LtD, 4 Chandos Street W1A 3BQ, London - UK.***Lead Molecular Design, S. L. , Fransesc Cabanes i Alibau, 1-3, 2-1, 08190 Sant Cugat del Valles, Barcelona – Spain

Failures due to bad metabolic properties are one of the major reasons of problems in drug companies. Biotransformation reactions are due to few CYP enzymes, thus it is unavoidable that different drugs will compete for the active site of a given P450.

Several aspects of these enzymes, such as the rate and site of metabolism, inhibition and so on, must be taken into account in the lead optimization process during the development of new therapeutic agents. To this end, it would be extremely valuable for the drug industry the design of computational predictive methods for each of these aspects.

The experimental elucidation of the site of metabolism is usually a high resource-demanding task, which requires several experimental techniques and consumes a considerable amount of compound. The recognition of the metabolic site *in silico* could be of great help to design new compounds with better pharmacokinetic profile as well as to avoid the presence of toxic metabolites.

The aim of the present paper is to report a new method, fast, easy and computationally inexpensive for predicting CYP 1A2, 2C9, 2C19, 2D6, and 3A4 site of metabolism, and CYP 2C9 2D6 and 3A4 isoform selectivity using human CYP X-ray structures and *ad hoc* developed 3D homology models. For the studied substrates, in more than 85% of the cases the atoms ranked in the first or second position were the experimentally reported one as the site of metabolism.

The computational procedure is fully automated and fast. The methods thus appear as a valuable new tool in virtual screening and in early ADME-Tox field where potential drug-drug interaction and metabolic stability information must be evaluated to enhance drug design efforts.

L48

Discovery of Agonists of the Glucose Dependent Insulinotropic Receptor, GPR119, a pancreatic beta-cell oGPCR, for the treatment of NIDDM

R. Jones

Director, Medicinal Chemistry, Arena Pharmaceuticals, San Diego, USA

Non-insulin dependent diabetes mellitus [NIDDM] is increasing worldwide in epidemic proportions. It's associated morbidity and mortality is imposing a major burden on the health care system. Worldwide figures are staggering: in 2000 the World Health Organization (WHO) reported a worldwide prevalence of 154.4 million diabetes patients. It is predicted that by 2010, 221 million people and by 2025, 324 million will be diabetic. The current cost of diabetes in the U.S. is estimated to be at $132 billion, which includes $92 billion of direct medical costs and $40 billion of indirect costs such as disability, work loss and premature mortality. Compelling scientific evidence indicates that lifestyle modification effectively prevents or delays the occurrence of type 2 diabetes. Recent clinical trials also demonstrate that success in the treatment of obesity, either surgically or pharmacological, leads to the prevention of type 2 diabetes among the obese. Hence

intense efforts in the discovery and development of more efficacious and safer diabetes therapies are underway.

The entero-endocrine gut hormone GLP-1 promotes normoglycemia acutely by enhancing post-prandial glucose-stimulated insulin release and chronically by maintaining pancreatic beta-cell mass. These effects occur via beta-cell-expressed, Class B G-protein coupled GLP-1 receptors which in turn mediate elevated intracellular cAMP. One drawback from a therapeutic standpoint, however, is that GLP-1 is rapidly inactivated by the dipeptidyl peptidase-4 (DPP-4) enzyme, and rapid clearance through the kidneys resulting in a short circulating plasma half-life of less than 2 minutes.

Two broad therapeutic strategies have been employed to exploit the physiology of GLP-1. One is to use injectable peptidic GLP-1 receptor agonists that have resistance to DPP-4 mediated degradation ["glutides"]. Another strategy is to specifically inhibit DPP-4, which prolongs the half-life of endogenously released GLP-1 ["gliptins"]. Both strategies enhance GLP-1 receptor function and have generated significant therapeutic promise in the clinic. Orally active, small-molecule GLP-1 receptor agonists have proven elusive, a feature that unfortunately is characteristic of class B GPCRs. Meanwhile, the spectrum of signaling peptides affected by inhibition of DPP-4 remains unclear.

It would therefore seem worthwhile to search for alternative therapeutic targets which afforded both the physiological selectivity of GLP-1 signaling and the opportunity for orally active treatment modalities. Since pancreatic beta-cell dysfunction is a hallmark event in the pathogenesis of type 2 diabetes we have sought to identify and focus on orphan class A GPCRs with robust beta-cell-restricted expression profiles.

In this presentation I will illustrate that the Ga_s-coupled receptor, GPR119, is largely restricted to insulin-producing beta-cells of pancreatic islets and functionally, GPR119 is a glucose-dependent insulinotropic 7TM receptor. Unlike receptors for GLP-1 and other peptides that mediate enhanced glucose-dependent insulin release, GPR119 appears to be a lipid responsive class A GPCR, responding to a subset of fatty acid amides, structurally distinct from the endocannabinoids. Early discovery work performed at Arena, directed at identifying a chemically tractable HTS hit for GPR119 will be outlined together with SAR studies that enabled us to arrive, some years ago, at the potent, highly specific and efficacious GPR119 agonist AR231453, as a proof of concept pharmacological tool. AR231453 significantly increased cyclic AMP accumulation in beta-cells *in vitro.* AR231453 also enhanced glucose-dependent insulin release *in vitro* and *in vivo*, and improved oral glucose tolerance in wild-type mice, but not in GPR119 knock out mice. Dosing in diabetic *KK/A^y* mice led to markedly improved glucose tolerance.

Orally active agonists of GPR119 may offer significant promise as novel anti-diabetics acting in a glucose-dependent manner. Since its actions are functionally analogous to those of the incretin receptors, we propose terming this receptor GDIR, or glucose-dependent insulinotropic receptor.

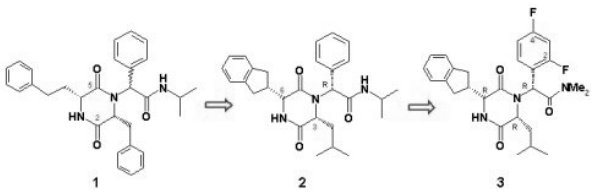
L49

2,5-Diketopiperazines - Novel Oxytocin Antagonists for Premature Labour. Discovery to Candidate Selection

A. Borthwick*, D. Davies**, A. Exall**, &. Hatley**, J. Hughes**, W. Irving**, D. Livermore**, S. Sollis**, F. Nerozzi**, K. Valko**, M. Allen**, S. Shabbir***, P. M. Woollard****

*GlaxoSmithKline Research and Development. **Departments of Medicinal Chemistry. ***Department of Assay Development and Compound Profiling, Harlow, U. K. ****DMPK, Cardiovascular and Urogenital Centre of Excellence for Drug Discovery, Stevenage, U. K

Premature (preterm) labour is a major clinical problem leading to death and disability in newborns. It accounts for 10% of all births and causes 70% of all infant mortality and morbidity. Identification of an oxytocin antagonist suitable for oral delivery that would delay labour safely for greater than 7 days represents a major medicinal chemistry challenge [1]. The only marketed oxytocin antagonist is atosiban, a peptide which is given by i.v. infusion. Our approach was based on optimizing the potency of the novel 2,5-diketopiperazine (DKP) template **1** (pKi = 6.5, hOT receptor binding). We developed a short, efficient and highly stereoselective synthesis of these chiral 2, 5-diketopiperazines derivatives. This gave *RRR* 3-Indanyl-6-isobutyl-DKP template **2** (pKi = 8.4), which however had poor rat oral bioavailability [2].



This talk will focus on the importance of the *RRR* stereochemistry of the 3 chiral centres for activity and on optimising the pharmacokinetic profile of this template using analogy and property based design. This rapidly led to the potent oxytocin antagonist the 2,4-diF-phenyl-dimethylamide **3** which has a high degree of selectivity toward the vasopressin receptors and has good oral bioavailability in both rat and dog. It is active in-vivo in our rat uterine contractility model and meets our target profile.

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L50

Teaching Medicinal Chemistry When the Times They Are A' Changing

D. Triggle

State University of New York at Buffalo and Center for Inquiry, Amherst New York USA

There has been until recently an implicit acceptance that the typical medicinal chemist practices within a single discipline largely determined by the boundaries of synthetic organic chemistry. This has nit, of course, been true for a long time. The progressive incorporation of the paradigms of molecular biology into chemistry and the realization that the molecular properties of a drug molecule are at least as important as the molecular biological activities has long rendered this simplistic view untenable.

These changes parallel those that have been taking place in university structures for the past three decades. Disciplines have burst out from their departmental straight jackets, and have rendered irrelevant the former hierarchical organization. Chemistry is practiced within disciplines from anthropology to zoology and the typical chemist (or biologist) proceeds through her career with a progressively enlarging toolbox of disciplinary achievements. Nonetheless, medicinal chemistry remains an essentially chemistry-based discipline, although its practice environment has changed substantially in the USA from a dominantly pharmacy environment. These changes will be discussed and speculations on the future of university-based science education offered.

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L51

Blending chemistry with other sciences

G. Ronsisvalle*

ABSTRACT IS NOT AVAILABLE

L52

Med Chem in Turkey: Teaching, Education and New Trends

N. Noyanalpan

Gazi Un. Fac. Of Pharmacy Med. Chem. Dept. Ankara Turkey

Teaching of Medicinal Chemistry in Turkey has started under the name “Pharmaceutical Chemistry” being governed particularly by french school. Consequently french technics of teaching have predominated. Stress has been given to synthesis of the compounds, chemical properties and some analytical aspects starting with physical appearance up to identification reactions involving almost no information regarding biological activity.

Time and incoming of younger generations who have been away from prevailing effects changed the course of teaching. More and more biological activity aspects of the drug molecule have been introduced to the curriculum. Structure activity relationship concept has been introduced a long time ago and ever since biological aspects kept increasing within the curriculum.

Today there are still some schools who regard the synthetic part of the course as a part of essentials. There are some schools which regard the synthetic part as less important and preponderate on molecular mechanism of action particularly their clinical applications being aware that future pharmacists may have a chance to serve as clinical pharmacist. More and more applicable information is rendered with enough underlying theoretical basis.

Schools in Turkey follow the foreign colleagues very closely and are aware that presently there are some pioneering research programs on several parts of the world which emphasize pharmacogenomics, bioinformatics and computational drug design. Almost all the faculties work on a similar program to recognize the curricula which comply with the contemporary educational model.

L53

Teaching Medicinal Chemistry: From a Pharmaceutical Industry Point of View

C. M. Timmers, C.A.A. Van Boeckel

Department of Medicinal Chemistry NV Organon, 5340 BH Oss, The Netherlands

The contemporary medicinal chemist is confronted with an expanding repertoire of synthetic chemical routes, structure-activity and structure-property relationships. Apart from that, medicinal chemistry is influenced more than ever by structural biology, *in silico* modeling and discovery techniques, and an increasingly detailed knowledge of disease mechanisms and pathologies is required. In order to be successful, pharmacokinetic, metabolic and toxicological considerations should concomitantly be integrated into the design of new molecular entities.

The medicinal chemist in the pharmaceutical industry is also increasingly influenced by the disciplines of molecular biology and cell biology and has to embrace the principles of assay development, high throughput screening, mining of corporate databases and intellectual property.

As a consequence of the multidisciplinary setting in an industrial environment the pivotal non-scientific skills and competencies of medicinal chemists in the pharmaceutical industry comprise strong communication, power to convince, multidisciplinary team building, organizing and sense of urgency.

As such the best learning environment is the pharmaceutical industry itself, while those chemists who have previously collaborated with other disciplines in the life sciences have the best prospects.

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L54

India's Quantum Leap Opportunities in Drug Discovery

N. De Souza

Noel J. de Souza Associates, 11, Sunita Nivas, S. V. Road, Santa Cruz (W), Mumbai 400054, India

In India the opportunities for medicinal chemists, whether budding or established or foreign-returned, have never been so favourable.

India's emergence as a world knowledge powerhouse is envisioned by the President of India through a convergence of bio-, informatics- and nano- technologies and their interconnectivities in grids including knowledge-, healthcare- and societal- grids. Significant funding initiatives by the Indian government in budgetary allocations for science and technology reflect an apparent committment to invigorate and promote excellence in scientific research and education.

The changed intellectual property scenario in India has critically influenced the dynamics of drug discovery initiatives, the demand for medicinal chemists and the orientation of teaching bodies. Concomitant emerging technologies in drug design, biotechnology, chemical biology/genetics, systems biology, different disciplinary methodologies and ancillary pharmacological disciplines also contribute to newer perspectives for drug discovery and development.

Consequently, the government, teaching bodies and research centres of domestic and multinational pharmaceutical companies are all engaged in approaches towards meeting the increase in demand for a newer breed of medicinal chemists. The newly-founded, public-funded, interdisciplinary, research Indian Institutes of Science Education & Research will find strong appeal to aspiring medicinal chemists. Medicinal Chemistry as a stand-alone department has begun to be introduced in a few institutes and universities, while in others continuing to remain as part of programmes of departments of pharmaceutical chemistry. The subject of natural products remains, too, in the teaching syllabi as a historical source of leads. The earlier mission-oriented natural products approaches to drug discovery have, however, lost ground to the so-called currently prevalent "analog" strategies. Domestic companies in the burgeoning pharmaceutical sector are successfully using such strategies to discover novel molecules of sufficient attraction to foreign pharmaceutical companies to license them. Seasoned expatriates are returning to help them. Collaborative links with academia and government are increasing.

A perspective view will be presented based on the speaker's 34-years of drug discovery/development experience with multinational and domestic pharma companies and interactions with academicians & the government.

L55

Immunoconjugates of Highly Potent Drugs for Cancer Therapy

P. Senter

Seattle Genetics, Bothell, WA 98021, USA

A great deal of interest has surrounded the use of monoclonal antibodies (mAbs) for the selective delivery of cytotoxic agents to tumor cells. Although the approach is conceptually appealing, several limitations have been identified, including the physiological barriers to mAb extravasation and intratumoral penetration, conjugate immunogenicity, non-specific conjugate uptake, low drug potency, and inefficient release of active drug. Several of these limitations were overcome in developing mAb-valine-citrulline-MMAE (mAb-vc-MMAE) conjugates. These are comprised of the highly potent antimitotic agent monomethyl auristatin E (MMAE), attached to mAbs through a cathepsin B cleavable vc linker. In this lecture, we will describe how this technology has been further optimized with newer generation drugs, linkers, and delivery agents. The results illustrate many of the key parameters in achieving both conjugate efficacy at well tolerated doses, as well as high intratumoral drug concentrations over sustained time periods. An overview of this therapeutic approach to cancer therapy will be provided.

L56

Novel Payload Chemistries for Application in Tumor-Targeted Antibody-Drug Conjugates (ADCs)

F. Vincent De Groot, P. Beusker, J. Joosten, H. Spijker

Syntarga B.V., Toernooiveld 1, 6525 ED, Nijmegen, The Netherlands

Antibody-Drug Conjugation (ADC): a rapidly emerging approach in anticancer therapy comprising the linkage of cell-killing agents to tumor-targeting antibodies in order to deliver the toxic payload specifically at the tumor site. Several novel chemistries developed in our laboratories to link cell-killing molecules to antibodies will be present-

ed. These chemistries are aimed at the creation of ADCs with an optimal therapeutic window, balancing the effects of potent cell-killing agents on tumor cells and healthy cells, respectively.

We have focused on the development of novel linker chemistries, novel drug chemistries and combinations thereof. For example, we are working on novel drug chemistries in the field of the duocarmycins (CC-1065 derivatives), a class of highly potent, cell-killing, DNA-damaging, minor groove binding agents. Several releasable linker chemistries (e.g. *SpaceLink*, *MultiLink*) and obtained biological results will be illustrated.

Suitable antibody payload chemistry plays a critical role in virtually all ADCs, whether on the market or in (pre)clinical development. Several issues that seem to have relevance for success in ADC will be discussed. Chemistries and biological data of (antibody) payload constructs incorporating different drugs will be presented. Finally, topics such as drug inactivation during conjugation, stability of the conjugate in the circulation and multivalency (i.e. number of drugs coupled per antibody molecule) will also be discussed.

L57

Discovery and development of MGCD0103 - an orally active HDAC inhibitor in Human clinical trials

Arkadii Vaisburg

MethylGene, Inc. Department of Medicinal Chemistry, 7220 Frederick-Banting, Montreal, Quebec H4S 2A1 (Canada)

Histone acetylation/deacetylation is essential for chromatin remodeling and regulation of gene transcription in eukaryotic cells. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes that catalyze the deacetylation (associated with transcriptional silencing) and acetylation (associated with transcriptional activation) respectively, of lysine residues located in the NH2 terminal tails of core histones. Perturbations of this balance in favor of histone deacetylation are often observed in human tumors. Thus, inhibition of HDACs has emerged as a novel therapeutic strategy against cancer with several molecules being evaluated in human clinical trials.

In our research program directed towards identification of novel HDAC inhibitors with high potency, low toxicity, favorable pharmaceutical characteristics and in vivo efficacy we identified several distinct series of such molecules. Further exploration of the lead series resulted in a discovery of N-(2-aminophenyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamide (MGCD0103). This compound inhibits a small set of recombinant human HDAC isoforms with IC50 values in the sub-micromolar range. In human cancer cells growing in culture it induces hyperacetylation of histones, causes the expression of the tumor suppressor protein p21WAF1/CIP1, inhibits cancer cell proliferation and is efficacious in vivo in various human tumor xenograft models in mice.

MGCD0103 is currently undergoing clinical evaluation as a single agent and in combination trials (Phase II trial expected to commence during second half of 2006) and is promising in treatment of hematological malignances and solid tumors.

L58

The identification of a novel series of N-hydroxybenzamides as potent HDAC inhibitors: synthesis, biological evaluation and structure activity relationships

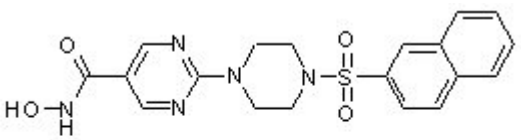
K.van Emelen[1], J. Arts[2], P. Angibaud[1], H. de Winter[3], V. Poncelet[1], A. Marien[2], S. van Brandt[1], W. Floren[2], M. Verdonck[1], J. van Dun[2], T. Geerts[2], L. Backx[1], B. Janssens[2], I. Pilatte[1], B. Roux[1], J. van Gompel[4], M. Carpentier[1], D. Corens[1], L. Meerpoel[1],P. Hellemans[5], E. Freyne[1] & M. Janicot[2]

[1] Department of Medicinal Chemistry, J&JPRD Europe. [2] Oncology Discovery Research, J&JPRD Europe. [3] Molecular Informatics, J&JPRD Europe. [4] ADME/Tox, J&JPRD Europe. [5] Experimental Medicine, J&JPRD Europe

Histone deacetylases (HDACs) represent a family of enzymes that compete with histone acetyltranferases (HATs) for modification of the nucleosomal histone proteins. Histone acetylation status modulates chromatin structure and thereby regulates transcriptional activity of a subset of genes.

Aberrant reduction in acetylation due to disruption of HDAC or HAT activity is associated with the development of cancer. Deregulated, sustained HDAC recruitment to the chromatin is observed in specific forms of leukaemia and lymphoma, such as APL and non Hodgkin's lymphoma. In agreement with a key role of HDAC activity in cancer, HDAC inhibitors from various structural families induce histone hyperacetylation, activate gene expression and consequently, inhibit the cell cycle, activate differentiation programmes or induce apoptosis. HDAC inhibitors have been described to exhibit potent antitumor activity in human xenograft animal models, suggesting that this class of compounds represents promising novel cancer therapeutic agents.

We have identified novel N-hydroxybenzamides showing potent HDAC inhibition and culminating in the identification of R306465 (JNJ16241199) as a nanomolar HDAC inhibitor with anti-tumor activity when dosed orally in human xenograft-bearing nude mice.



R306465 (JNJ16241199)

IC_{50} HDAC = 6 nM (HeLa nuclear extract)
IC_{50} A2780 = 30 nM (cell proliferation)

In this contribution, the strategy, synthesis and pharmacological and preclinical evaluation leading to the identification of R306465 will be discussed in detail.

R306465 is currently undergoing Phase I clinical trials.

L59

New insights on receptor activation and receptor-receptor interactions

N. Birdsall*, A. Baig*, B. Birdsall*, C. Browning*, M. Buccioni*, J. Corrie*, J. Hern*, R. Leppik*, S. Lazareno**, G. Mashanov*, J. Molloy*

*Division of Physical Biochemistry, MRC National Institute for Medical Research, Mill Hill, London, UK. **MRC Technology, Mill Hill, London, UK

The initial events in the G protein-coupled receptor (GPCR) signalling cascade can be monitored readily by radioligand binding to membrane-bound receptors and their associated G-proteins and by measurement of early biochemical or electrophysiological signals. These studies have given rise to a number of valuable models that cannot simulate all elements of the binding phenomena, possibly because the molecular composition of the receptor (monomer/dimer) and receptor-G complex(es), and their clustering in the plane of membrane, are not known.

In equilibrium and kinetic radioligand binding studies and in functional studies we have been able to detect evidence of cooperative behaviours compatible with the presence of receptor-receptor interactions in agonist-receptor-G protein complexes. For the example of A_1 adenosine receptors, such interactions are a function of receptor concentration, suggesting that there could be reversible formation of dimers. Cooperative interactions are not detectable with orthosteric antagonists but can be observed with allosteric ligands.

For a direct visualisation of GPCRs and their dimerisation status, we have used total internal reflectance fluorescence microscopy to track the motion, on a millisecond time scale and with nanometre spatial resolution, of individual molecules of M_1 muscarinic acetylcholine receptors on living CHO cells. It is possible to measure any clustering of the muscarinic receptors, their dimerisation, and their rates of diffusión on the cell surface.

L60

Using PREDICT 3D models of GPCRs for rapid drug discovery: The discovery of a 5-HT4 agonist as a potential drug for Alzheimer's disease

Y. Marantz[1], B. Inbal[1], M. Lobera[2], M. Pradyumna[2], D. Chen[2], S. Shacham[2], S. Noiman[1], O. Becker[1]

1) Predix pharmaceutical Ltd. 3 Hayetzira St, Ramat Gan, Israel 52521. 2) Predix pharmaceutical, 4 MaguireRd, Lexington MA 02421

GPCR constitute a major family of drug targets involved in many physiological responses. Structure based drug discovery for the GPCR family has been limited by the existence of only one GPCR x-ray structure (i.e. bovine rhodopsin). In this presentation we will demonstrate our structure-based drug discovery process for GPCR targets which is based on a set of novel computational approaches, allowing discovery and development of drug candidates in very short time periods. This process starts with the PREDICT de novo modeling algorithm, continues with high-throughput structure-based in-silico screening, which is then followed by integrated computational & medicinal-chemistry lead optimization. We will discuss the discovery and lead optimization of a novel 5-HT4 partial agonist for the treatment of Alzheimer disease, which is currently under clinical development.

L61

Physicochemical Compound Profiling and PLS Analysis: Tools to Drive Lead Optimization Programs

A. Gilbert

Exploratory Medicinal Chemistry, Chemical and Screening Sciences, Wyeth Research, 401 North Middletown Road, Pearl River, NY 10965

Given the challenge of expedited Discovery timelines, pharmaceutical companies are optimizing physicochemical compound properties (i.e. aqueous solubility, permeability, CYP inhibition, microsomal stability) in addition to biological activity during the "hit to lead" (HTS hits to validated chemical lead series) phase of compound/series optimization. Since libraries of compounds are being prepared and biological/physicochemical properties measurements are being generated robotically, a tremendous amount of data is created that can be difficult and time consuming to interpret. The analysis of bioassay and physicochemical data is facilitated using multivariate analysis (MVA), specifically PLS (projection to latent structures by means of a partial least squares analysis). In this presentation, examples will be discussed showing how MVA is being used to efficiently drive "hit to lead activities in several of Wyeth's hit to lead programs.

L62

Potency and PKDM Profile of 2,3-Substituted Quinazolin-4-ones as Potent CXCR3 Antagonists and the Discovery of AMG 487

M. Johnson*, A. Li*, T. Collins*, Z. Fu*, J. Liu*, A. Huang*, G. Tonn*, D. Dairaghi*, T. Schall**, T. Sullivan**, J. Medina*

*Amgen SF LLC, 1120 Veterans Blvd. , South San Francisco, CA 94080. **ChemoCentryx, 850 Maude Ave. , Mountain View, CA 94043, USA

CXCR3 is a chemokine receptor associated with the recruitment of leukocytes from the peripheral blood into inflamed tissue. Blockade of CXCR3 may play a beneficial role in the treatment of inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and other immune-mediated diseases. This presentation will summarize the discovery and improvement of a series of quinazolinone analog antagonists of CXCR3, with emphasis on the optimization of PK parameters that afforded the clinical candidate AMG 487. The efficacy of these compounds in *in vivo* models will also be discussed.

L63

PPAR-alpha/gamma: Design and Synthesis of Potent and Balanced PPAR-alpha/gamma Dual Agonists for the Treatment of Type II Diabetes and Dyslipidemia

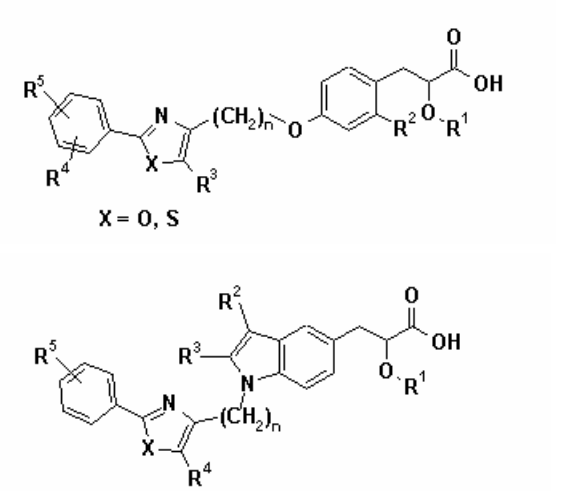
H. Märki, A. Bénardeau, J. Benz, A. Binggeli, U. Grether, H. Hilpert, B. Kuhn, M. Meyer, P. Mohr, K. Puentener, S. Raab, F. Ricklin, A. Ruf, U. Sprecher, N. Wyttenbach

Pharmaceuticals Division, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland

Type II diabetes is a metabolic disease characterized by insulin resistance and hyperglycaemia which is often accompanied by hyperlipidemia. Two classes of compounds were empirically discovered decades ago, known as the thiazolidinediones (TZDs) and the fibrates. While the TZDs lower blood glucose as well as insulin levels and improve insulin sensitivity, the fibrates are effective at lowering serum triglycerides and raising HDL cholesterol levels.

Recently, the ligand-dependent transcription factors Peroxisome Proliferator Activated Receptor-gamma (PPAR-gamma) and –alpha (PPAR-alpha) have been identified as being the primary molecular targets for the antidiabetic TZDs and the lipid lowering fibrates, respectively. This has provided new opportunities for the treatment of type II diabetes, since the profile of a PPAR-alpha/gamma dual agonist appears well suited for addressing both hyperglycaemia as well as the enhanced cardiovascular risk of diabetic patients.

The identification of two novel classes of PPAR-alpha/gamma dual agonists, alpha-alkoxy-phenyl-propionic acids as well as indolyl-alkoxy-propionic acids, is described. Both series have been identified with x-ray structure and modeling guidance making use of design constraints inferred from known PPAR-alpha and -gamma selective agents and show a wide range of PPAR-alpha/gamma ratios within a rather narrow structural space. The optimization of the relative PPAR-alpha/gamma/delta potencies and the rationalization of the SAR within the protein structure context are described. Further profiling of advanced compounds in animal models of type II diabetes and dyslipidemia will be presented.



L64

FXR Agonists from Bench to Clinic. The case of 6ECDCA (INT-747)

R. Pellicciari

Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Via del Liceo 1, 06123 Perugia, Italy

Deorphanized not earlier than seven years ago as a physiological sensor for bile acids, the Farnesoid X Receptor (FXR) has rapidly became an attractive target for drug discovery in view of its pleieotropic role in a variety of physiological processes and in view of its possible implication in several diseases and syndromes. Thus, upon activation, FXR heterodimerizes with RXR and regulates the expression of a cohort of genes implicated in the repression of bile acid synthesis and import in hepatocytes; in the stimulation of bile acid export from hepatocytes; in the protection of hepatocytes from bile acid toxicity. The effect of FXR's activation is not limited to regulation of bile acid levels, but also impacts the transcription of genes involved in gluconeogenesis and lipidogenesis, thus affecting, in concert, bile acids, lipids and metabolism.

The availability of potent and selective FXR modulators has helped to a great deal the understanding of the pathophysiological role of FXR. In 2002 we reported the synthesis and the preliminary evaluation of 6ECDCA (INT-747) as a potent and selective FXR agonist, endowed with protective properties against cholestasis and liver fibrosis when administered in in vivo animal models. Since then, 6ECDCA (INT-747) has served as a powerful tool for the elucidation of the FXR' s structure, pharmacology and patho-physiological role.

Orally active, 6ECDCA (INT-747) has a great potential for proving effective in reducing and possibly reversing the liver damage caused by fibrosis or cholestasis, a condition that may eventually lead to liver cirrhosis and liver failure in the millions of patients afflicted with chronic liver diseases. Phase Ia clinical trials have shown that 6ECDCA (INT-747) is well tolerated in a single dose in

healthy volunteers and the compound is expected to enter soon Phase II trials.

In this paper a survey of the steps that have lead from the discovery of 6ECDCA (INT-747) to its current clinical evaluation will be reported.

L65

Computational Prediction of Human Drug Metabolism

S. Ekins

GeneGo, 500 Renaissance Drive, Suite 106, St. Joseph, MI 49085. USA and School of Pharmacy Department of Pharmaceutical Sciences, University of Maryland

Since the early 1990s various in vitro experimental approaches have enabled investigation of human cytochrome P450s (CYP), providing considerable insights into their function and substrate/inhibitor specificity. In parallel the development of various computer models has provided visual images to explain the empirical data. Various computational techniques such as homology models, initially developed with X-ray structures from bacterial CYPs as the template, were used to dock molecules in the position necessary to explain the metabolites observed or to explain the site directed mutagenesis results. The development of pharmacophore and quantitative structure activity relationships for the various CYPs made use of the experimental data being generated in increasing quantities as a consequence of earlier drug-drug interaction studies and high throughput screening. The key molecular properties or features and their orientation required for interaction with the major CYPs was described. With the recent X-ray structures for human CYPs we now have a wealth of computational tools and models potentially available for rapid prediction of binding to CYPs. The effective combination of approaches considering the molecular properties of the compounds as well as the proteins themselves represents a method for the evaluation of potential CYP selectivity for molecules being considered as possible therapeutic agents. This talk will discuss the development and application of these approaches and describe our product MetaDrug. This new tool is used to; 1) predict metabolites for molecules based on their chemical structure, 2) predict the activity of the original compound and its metabolites with various ADME/Tox models, 3) incorporates the predictions with human cell signaling and metabolic pathways and networks and 4) integrates networks and metabolites, with relevant toxicogenomic or other high throughput data.

L66

Progress on tools for prediction and simulation of pharmacokinetics and drug-drug interactions: An integrated approach

A. Rostami-Hodjegan

Academic Unit of Clinical Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Glossop Road, Sheffield, S10 2JF, United Kingdom

New challenges in drug development have increased the need for the application of *in silico* approaches to avoid unnecessary clinical studies. Although modelling and simulation (M&S) is gradually finding its rightful place within drug discovery and development departments in the majority of pharmaceutical industry, it is becoming clear that the knowledge requirement to test compounds in "virtual populations of patients" is much greater than originally thought. Moreover, the value of an integrated approach is not well recognised and appreciation of the vital distinction between a useful "*simulation"* and a precise "*prediction"* is often lacking (Rostami-Hodjegan & Tucker 2004).

The possibility of extrapolating observations from *in vitro* systems to the *in vivo* pharmacokinetics (IVIVE) has now become a reality and it has moved from being a "purely academic exercise" to "day-to-day practice" in many leading pharmaceutical companies. In particular, advances has been made in *a priori* assessment of metabolic routes for candidate drugs, the influence of genetics on overall metabolism and the extent of likely metabolic drug-drug interactions (mDDI). These have provided guidance on decisions to (dis)-continue development of candidate drugs. Although *in vitro* data are routinely generated during drug development, they are often not integrated efficiently within a single platform to facilitate communication between discovery, pre-clinical and clinical development teams. Efforts within the a Global Consortium of pharmaceutical industry (www.simcyp.com) will be described to show the applications of integrated IVIVE on some areas such as those listed below:

- Assessing the impact of inter-individual variability, potential inter-ethnic differences and effect of age related differences in paediatrics
- Implications of plasma protein binding displacement, experimental design of *in vitro* studies and inter-individual differences for quantitative assessment of metabolic drug-drug interactions
- Assessing Propagation of PK Variability into Pharmacodynamics (PD)

[1] Rostami-Hodjegan & Tucker *"In silico"* simulations to assess the *"in vivo"* consequences of *"in vitro"* metabolic drug-drug interactions. *Drug Disc Today Technol,* 2004, **9:** 441-448

L67

Handing over the baton - Connecting Medicinal Chemistry with Process R&D

H. Federsel

Global Process R&D, AstraZeneca, 151 85 SÖDERTÄLJE, Sweden

Once an interesting molecule has been identified that is considered worthwhile for further development into a new medicine and, eventually, a launch on the market does this mean the end of the more chemistry-oriented activities in a drug project? Quite the opposite as it still remains to design and develop a route that has to prove its scalability and suitability for delivering the active ingredient in required amounts at the right quality. This “piece in the puzzle” is the responsibility of Process R&D which, thus, represents the unit that bridges small scale laboratory experimentation with multi-kg pilot plant production and even commercial manufacture [1]. Of the plethora of activities that an organization of this kind is involved with suffice it to mention a few examples such as identifying the best synthetic route to a given target compound, addressing cost of goods issues, determining quality criteria for starting materials and intermediates, optimising yields, mapping and eliminating process-related safety, health, and environmental problems, and turning a sequence of chemical steps into a full-fledged process. And all this has to be achieved in a short period of time with limited resource whilst feeling a continuous pressure to avoid delaying delivery of the requested product which might severely hamper the ability to conduct crucial trials, for example testing in man [2].

The increased focus over the years on timelines to reach certain pivotal stages or milestones along the R&D pipeline – for example first time in man, proof of concept, market launch – has made the pharmaceutical industry aware of the need to initiate work earlier and earlier. Instead of conducting activities in a largely sequential order there will inevitably have to be a certain degree of timely overlap, which leads to interdependent work being carried out in parallel. Furthermore, this frontloading approach has to take a substantial risk into account that virtually all projects are exposed to during the early phases, as it is here where the attrition is most severe [3]. Hence a balanced way to operate this concept under high degree of uncertainty seems to be more and more accepted as the best way to ensure that drug discovery and development will remain a sustainable and prosperous business.

The Medicinal Chemistry – Process R&D interface represents one area where there is a need for extensive interactions during the early part of the project life cycle. To be successful in this regard an open climate of information sharing and mutual understanding is vital and this can only be achieved when signs of “silo” mentality are actively counteracted. Structures of molecules that might end up as the next candidate drug need to be exchanged as well as thoughts on how these might be synthesized. There is always a good possibility to have a stimulating discussion on how feasible the scale-up of the first preparative method from the laboratory would be and to use imagination or intelligent computer software tools to suggest various likely or less likely routes [4]. With increased molecular complexity, the incorporation of rather unorthodox structural motifs, and the presence of multiple stereogenic centres (to date targets with up to at least 13 of these have been produced on scale) it has become even more important to address the challenges early on. Only by always having access to best available technologies and methods will it be possible to prepare the desired molecules at a competitive cost that meets high expectations [5,6]. Sharing and discussing aspects on how to handle these complex interrelations constitutes the main topic of this lecture.

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[3] Federsel, H.-J. *PharmaChem* **2003**, *2*(9), 28-32

[4] Federsel, H.-J. *Comb. Chem. High Throughput Screen*. **2006**, *9*(2), 79-86

[5] Federsel, H.-J. *PharmaChem* **2005**, *4*(11/12), 4-9

[6] Federsel, H.-J. *Drug Discov. Today* submitted

L68

Automation in Chemical Process Research and Development

H. Weinmann

Schering AG, Research Center Europe, Medicinal Chemistry I D-13342 Berlin, Germany

In modern pharmaceutical industry a smooth transfer of new drug candidates from drug discovery to development functions is crucial in order to shorten development timelines. Therefore a close interaction between Medicinal and Process Chemists is of utmost importance for a successful drug development.

The steadily increasing use of automation in chemical development has several advantages *e.g.* faster optimization of chemical processes enables a faster supply of larger amounts of drug substance for other development functions. Furthermore, a large number of synthesis parameters usually has to be optimized manually in the process research laboratories with huge time efforts for each single reaction.

Given these advantages, many automated systems have been designed and established in Chemical Process R & D as a major trend in the last few years [1,2]. This lecture will give an overview on various customized robotics systems which have been successfully implemented at Schering AG for reaction screening, dissolution and crystallization experiments, reaction optimization and fine-tuning of reaction and work-up parameters.

Successful chemical examples of automated reaction screening and optimization will be discussed [3]. These automated process research devices have also proven to be of high value for the support of Medicinal Chemists in late stage drug discovery projects.

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[2] Cork DG, Sugawara T. Laboratory Automation in the Chemical Industries. Marcel Dekker Inc. New York, 2002; ISBN 0-8247-0738-9.

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L69

New structural features involved in ligand recognition and activation of rhodopsin-like histamine and chemokine receptors

R. Leurs

Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, Vrije Universiteit-FEW, Amsterdam, the Netherlands

Using a combination of molecular modeling and protein mutagenesis we investigate basic mechanisms of G-protein coupled receptor (GPCR) function, i.e. ligand binding and GPCR activation. In aminergic GPCRs, including the histamine H_1 receptor (H_1R), the ligand-binding pocket is thought to reside in a hydrophilic cleft formed by the seven transmembrane domains (TMs). Small molecule binding to the aminergic receptors is currently considered to mainly occur between the TMs 3 (containing the conserved an aspartate (Asp), 5 and 6. Using the natural variation in GPCR sequence we recently obtained evidence that for some H_1R ligands, the binding pocket is not only limited to TMs 3, 5, and 6 but also comprises an additional pocket formed by TMs 2 and 7, that is not targeted by most developed H_1 antagonists. Using a similar approach we discovered a role for the second extracellular loop of the histamine H_4 receptor in the binding of H_4 agonists, but not antagonists.

The detailed molecular mechanism for agonist-induced activation of rhodopsin-like GPCRs has not yet been described. Activation of GPCRs is thought to involve disruption of intramolecular interactions that stabilize their inactive conformations. Such disruptions are induced by agonists but may also be induced upon mutation of the receptor. Recently, we characterized important steps in the activation of H_1R. Both Ser3.36 and Asn7.45 are important links between histamine binding and previously proposed conformational changes in helices 6 and 7. Ser3.36 acts as a rotamer toggle switch that, upon agonist binding, initiates the activation of the receptor through Asn7.45. The proposed transduction involves specific residues that are conserved among rhodopsin-like GPCRs. To further investigate the activation process of the rhodopsin-like family of GPCRs we created (through random mutagenesis) and characterized constitutively active mutant histamine H_1 receptors. Several highly constitutive active mutants were obtained and their action can be rationalized by the existence of a conserved hydrophobic motif in the TM pocket, which is stabilizing the inactive receptor state. Finally, we recently studied the role of helix 8 in the activation of the viral chemokine receptor ORF74. While chemokines interact with the extracellular amino-terminus and loops of the receptor, we demonstrate that helix 8 (Hx8) in the intracellular carboxyl tail (C- tail) of ORF74 directs chemokine binding. We propose that the conserved residues in helix 8 exert a key role in directing the ligand binding profile of ORF74 and likely also that of other class A GPCRs.

L70

The 5-HT3 receptor - undertanding the binding site and the transduction pathway.

S. Lummis*, K. Price*, D. Beene**, A. Thompson*, L. Lee**, H. Lester***, D. Dougherty**

*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1GA, United Kingdom. **Divisions of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA. ***Divisions of Biology , California Institute of Technology, Pasadena, CA 91125, USA

The $5HT_3$ receptor is a member of the Cys-loop family of ligand-gated ion channels [1]. Cryo-electron microscope images of the related nACh receptor have led to a model of channel opening in this family of proteins: agonist binding to the extracellular domain causes a rotation which is then transduced via the M2-M3 loop to the pore lining domain, M2 [2]. We have created a homology model of this receptor and docked 5-HT and granisetron into the binding pocket. Mutagenesis, radioligand binding and functional experiments have revealed the amino acids involved in ligand binding, and the orientations of ligands in the binding site. We have also explored the roles of critical amino acids in the transduction pathway of the receptor. Of particular significance has the been the use of unnatural amino acids, firstly to distinguish the role of H-bonds and aromatic interactions in receptor binding, and secondly to probe the critical features of a proline residue located at the apex of the M2-M3 loop. The data from the binding site show distinct roles of different aromatic residues in the binding pocket [3]. The data from the M2-M3 loop are consistent with a cis-trans isomerisation of the proline at the apex of this loop providing the switch that interconverts the open and closed states of the channel [4].

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L71

Nvp-Aeb071: First Protein Kinase C Inhibitor Prolonging Graft Survival

R. Albert, C. Beerli, C. Bruns, M. Bigaud, V. Brinkmann, C. Burkhart, N. Cooke, J. Evenou, S. Cottens, P. Guitard, P. Heining, A. Maibücher, P. Marbach, B. Metzler, R. Morris, C. Pally, C. Papageorgiou, C. Rordorf, R. Sedrani, W. Schuler, P. von Matt, M. van Eis, J. Wagner, G. Weckbecker, G. Zenke.

Transplantation Research - ATDA and Global Discovery Chemistry, NIBR, Novartis Pharma AG, Basel, Switzerland

Background: Immunosuppressants with an improved therapeutic window represent a high medical need. The search for novel approaches to block T-cell activation led to NVP-AEB071, a selective inhibitor of protein kinase C (PKC). We show here for the first time that NVP-AEB071 exerts its immunosuppressive activity *via* a new mode of action, prevents graft rejection in preclinical models and is well tolerated in a single dose healthy volunteer study.

Results: A medicinal chemistry program at Novartis identified NVP-AEB071 as a potent inhibitor of classical and novel PKC isoforms with K_i values in the low nM range. T-cell activation is effectively blocked as determined by inhibition of IL-2 production (IC_{50} ~ 5 nM). In contrast, IL-2-dependent T cell proliferation is not affected. With a MLR (two-way mixed lymphocyte reaction) / BM (bone marrow proliferation assay) IC_{50} ratio of > 20, NVP-AEB071 is strongly immunosuppressive, but without anti-proliferative effects on bone marrow cells. NVP-AEB071 effectively prevents graft rejection in rodents and monkeys either in monotherapy or in combination with other immunosuppressants. In a single dose healthy volunteer trial, no severe adverse effects were seen up to exposures levels which would correspond to an efficacious exposure range based on non-human primate and pharmacodynamic data.

L72

Design and Structure-Activity Relationship of novel, potent, highly selective dual-specific Src and Abl kinase inhibitors

L. F. Hennequin, A. Jack, C. Gerard, F. Mike, G. Tim, J. Vivien, M. RéMy, O. Annie, P. Patrick

AstraZeneca, Parc Industriel Pompelle, Reims Cedex 2 France

Normal cell growth, activity and function are regulated by a complex network of signalling pathways. Aberrations in these signalling pathways are a hallmark of tumour development. Src kinase, a non-receptor tyrosine kinase, is a signal transduction modulator and is a critical component of many of the key signalling pathways currently thought to be pivotal in carcinogenesis. In contrast to its highly regulated role in normal cells, Src kinase has been shown to be de-regulated and its activity significantly increased in many human tumours. Recent publications have highlighted the critical role of Src kinase in tumour cell migration and invasion. Inhibition of Src kinase has the potential to have a significant impact as a treatment for cancer

AZD0530, a novel anilinoquinazoline, is a potent dual specific inhibitor of cSrc kinase (IC_{50}: 2.7 nM) and the related Abl kinase (IC_{50}: 30nm). AZD0530 is highly selective for Src and Abl kinases against a large range of tyrosine and serine-threonine kinases.

AZD0530 exerts its activity through ATP competitive and reversible inhibition of the target enzyme. SAR are presented focusing in particular on the critical role played by the aniline substitution pattern and the effect of the C5–C7 quinazoline substitution pattern.

AZD0530 possesses excellent properties for a small molecule inhibitor of Src kinase that translate into excellent ADME properties in pre-clinical animal species. AZD0530 is orally available and is suitable for once daily administration to humans.

AZD0530 causes dramatic inhibition of human breast cancer cell migration *in vitro*. Following oral administration at single daily doses of 6 mg/kg, AZD0530 completely prevents the growth of c-Src-NIH 3T3 xenografts grown in nude rats and at 25mg/kg/d significantly increases survival in an orthotopic model of human pancreatic cancer.

AZD0530 has the potential for activity in a wide range of tumours and is currently in early clinical development

L73

Carrier-linked prodrugs for targeting cancer cells

F. Kratz

Tumor Biology Center, Breisacher Straße 117, 79106 Freiburg, Germany

Designing and developing truly tumor-specific prodrugs remains a challenge in the field of cancer chemotherapy. On the one hand, active targeting strategies aim at exploiting membrane-associated receptors or antigens for drug delivery, on the other hand, the enhanced vascular permeability and retention of macromolecules in tumor tissue substantiates the concept of passive targeting.

Consequently, research efforts have concentrated on conjugating anticancer agents with a wide spectrum of macromolecules including antibodies, peptides, and growth factors as well as polysaccharides, serum proteins, and synthetic polymers. Despite attaining proof of concepts for numerous prodrug approaches in the preclinical setting, only a few carrier-linked prodrugs with anticancer agents have reached an advanced stage of clinical testing.

This overview gives an update of anticancer prodrugs that are being assessed in clinical trials. In addition, it describes the development of prodrugs with the anticancer drug doxorubicin, an anthracycline that has been used widely in the development of antitumor drug delivery systems [1]. The various prodrugs of doxorubicin, e.g. with antibodies, peptides, serum proteins or synthetic

polymers, exemplify the salient features of the respective drug delivery system and shed light on some of the pitfalls that are encountered when attempting to realize Paul Ehrlich´s vision of "the magic bullet" that he proclaimed at the beginning of the last century.

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L74

Bioreactive prodrugs for targeting cancer cells

L.H. Patterson, K. Pors, P.M. Loadman

Institute of Cancer Therapeutics, University of Bradford, West Yorkshire, BD7 1DP, UK

Cytochrome P450s (CYP's) are a superfamily of mixed function oxidases responsible for metabolising drugs and xenobiotics. Metabolism by the CYP 1-3 family is generally viewed as a route to drug detoxification and increased elimination but they also have the potential for cancer drug activation. There is considerable evidence demonstrating expression of a wide range of CYPs in all the major clinically derived solid tumours and even over-expression of selected isoforms. Spirocyclopropacyclohexadienone is the reactive unit of several families of natural products that promote stalling of replication forks, DNA double strand breaks and cell death, however, they are not tumour selective. We have synthesised a library of analogues lacking the hydroxyl group crucial to the initiation of DNA covalent binding. Screens have been developed to identify regioselective hydroxylation by tumour expressed CYPs and have identified several agents suitable for further development. A proof-of-principle compound, DP-7, produced IC_{50}s >50, 5, 0.1mM in CYP-null cells and transfectants over-expressing CYP1B1 or CYP1A1 respectively. This activity is similar to that obtained from DP-7 activation by bactosomes containing either CYP1A1 or CYP1B1. This suggests that DP-7 is predominantly metabolised by CYP1A1. This is further supported by HPLC data that reveals that CYP1A1 bactosomes metabolise DP-7 to a greater extent than CYP1B1 bactosomes. To exclude possible hepatic damage, DP-7 was incubated in the presence of mouse liver homogenates which produced a low level of metabolites and no cytotoxicity suggesting that DP-7 is not hydroxylated by all CYPs and may preclude liver activation and elimination.

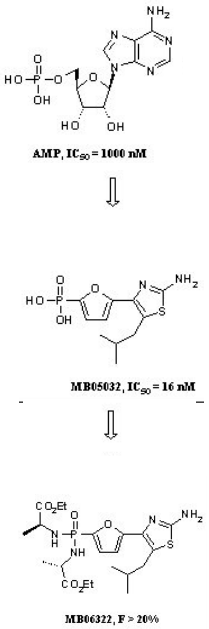
L75

Discovery of Potent and Selective Inhibitors of Fructose-1,6-bisphosphatase with Potential as a New Class of Agents to Treat Type 2 Diabetes

Q. Dang, M. Erion, K. Reddy, S. Kasibhatla, M. Reddy, P. Poelje

Metabasis Therapeutics, Inc., 11119 N. Torrey Pines Rd., La Jolla, CA 92037, USA

Fructose-1,6-bisphosphatase (FBPase) is a key rate-limiting enzyme of gluconeogenesis (GNG), which has been shown to contribute significantly to the up-regulated hepatic glucose output in type 2 diabetes (T2DM). Consequently, inhibition of FBPase has been explored as a potential approach to control blood glucose levels in T2DM. However, past drug discovery programs targeting the AMP binding site of FBPase have not identified potent and selective FBPase inhibitors as clinical candidates, which can be attributed to the highly hydrophilic nature of the AMP site and the fact that AMP is often used to regulate enzymes and receptors naturally.



Using a structure-guided drug design strategy, we discovered a series of potent and selective FBPase inhibitors that mimic AMP but with little structural resemblance. Moreover, a novel bisamidate prodrug approach was introduced to deliver these phosphonates orally. MB06322 (CS-917) was identified and is in Phase IIB clinical trials as a potential treatment for T2DM. The medicinal chemistry aspects on the design and SAR of our FBPase inhibitor program will be presented.

L76

Looking for the future of phosphatase inhibitors in inflammatory / auto-immune diseases

A. Bombrun

Serono Pharmaceutical Research Institute – Geneva, Switzerland

Reversible phosphorylation of tyrosine is a well-known aspect of signal transduction, with protein tyrosine

kinases (PTKs) and protein tyrosine phosphatases (PTPs) as opposing players in the game. Their respective actions need to be well-adjusted, as any imbalance can lead to various diseases states. The idea to treat such diseases by selectively modulating the tyrosine kinase/phosphatase balance has made these enzyme families favored targets for the drug industry. But while the inhibition of PTKs has been extensively (and successfully) studied, only few academic and industrial groups have studied PTP inhibitors as possible treatments for inflammation/auto-immune diseases.

Already in the late 1980s, sodium orthovanadate, a potent competitive inhibitor of tyrosine phosphatases was demonstrated to play a key role in lymphocyte activation. In recent years PTPs have received growing interest, but many compounds suffered from a lack of druglikeness and were difficult, if not impossible, to develop as drugs.

This talk will focus on inhibitors of PTPs directly involved in the human immune system, e.g. SHP1, SHP2 and GLEPP-1, discuss screening technologies involved and illustrate the optimization of inhibitors.

L77

The Path to In Silico Pharmacology

J. Mestres

Chemogenomics Laboratory, Research Unit on Biomedical Informatics, Institut Municipal d'Investigació Mèdica and Universitat Pompeu Fabra, Dr Aiguader 88, 08003 Barcelona, Catalonia, Spain,

One of the grand challenges in chemical biology is identifying a small-molecule modulator for each individual function of all human proteins. For pharmaceutical research, this has the potential to provide molecules that may then be used as chemical probes for protein validation and as initial hits for lead generation in target and drug discovery programs, respectively. Vital to this aim is the ability to produce quantitative data on the response of biological systems to the presence of chemical compounds. Pharmacologists have been gathering this type of data for over a century. However, it has not been until recently that the technological advances produced in combinatorial chemistry and high-throughput screening have made possible to collect these data in a more automatic and systematic manner, opening an avenue towards determining experimentally the pharmacological profile of compounds. Nonetheless, in spite of the significant progress made towards improving the capacity for chemical synthesis and biological testing, any aspiration of being able to make and store every synthetically feasible molecule and test it on every assayable protein remains to date unreachable and thus complementary strategies for massive pharmacological profiling of large compound collections need to be explored.

This presentation will introduce some of the computational approaches to pharmacological profiling of chemical libraries developed recently in our laboratory and their application to family-directed drug discovery.

L78

Novel Methods for Molecular Similarity Analysis and Ligand-based Virtual Screening

J. Bajorath

Department of Life Science Informatics, B-IT, Rheinische Friedrich-Wilhelms-Universität. Dahlmannstr. 2, D-53113 Bonn, Germany

Different methodologies are introduced for the evaluation of molecular similarity relationships and application in large-scale virtual screening. Mapping algorithms are designed to map database compounds to activity-dependent consensus positions in chemical space or, alternatively, to combinations of activity class-specific descriptor value ranges. These algorithms operate in high-dimensional chemical space representations generated from binary-transformed or original molecular descriptors. During compound mapping, the dimensionality of reference spaces is gradually increased in order to remove inactive compounds from groups of molecules sharing similar activity. In virtual screening trials, mapping algorithms produce significant hit and recovery rates and recognize remote molecular similarity relationships. Active molecules are often enriched in small selection sets consisting of only 10-50 database compounds. Furthermore, novel molecular fingerprints are designed that incorporate compound class-specific features. In similarity search calculations based on multiple active template compounds, these fingerprints produce encouraging results on structurally diverse activity classes.

L79

The Discovery of the Cholesterol Absorption Inhibitor Ezetimibe

W. Greenlee, D. Burnett, M. Caplen, S. Rosenblum, J. Clader, T. Huynh, A. Afonso, H. Davis, M. Van Heek, N. Yumibe, K. Alton, E. Veltri

Schering-Plough Research Institute, Kenilworth, NJ 07033, USA

Cardiovascular disease (CVD), especially heart attack and stroke, is a major cause of mortality in the United States and Western Europe. Elevated cholesterol is recognized as a major risk factor for CVD, and the use of cholesterol biosynthesis inhibitors (statins) to reduce LDL cholesterol levels is now well-established. Nevertheless, there is a continuing need for development of new agents for treatment of elevated cholesterol. During a program to discover inhibitors of the enzyme ACAT, a series of cholesterol absorption inhibitors was identified. Characterization of SAR in this series led to the discovery

of the potent cholesterol absorption inhibitor ezetimibe as a development candidate. As monotherapy (Zetia™) or in fixed-dose combination with the statin Zocor™ (Vytorin™), ezetimibe is effective in lowering LDL cholesterol, and is well-tolerated. The discovery of ezetimibe, and studies leading to the elucidation of its mechanism of action, will be presented.

L80

Opening the gate to novel ion channel modulators and innovative anti-arrhythmics

P. Blom, D. Leysen

Department of Medicinal Chemistry, Devgen, Technologiepark 30, 9052 Zwijnaarde, Belgium

Anti-arrhythmic research has failed to produce any major innovations over the last 25 years. Current therapies are poorly effective and prone to life threatening side effects unless administered in a clinical setting. A significant bottleneck in finding new drugs active on novel ion channel targets, such as **Kv4.3,** is the availability of cost-effective, high-throughput but **functionally relevant** screens. Devgen's **proprietary** technology overcomes this constraint through whole animal ion channel screening in *C. elegans*.

Using this screening technique, Devgen identified **novel** classes of Kv4.3 inhibitors. Two series were selected and optimized for inhibitory **potency** (< 1 μM) for I_{to} in human atrial tissue, and **selectivity** versus I_{Na}, I_{Kr} and I_{Ca}.

The screening technology, specific approaches adopted in medicinal chemistry and the compelling data obtained in several animal models of cardiac safety and efficacy will be presented.

This work was supported by an IWT grant: IWT 020385

L81

New Screening and Hit Characterization Strategies for Natural Products-Based Drug Discovery

F. Koehn

Natural Products Discovery- Chemical and Screening Sciences, Wyeth Research, Pearl River, NY, USA

The drug discovery environment of today often calls for rapid screening, hit characterization and hit-to-lead development. Traditional natural products programs based on extract library screening, bioassy-guided isolation and structure elucidation are often challenged to meet the shortened timelines imposed by today's environment. Moreover, the unique attributes of natural products libraries call for somewhat different screening strategies and hit characterization methods from those used in the past. These approaches, when combined with important new developments in analytical technology, make it possible for natural products programs to meet the sense of urgency found in the modern drug discovery programs. This talk will describe new approaches which greatly accelerate the major components of natural products lead discovery including library generation, hit validation, dereplication, and structure elucidation.

L82

Bioassay-Guided Isolation Procedures For The Discovery Of Drug Canditates From Plants

E. Yeşilada

Yeditepe University Faculty of Pharmacy, Kayışdağı 34755 Istanbul/Turkey

A great diversity of compounds are synthetized in plant kingdom through photosynthesis. Random screening of plants with the aim of discovering -new drug candidates or ingredients, is an expensive and tedious task. Though recent techniques, such as semi-random or high throughput screening tests and computational techniques have been helpful up to date, a limited success was achieved.

Traditional remedies are regarded as reliable and effective sources for the discovery of drug canditates from plants. Bioassay-guided fractionation and isolation procedures (BGFI) which comprise successive applications of chemical, chromatographical and pharmacological (in vitro or in vivo) procedures, are tools presently used for investigating and evaluating the therapeutical potential of plants.

Turkey has a rich flora as well as cultural diversity which yielded the accumulation of a notable tradition originated remedic information. Several chemical and pharmacological studies have been conducted in order to evaluate this wealth of information and through application of BGFI procedures a number of molecules were isolated with anti-inflammatory, antinociceptive, antidiabetic, anti-Helicobacter, IL-2 inducing, antihepatotoxic, antiulcerogenic, antioxidant or antiviral effects. The potential of these molecules as drug candidates will be discussed.

L83

Exploiting Plasticity in the Nuclear Receptor Ligand Binding Domain for Drug Discovery

T. Willson[1], R. Nolte[2], L. Wang[2], R. Xu[2], N. Campobasso[2], K. Madauss[2], A. Miller[2], M. Lambert[2], S. Williams[2]

[1]High Throughput Chemistry, and [2]Computational, Analytical and Structural Sciences, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA

The Nuclear Receptors (NRs) are a family of ligand-regulated transcription factors that are effective drug targets for a wide range of human diseases from osteoporo-

sis to asthma to diabetes [1]. The Human genome contains four dozen NRs. Among the best characterized are the estrogen, androgen, and glucocorticoid receptors that function as cellular targets for sex steroid and corticosteroid hormones. There are also many orphan NRs for which the cognate hormone was unknown at the time of their identification. We have solved high resolution X-ray crystal structures of the ligand binding domain for most of the NRs, including all of the steroid hormone receptors and many of the orphan receptors. Our structures revealed the ligand binding domain in different states of activation: bound to agonists, antagonists or in the absence of a ligand. Conformational changes were seen that define the molecular relationship of ligand structure to functional activity and explain the promiscuity of certain receptors for ligands of varying sizes. Examples will be presented where we have exploited insights into the plasticity of the ligand binding domain to design new ligands with improved receptor selectivity and modified functional activity.

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L84

Lead-discovery at fluxional sites in proteins through Tethering

D. Erlanson

Sunesis Pharmaceuticals, Inc., 341 Oyster Point Blvd., South San Francisco, CA 94080 USA

Tethering, a fragment-based lead discovery technology, experimentally identifies fragments that bind site-specifically to a target of interest. A fragment's affinity for the protein target is boosted by a reversible covalent bond, allowing even weak-binding compounds be identified through mass spectrometry. Structural characterization of fragment-protein complexes often reveals new and unanticipated modes of binding that exploit previously unrecognized conformational plasticity on the protein. A second-generation technology, Tethering with Extenders, facilitates not only identification of fragments but also their assembly into inhibitors using dynamic combinatorial chemistry. I will discuss how we have used these methods to discover potent small molecule inhibitors of IL-2, proteases, and kinases.

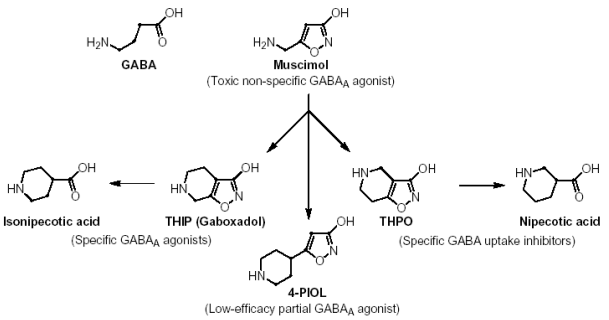
L85

Conversion of the Mushroom Toxin Muscimol into THIP (Gaboxadol), a Novel Type of Hypnotic and Non-Opioid Analgesic

P. Krogsgaard-Larsen

Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences 2, Universitetsparken, DK-2100 Copenhagen Ø, Denmark

Muscimol is a heterocyclic 3-isoxazolol bioisostere of GABA, showing high affinity for the GABAA receptors. Muscimol is toxic, but it is an interesting lead structure for the design of specific GABAergic drugs. THIP is a specific GABAA agonist, whereas THPO is a specific GABA uptake inhibitor. The isosteric amino acids isonipecotic acid and nipecotic acid were shown to possess potent and highly selective GABAA agonist and GABA uptake inhibitor effects.



THIP is a partial GABAA agonist showing different levels of efficacy at GABAA receptors with different subunit combinations. The zwitterionic structures of muscimol, THIP, and THPO do not prevent these compounds from entering the brain after systemic administration. Zwitterionic THIP enters the brain very quickly, even after oral administration. THIP is well tolerated in man. THIP (under the name Gaboxadol) is now in phase III clinical trials as an agent showing potent non-opioid analgesic effects and an ability to re-establish normal sleep architecture in patients with disturbed sleep patterns.

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Small Molecule-Kinase Interaction Maps as a Tool for Drug Discovery

S. Bhagwat

Ambit Biosciences, 4215 Sorrento Valley Boulevard, San Diego, CA 92121, U.S.A.

Kinases are currently one of the most important classes of drug targets. A large majority of kinase inhibitors

are designed to inhibit substrate phosphorylation by binding to the ATP-site of the enzyme. An inhibitor designed for one kinase, therefore, is found to inhibit a number of other kinases depending on how well it can fit into the ATP-site of other kinases. This promiscuity of kinase inhibitors becomes a critical parameter during lead optimization and calls for extensive profiling of compounds in a large panel of kinases for driving the structure activity relationship (SAR) towards inhibitors with a more desirable specificity profile. Additionally, the inherent promiscuity of kinase inhibitors binding to the ATP-site is an ideal rationale for the application of chemogenomics to discover inhibitors of novel kinases and to discover novel scaffolds to overcome intellectual property issues. Thus, application of chemogenomics with profiling compounds in a broad panel of kinases helps address as well as harness the promiscuity of kinase inhibitors.

KinomeScan, a phage display technology developed by Ambit, allows one to rapidly profile individual compounds as well as kinase-focused libraries in a panel of over 225 kinases. The resulting kinase interaction profile, which can be displayed in the form of a heat-map, is a powerful tool for understanding SAR across the entire panel of kinases. This presentation will describe how KinomeScan, coupled with the power of chemogenomics, can be used to discover novel kinase inhibitors. The discovery of potent, specific and orally efficacious inhibitors of Type-III receptor tyrosine kinases (RTKs) will be described to highlight the application of this approach. This promiscuity of kinase inhibitors becomes a critical parameter during lead optimization for driving the structure activity relationship (SAR) towards inhibitors with a more desirable specificity profile as well as for identifying structural features responsible for inhibiting novel kinases, both of which require extensive kinase profiling. Additionally, the inherent promiscuity of kinase inhibitors binding to the ATP-site becomes an ideal rationale for the application of chemogenomics to discover inhibitors of novel kinases or to discover novel scaffolds to overcome intellectual property issues. Can be addressed as well as harnessed by combining the power of chemogenomics with profiling compounds in a broad panel of kinases. The issue of promiscuity of kinase inhibitors can be addressed by profiling compounds in a broad panel of kinases to help drive the SAR towards inhibitors with a more desirable specificity profile as well as to discover structural features responsible for inhibiting novel kinases. combining the rich knowledge derived from profiling compounds in a broad panel of kinases and calls for extensive profiling in a large panel of kinases.

L89

The Diarylquinoline (DARQ) series A new class of anti-tuberculosis agents

J. Guillemont*, A. Koen*, V. Peter*, L. Nacer*, J. Vincent**

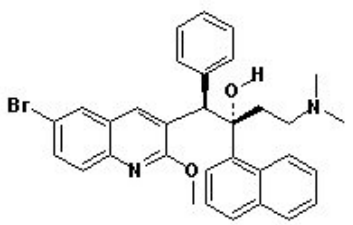
*Johnson-Johnson. **Pitié-Salpêtrière School of Medecine (France)

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* affects more than 8 million people worldwide and unfortunately kills about 2 million people annually. The World Health Organization (WHO) estimated one third of the world population to be infected with *M. tuberculosis*. The emergence of multidrug-resistant tuberculosis (MDR-TB) and its association with HIV in immuno-compromised patients have worsened the picture.

However, over the past 40 years, no major new class of anti-TB chemotherapeutics has been introduced. Consequently, there is an urgent need to identify compounds working via new mechanisms of action, to be able to reduce treatment duration, to improve the treatment of MDR-TB and to fight against latent TB.

Based on *Mycobacterium smegmatis* (MSM), a surrogate of *M. tuberculosis*, a high throughput-screening (HTS) platform has been put in place to test more than 70,000 chemical compounds. A new chemical entity giving birth to the diarylquinoline class (DARQ), (1-[6-bromo-2-methoxy-quinolin-3-yl]-4-dimethyl-amino-2-phenyl-1-phenyl-butan-2-ol, was identified to start a lead optimization process. The structure-activity relationship (SAR) study from this DARQ derivative has led to the selection of R207910 (TMC207) as the most promising candidate of this class.

With a novel mechanism of action, R207910 attacks an essential membrane-bound enzyme, ATP synthase, and consequently blocks the energy source for the bacteria. The synthesis and SAR elements of the DARQ class as well as biological activities of R207910 will be presented.



R207910 (TMC207)

L90

The Drug-Hybrid Approach to Antimalarial Chemotherapy

N. Araujo

University of the Algarve, FCT, Faculty of Science and Technology, Faro, P-8005-139, Portugal

Malaria parasite resistance development to chemotherapeutic agents has prompted the call for the use of drug combinations as standard practice in malaria chemotherapy. The current debate strongly supports the position that one component of such a combination

should be a peroxide containing artemisinin derivative due to the ability of this class to rapidly reduce parasite biomass (100,000 fold per asexual life cycle compared to 100-1000 for other available drugs).[1]

In this presentation the concept of combining two antimalarially active components within a single chemical entity will be described. Our alternative strategy is based on the ability of Fe(II) to selectively cleave the peroxide bridge, a process which appears to be restricted to the malaria parasite. We have designed synthetic routes to produce antimalarial drug hybrids that, as a function of haem (or ferrous iron dependent) peroxide cleavage, will liberate not only free radicals (artemisinin type of action) but also a second antimalarial drug with an independent mechanism of action.[2] As a paradigm for this potentially generic approach to combination chemotherapy, the presentation will focus on two classes of peroxides incorporating chalcone and peptidic cysteine protease (falcipain 2) inhibitors. The talk will conclude with a brief overview of single cell confocal imaging studies[3] that confirm the role of free iron in the antimalarial mechanism of action of synthetic and semi-synthetic endoperoxide derivatives.

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L91

Overcoming the inadequacies or limitations of experimental structures as drug targets by molecular modeling and molecular dynamics simulations

F. Gago

Department of Pharmacology, University of Alcalá, E-28871 Madrid, Spain

X-ray crystallography, NMR spectroscopy, and electron cryomicroscopy stand out as powerful structural biology tools that enable us to obtain atomic detail about biomolecules that can be potentially targeted by drugs. This knowledge is essential if virtual screening or structure-based ligand design methods are going to be used in the drug discovery effort. However, not always is the macromolecule of interest amenable to this type of experiments or, as is often the case, the conformation found experimentally cannot be used directly for docking studies because of significant changes between apo and bound forms. Furthermore, the desired insight into the binding mechanism cannot be gained sometimes because the structure of the ligand-receptor complex, not having been time-resolved, represents the end-point of the binding process and therefore retains little or no information about the intermediate stages that led to its creation.

Molecular dynamics (MD) simulations have been applied for almost 30 years now [1] to the study of biomolecular systems with the aims of sampling configuration space and better understanding both the factors that determine structural stability and relevant processes such as protein folding, ligand binding or enzymatic reactions. This field has matured significantly in recent years and strategies have been devised (e.g. activated, steered or targeted MD) to bias the trajectories in attempts to properly shape a binding pocket or simulate large-scale motions involving one or more protein domains. Application of these methods in my laboratory to medicinal chemistry and *in silico* pharmacology endeavours will be discussed [2-5].

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L92

Applications of siRNA induced gene silencing in drug discovery

W. Marshall

Dharmacon Inc. Technology and Business Development Fisher Biosciences Group, Lafayette, Colorado 80026, USA

RNA interference (RNAi) has the potential to greatly accelerate the pace of discovery biology by allowing for rapid, reliable reverse genetic analysis of gene function. In addition to applications in target discovery and validation, the method can be leveraged to streamline a variety of steps in the drug discovery process by coupling gene specific knockdown with other enabling technologies.

Innovations in sequence selection methods, modification strategies and coupling siRNA knockdown with sophisticated profiling technologies will be discussed. The development of predictive methods for creating high potency gene silencing reagents and the ability to modify them to enhance their biological properties should greatly facilitate high throughput, genome-wide gene functional analyses.

Oral Presentations

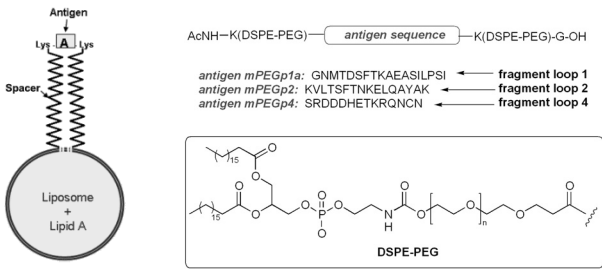
O1

Pegylated lipopeptides in liposomes as MDR therapeutic vaccine

P. Lopez[1], Claudie Madoulet[2], David Hickman[1], Pierre-François Tosi[2], Andrea Pfeifer[1], Andreas Muhs[1] & Claude Nicolau[3]

[1]ACImmune S.A., EPFL-PSE B, 1015 Lausanne, Suisse. [2]IFR53, UFR Pharmacie, 3 avenue du Maréchal Juin, 51096 Reims Cedex, France. [3]Tufts University, Friedman School of Nutrition Science and Policy, Boston, MA 02115, USA

Active immunisation of mice with P-glycoprotein-derived peptides in liposomes has shown to produce a strong immune response specific for the Pgp170 efflux pump[1]. The antibodies raised against these tetrapalmitoylated peptides were capable of reverting the MDR phenotype *in vitro* and increasing the efficiency of chemotherapy *in vivo*. With the aim to improve antigen presentation, we have introduced a polyethylene glycol linker between the peptide and the lipid anchor to mimic the loop conformation adopted by the membrane protein. In contrast to the tetrapalmitoylated antigen resting flat on the surface, in this new construct the peptide is sufficiently far located from the liposome bilayer to be independently paralleled by an enhanced and linker-lasting immune response.



Peptides bearing the extracellular sequences of the murine *mdr1* protein, with two Lys(ivDde) residues at each N- and C-terminal end, were synthesized on the 2-chloro-trityl resin using standard peptide chemistry. After chemoselective deprotection and mild cleavage from the resin, the internally protected peptides underwent site-specific conjugation to the DSPE-PEG group on the terminal free lysines. Antigen constructs obtained after side-chain deprotection and HPLC purification were characterized by MALDI-TOF mass spectrometry. Intraperitoneal inoculation to mice of these lipopegylated peptides reconstituted in liposomes containing Lipid A, elicited significant and long-lasting titers of anti-Pgp170 antibodies.

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