30th Noordwijkerhout-Camerino-Cyprus Symposium

Trends in Drug Research

May 13th - 17th, 2012

Royal Tropical Institute Amsterdam

Programme
and Abstract Book

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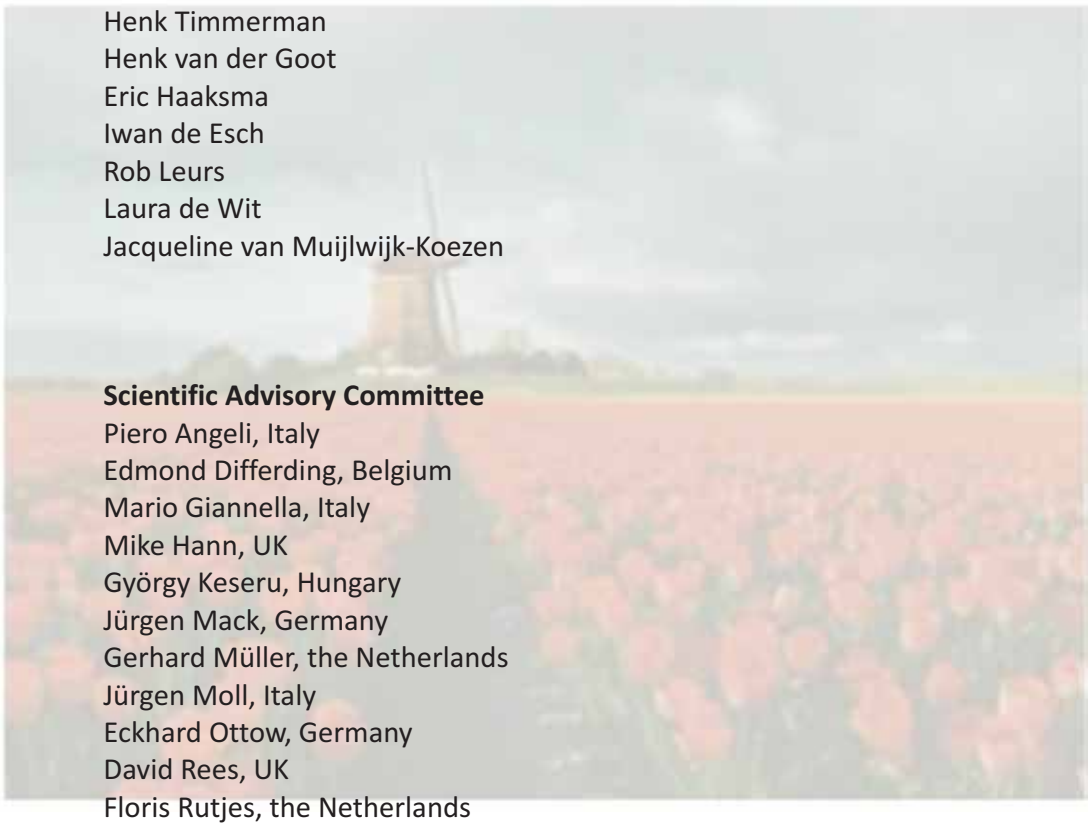

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Welcome

Welcome to the Netherlands, welcome especially to the 30th Noordwijkerhout-Camerino-Cyprus (NCC) Symposium "Trends in Drug Research". The first medicinal chemistry symposium in the Netherlands dates back to 1974, when the 4th EFMC International Symposium on Medicinal Chemistry took place in Noordwijkerhout. Thereafter the Dutch contribution to the present series started. In the 1980's a collaboration with "Camerino" started. The consortium was completed in the early years of the present century when the organizers of the Cyprus symposia joined. Currently, every year a symposium in the NCC series is organized, in the sequence, the Netherlands, Italy, Cyprus.

The programme we have prepared this year is again a mix from all disciplines which together constitute medicinal chemistry. Topics have been selected in such a way that important current issues in the processes leading to new and better medicines will get full attention. The symposia will focus on Epigenetics, New synthetic technologies, Fragment- based drug discovery, Structural biology of membrane proteins, Antibody conjugates, Drug resistance issues, Kinetic end thermodynamics aspects of drug-protein interactions. Considering the turbulent state of pharmaceutical sciences (both public and private), a special session to discuss "the medicinal chemistry arena in 2020" will be held. Interactive posters sessions complete the program.

This year we have changed the venue, from a centre surrounded by tulip fields to one in the heart of attractive old Amsterdam. Though the programme is full of interesting lectures and activities, there will be time to visit well-known spots. The centre of Amsterdam is rather small and the efficient public transportation will bring you fast to the places you selected for a visit.
On Monday evening you have the chance to attend for a low fee, a concert in the world-famous "Concertgebouw" and Wednesday evening the symposium dinner takes place in an attractive old "Heerenhuis" located at the Keizersgracht in the heart of the old city.
Tuesday evening is free for strolling individually through the city, along the canals.

The organizers appreciate the constructive and friendly cooperation with the colleagues of the teams organizing the meeting in Camerino and on Cyprus.

The organizers hope that the meeting will bring you what you expected: excellent science, great possibilities for making friends and pleasure as well, enjoying the Netherlands and its capital city Amsterdam.

Let us make together a productive, interactive and also enjoyable meeting!

The organizers

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General Information

Venue

Koninklijk Instituut voor de Tropen (KIT)

Mauritskade 63

1092 AD Amsterdam

The Netherlands

Registration information

At the registration desk, you will receive a congress bag containing the detailed final programme and abstract book, information provided by sponsors, a notepad and a pen. The package will also include a personal envelope with your badge, ticket for the concert and the symposium dinner (if applicable)

The registration desk is located in the Hall of the Koninklijk e Instituut voor de Tropen and opens on

Monday May 14 - 08.30 – 17.00

Tuesday May 15 - 09.00 – 17.00

Wednesday May 16 - 10.00 – 17.00

Thursday May 17 - 10.00 – 16.00

Conference Attendee Name Badge

At registration, together with your welcome package, you will receive your conference name badge. Your badge must be worn and visible for admittance to the sessions and other functions.

Language

The official language of the Congress is English. No simultaneous translation will be provided.

Transportation

Koninklijk Instituut voor de Tropen can be reached by :

From Central Station - tram 9 or bus 22; from Muiderpoort station - tram 3 or 7;

From Dam - tram 9 or 14; from Leidseplein - tram 7 or 10; Museumplein - tram 3

You can buy daytickets at the local transport organization (GVB) :

- Driver and conductor (only 24 hrs card)
- Ticket machine in metrostations
- VVV desk or info GVB ticket office (opposite Central Station)
- Some hotels

Number of days/hours	price
day - 24 hr	7,50
2 days 48hr	12.00
3 days - 72 hr	16,00
4 days - 96 hr	20,50

Attention!!!!!

With these public transportation tickets, you have to check in-and check out every time you get in or/ and out tram, bus and metro.

Registration package

The registration package includes:

- Admission to all scientific sessions and exhibition
- Programme and abstract book
- Certificate of attendance – on request only
- Welcome reception
- Lunches on Monday, Tuesday, Wednesday and Thursday
- Refreshment at breaks
- Wifi: free available at the venue

SOCIAL PROGRAMME

Please note that only participants and registered accompanying persons carrying a symposium badge have access to the social programme.

The social programme of the Symposium for participants and accompanying persons include:

Sunday May 13 *Social gathering* at 17.00 hr

Drinks sponsored by Mercachem

All participants are invited to the welcome reception at NH Carlton hotel – Vijzelstraat 4 – Amsterdam

Monday May 14 *Concert* (ticket required) at the Concertgebouw

19.40 - Spiegelzaal, Concertgebouw

Interview withconductor and soloist

20.15 - concert in Grote Zaal (Royal Concertgebouw)

Tram 2, 5, 16, 24, 12, bus 170

Wednesday May 16 *symposium dinner* (ticket required)

1930: - symposium dinner at Felix Meritis, Keizersgracht 324, Amsterdam

Tram 1,2,5 – stop Spui or tram 13, 17 stop Westermarkt

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SCIENTIFIC PROGRAMME

Monday May 14th, Royal Tropical Institute

8.30 – 17.00	Registration

Opening

9.00 – 9.05	Henk Timmerman, symposium chair

Forum on the MedChem 2020 arena (chairs Henk Timmerman & Dave Swinney)

9.05 – 9.15	Introduction by **Dave Swinney** (iRND3, USA)
9.15 – 9.45	**Gerhard Müller** (Mercachem, NL) *Medicinal Chemistry Services within "small pharma": from molecular understanding to complex chemistry*
9.45 – 10.15	**Anthony Wood** (Pfizer, US) *Challenges and Opportunities for Medicinal Chemistry*
10.15 – 10.30	**György Keseru** (Gedeon Richter, Hungary) *Finding the sweet spot: the role of nature and nurture in Medicinal Chemistry*
10.30 – 11.00	Discussion with **Gerhard Müller, Anthony Wood**, **Mike Hann, György Keseru** and the audience - *moderated by **Henk Timmerman** & **Dave Swinney***
11.00 – 11.30	Coffee break

Session 1: New synthetic technologies for medicinal chemistry (chair: F. Rutjes, NL)

11.30 – 11.35	Introduction by **Floris Rutjes** (Radboud University Nijmegen, NL)
11.35 – 12.15	Keynote lecture **Thomas Franch** (Nuevolution, Danmark) Bi*llion member DNA-tagged screening libraries. From theory to practical application in medicinal chemistry.*
12.15 – 12.45	**Floris Rutjes** (Radboud University Nijmegen, NL) *Recent advances in flow chemistry*

12.45 – 13.15	**Daniel Obrecht** (Polyphor, Switserland) *Medium-sized macro cyclic PEM molecules: synthetic challenges and evaluation for targeting challenging and therapeutically relevant GPCRs*
13.15 – 14.15	Lunch break & poster viewing

Session 2: Fragment-based drug discovery (chairs D. Rees, UK, I. De Esch, NL),

14.15 – 14.55	Keynote lecture: **David Rees** (Astex, UK) *The Rise of Fragment Based Drug Discovery*
14.55 – 15.25	**Mike Hann** (GSK, UK) *Molecular Obesity, potency and other addictions in medicinal chemistry.*
15.25 – 15.55	**Herbert Nar** (Boehringer Ingelheim, Germany) *Linking HTS-based and fragment-based approaches to a productive lead identification process using structural biology and biophysics*
15.55 – 16.10	**Carol Austin** (Selcia, UK) *Fragment screening using capillary electrophoresis*
16.10 – 16.25	**Gregg Siegal** (Leiden University, NL) *Effective structure determination of protein-fragment complexes using NMR*
20.15	Social program – **Concert** (ticket required) in **Royal Concert Hall**

Tuesday May 15th, Royal Tropical Institute

9.00 - 17.00	Registration
9.15 – 9.55	**Special lecture: Anette Beck-Sickinger** (Univ. Leipzig, Germany) *Peptide Drugs – State of the Art and Innovative Applications*

Session 3: Structural biology of membrane proteins (chair: Fiona Marshall, UK)

9.55 – 10.35	Keynote lecture: **Fiona Marshall** (Heptares, UK) *Structure and fragment based drug discovery applied to G-protein coupled receptors*
10.35 – 11.05	**So Iwata** (UCL, UK) *Structural studies on G-protein coupled receptors*
11.05 – 11.30	Coffee break
11.30 – 12.00	**Gebhard Schertler** (Paul Scherrer Institute, Switzerland) *Structures of constitutively active G-Protein Coupled Receptors*
12.00 – 12.30	**Chris de Graaf** (VU University Amsterdam, NL) *Fragments, Fits, Fingerprints: Structure-based virtual screening for fragment-like GPCR ligands*
12.30 – 13.00	**Marijn Sanders** (Radboud University Nijmegen, NL) *Snooker: multiple sequence alignment derived pharmacophores and its application in drug design*
13.00 – 14.00	Lunch break
14.00– 14.30	**Margot Ernst** (University of Vienna, Austria) *GABAA receptor subtypes – exciting targets for the development of clinically important drugs*
14.30 – 15.00	**Chris Ulens** (University of Leuven, Belgium) *Structural principles of agonist and modulator recognition in a bacterial GABA-gated ion channel*
15.00 – 15.15	**Rogier Smits** (Griffin Discoveries, NL) *Long receptor residence time and H_4R antagonism*
15.15 – 15.30	**Fabio Del Bello** (University of Camerino, Italy) *Might Allyphenyline and cyclomethyline become profitable multifunctional tools in the opioid addiction management?*
15.30 – 15.45	**Maikel Wijtmans** (VU University Amsterdam, NL) *Bisaryl-type Ligands: a Thin Line Between Antagonism and Agonism at Chemokine Receptor CXCR3*
15.45 – 17.00	Drinks & poster discussions with presenters (please be present at your poster!!)

Wednesday May 16th, Royal Tropical Institute

9.00 – 17.00	Registration
9.15 – 9.55	**Special lecture: Martine Smit** (VU University Amsterdam)
	Therapeutic targeting of chemokine receptors with Nanobodies (VHH immunoglobulin-based single variable domains)

Session 4: Target tumor therapy via antibody-drug conjugates (chair: R. Jautelat, Ger)

9.55 – 10.00	Introduction by chair **Rolf Jautelat** (Bayer, Germany)
10.00 – 10.40	Keynote lecture: **Hans-Georg Lerchen** (Bayer, Germany)
	Antibody Drug Conjugates: Challenges in the interface between proteins and small molecules
10.40 – 11.15	Coffee break
11.15 – 11.45	**Vincent de Groot** (Synthon, NL)
	Preclinical Development Progress of ADCs based on Novel Potent Payload Linker-Drug Technology
11.45 – 12.15	**David Satijn** (Genmab, Denmark)
	Development of antibody drug conjugates against Tissue Factor for the treatment of solid tumors.
12.15 – 13.15	Lunch break

Late breaking topics

13.15 – 13.45	**Eelco Ruijter** (AIMMS, VU University Amsterdam, NL)
	Multicomponent reactions – opportunities for medicinal chemistry
13.45 – 14.15	**Thiery Langer** (Prestwick, Chemical SAS France)
	3D Chemical Feature-based Pharmacophores: Efficient Tools In Lead Optimization and Profiling
14.15 – 14.30	**Kristina Orrling** (TI Pharma consortium T4-302 Swe)
	From fragments to trypanocidals – PDE inhibitors as potential new treatment of African Sleeping Sickness
14.30 – 15.00	Coffee break

Session 5: Epigenetics: from chemical biology to new therapeutics (chair: M. Hann, UK)

15.00 – 15.05	Introduction by chair **Mike Hann** (GSK, UK)
15.05 – 15.45	Keynote lecture by **John Trzupek** (Pfizer, USA)
	Chemical Probes for Epigenetics
15.45 – 16.15	**Jian Jin** (University of North Carolina, USA)
	Discovery of Chemical Probes for Methyl Writers of the Histone Code
16.15 – 16.45	**Chun-wa Chung** (GSK, USA)
	Hedging your BETs: from phenotypic screening to fragment based drug discovery

19.30	**Symposium diner** at **Felix Meritis, Keizersgracht 324** (ticket required)

Thursday, May 17th Royal Tropical Institute

9.00 – 17.00	Registration

Session 6: The drug resistance issue (chair: J. Moll, Germany)

9.15 – 9.20	Introduction by chair **Jürgen Moll** (Boehringer Ingelheim, Germany)
9.20 – 10.00	Keynote lecture: **Doriano Fabbro** (Novartis Pharma AG, Switzerland)
	Drug resistance mechanisms for kinase inhibitors
10.00 – 10.30	**Ivana Scovassi** (IGM-CNR, Italy)
	Search for anticancer drugs: A look to apoptosis but also to autophagy!
10.30 – 11.00	**Anne Gougeon** (University of Rennes, France)
	Antibiotics and bacteria in biofilms: a daily fight
11.00 – 11.30	Coffee break

Session 7: Kinetic and thermodynamic aspects of ligand binding (chair G. Keseru, Hungary)

11.30 – 11.35	Introduction by the chair **György Keseru** (Gideon Richter, Hungary)
11.35 – 12.15	Keynote lecture **Dave Swinney** (iRDN3, USA)
	The *value of understanding and applying binding kinetics to drug discovery*
12.15 – 12.45	**Glyn Williams** (Astex, UK)
	Mapping the Thermodynamic Landscape of Fragment-Based Drug Discovery
12.45 – 13.45	Lunch break
13.45 – 14.15	**Ron Dror** (DE Shaw Research, USA)
	How drugs bind and control their targets: characterizing GPCR signaling through long-timescale simulation
14.15 – 14.45	**Xavier Barril** (University of Barcelona, Spain)
	Water-shielded hydrogen bonds as structural determinants of binding kinetics
14.45 – 15.15	**Peter Brandt** (Beactica, Sweden)
	Importance of binding kinetics and Quantitative Structure-kinetics modelling
15.15 – 15.45	**Iwan de Esch** (VU University Amsterdam, NL)
	Fragment screening and growing in acetylcholine binding protein, structural insights and thermodynamic considerations
15.45 – 16.15	**Anna Tigerström** (AstraZeneca R&D, Sweden)
	Competitive single injection ITC, a tool for thermodynamic profiling of fragments
16.15	**Closing of the symposium**

Notes

ABSTRACT LECTURES

MONDAY, May 14 2012

Forum on the MedChem 2020 arena

Gerhard Müller (Mercachem, NL)

Medicinal Chemistry Services within "small pharma": from molecular understanding to complex chemistry

Within "small" medicinal chemistry focused companies it is mandatory to elaborate and mature research topics into novel offerings that anticipate the tomorrow's needs of "big pharma", thus providing a competitive edge for "small pharma".
At Mercachem, target family-centric approaches employing the privileged structure concept are pursued to offer novel chemical matter for lead finding and optimization campaigns. Today's most relevant targets cluster into densely populated families which exhibit structural and functional commonalities in terms of molecular recognition. Thorough analysis of these family-wide shared features allows for the definition of key pharmacophoric elements.
We have focused our interest on the multi-member target classes of PDEs, kinases, GPCRs, and epigenetic target proteins and analyzed the family-wide recognition elements that can be exploited for small-molecule design.
Special emphasis will be laid on the design principle of "escape from flatland", i.e. generating compounds with an increased fraction of fully saturated sp^3 carbons. This usually aligns with more complex chemistry, but is addressing one of the main shortcomings within the last 20 years of medicinal chemistry, i.e. the trend of making increasingly flat molecules.

Anthony Wood (Pfizer, US)

Challenges and Opportunities for Medicinal Chemistry

György Keseru & Mike Hann (Gideon Richter, Hungary and GSK, UK)

Finding the sweet spot: the role of nature and nurture in Medicinal Chemistry

Given its position at the heart of small-molecule drug discovery, medicinal chemistry has a key role in tackling the well-known productivity challenges in pharmaceutical research and development. In recent years, extensive analysis of successful and failed drug compounds has improved our understanding of the role of physicochemical properties in drug attrition, and clarified the difficulties in finding the 'sweet spot' in lead discovery and optimization. Here, we discuss scientific, strategic, organizational and cultural considerations that could help transform present medicinal chemistry practices by promoting the more effective use of what is already known and the wider appreciation of the risks of pursuing sub-optimal compounds.

Session 1: New Synthetic technologies for Medicinal Chemistry

Thomas Franch (Nuevolution, Denmark)

Billion member DNA-tagged screening libraries. From theory to practical application in medicinal chemistry.

Current fragment based methods typically allow only a few thousands of fragments to be screened against a target. Following such screening, any low potent fragments identified, must be optimized. This involves the further elaboration (evolution) of such fragments into bigger molecules with higher potency. The optimization is often achieved by attachment of additional chemical moieties to the originally identified fragment (*i.e.* by combination of fragments). This is a complex and often not a straight-forward process.
Since 2001, Nuevolution has researched methods for producing DNA-tagged small molecule libraries, wherein such small molecules comprises fragments and fragments in combination. The DNA is used to encode the synthetic history and chemical structure of the small molecule produced from a split-and-mix synthesis process. The use of DNA offers a unique opportunity for storage of chemical information. The DNA-tag provides essentially single-molecule detection by allowing amplification and sequencing for hit/lead identification from *in vitro* affinity screening of compound libraries in the size of millions to billions of molecules per library. During the presentation, we will discuss the lesions and scientific solutions discovered during ten years of development and application of this technique for small molecule lead discovery.

Floris Rutjes (Radboud University Nijmegen, NL)

Recent Advances in Flow Chemistry

In the fine chemical and pharmaceutical industry, conventional chemical production processes are scaled up by increasing the physical size of the reactors, resulting in time consuming and costly process optimization per scale up step. Microreactor flow technology allows optimal control over reaction conditions due to the small internal dimensions, leading to more efficient, economical and safer chemistry. Furthermore, the technology is ideal for reaction screening, since it allows testing of reaction parameters in a fast and efficient way. In the recent past, we have developed a plug-and-play microreactor platform that can be routinely used for optimization of a diverse array of chemical reactions. By scaling up the internal dimensions, followed by numbering out the reactors, the optimized reaction can be readily scaled up as well. Such a continuous flow optimization–production approach offers a fast and therefore valuable trajectory for product development and eventual manufacturing. In this contribution, an overview will be provided of the general scope and limitations of flow chemistry, with a focus on application in the pharmaceutical industry. In addition, recent advances in the development of fully automated flow equipment for the synthesis of PET-tracers will be highlighted.

Daniel Obrecht (Polyphor, Switserland)

Medium-sized macrocyclic PEM molecules: synthetic challenges and evaluation for targeting challenging and therapeutically relevant GPCRs

Protein Epitope Mimetics (PEMs) are fully synthetic, medium-sized macrocyclic peptide-like molecules designed to address challenging therapeutically relevant targets. PEM molecules mimic protein epitopes such as the -hairpin and the -helix. By integrating pharmacophores derived from peptidic natural products (e.g. peptidergic GPCR ligands, host defense peptides, natural toxins) into different PEM scaffolds, a library of PEM molecules (PEMfinder® Library) was designed, synthesized and screened on challenging GPCRs, in particular chemokine receptors. Potent hits on CXCR4, CXCR7 and CCR10 were discovered. By applying PEM Technology [1], activity, selectivity and ADMET properties of PEM hits could be optimized in rapid iterative cycles. A CXCR4 case study

which led to the discovery of POL6326 will be discussed in more detail. The chemokine receptor CXCR4 is expressed on many cell types such as stem cells, peripheral blood lymphocytes (PBL), monocytes, endothelial cells as well as tumor cells, and is implicated in several human diseases. Antagonists of CXCR4 have broad applications in stem cell transplantation, tissue repair, oncology and inflammation. POL6326 is currently in a Phase II clinical trial in hematopoetic stem cell transplantation in multiple myeloma patients, thus providing an important clinical validation of PEM Technology.

[1] J. A. Robinson, S. DeMarco, F. Gombert, D. Obrecht, *Drug Discov. Today* **2008**, *13*, 944-951.

Session 2: Fragment-based drug discovery

David Rees (Astex, UK)

The Rise of Fragment Based Drug Discovery

Mike Hann (GSK, UK)

Molecular Obesity, potency and other addictions in medicinal chemistry

Despite the increase in global biology and chemistry knowledge, the discovery of effective and safe new drugs seems to become harder rather than easier. Some of this challenge is due to increasing demands for safety and novelty, but some of the risk involved in this and the consequential attrition should be controllable if we had more effectively learnt from our failures. This presentation reflects on some of the learnings of recent years in relation to the causes of attrition.
The term Molecular Obesity is introduced to describe our tendency to build potency in the molecules by the inappropriate use of lipophilicity which leads to the premature demise of drug candidates. The tendency to use lipophilicity to drive potency and the reasons why we do this are discussed. Suggestions are made for how to control these tendencies through the use of indices and cultural influences.

Herbert Nar (Boehringer Ingelheim, Germany)

Linking HTS-based and fragment-based approaches to a productive lead identification process using structural biology and biophysics

Linking HTS-based and fragment-based approaches to a productive lead identification process using structural biology and biophysics" The fragment-based drug discovery activities at Boehringer Ingelheim will be described. Further, the organization of structural and biophysical support during hit evaluation and hit-to-lead and its project applications will be presented.

Carol Austin (Selcia, UK)

Fragment screening using capillary electrophoresis

Fragment-based screening (FBS) is now a recognised and successful complementary approach to high throughput screening. FBS requires technqiues that can detect the weak affinity fragment/target interactions and, in addition, those fragment hits need to be confirmed by more than one technique to obtain validated fragment hits. Selcia have developed a capillary electrophoresis method (CEFrag^{TM} screen) to detect weak fragment binding interactions with soluble protein targets in solution, without the need to immobilise the target protein(s).

CEFrag^{TM} is a microscale, high resolution, separation technique that can be used either as a primary screen or as an orthogonal screening tool to confirm fragment hits obtained by other methods. The principle of the technique uses CE to detect a reduction in the interaction of a probe ligand with its target protein in the presence of a binding fragment. The competitive nature of this interaction ensures that fragment "hits" bind in a defined manner and avoids the problem of false positives caused by non-specific binding. The technique is broadly applicable to a wide range of targets with low consumption of protein and other reagents. Case studies of several different targets, including protein-protein interactions, screened against the Selcia fragment library, will be presented.

Gregg Siegal (Leiden University, NL)

Effective structure determination of protein-fragment complexes using NMR

As Fragment-Based Drug Discovery has matured and been integrated into the pipelines of nearly every major pharmaceutical company, it has been applied to an ever greater array of targets. Being held under the microscope has exposed some of the weaker points in the process. One particularly challenging problem is the lack of structural information concerning protein-fragment complexes, especially during the early stages of a drug discovery project. While X-ray crystallography is most often used to obtain this information, the success rate is at best moderate with the low affinity hits generated in a fragment screen. In principle NMR, which is extremely sensitive to weak protein-fragment interactions, can be used to fill this gap. However, NMR projects based on complete determination of the protein structure are both slow and limited to proteins of moderate size. The key then is to develop a method to elucidate the NMR structure of a protein-fragment complex rapidly yet reliably.

Quite frequently the structure of a protein target (or closely related homolog) is available at the beginning of a drug discovery project. When this is the case, the available structural information can be combined with advanced isotopic labeling schemes such as that proposed by the group of Lew Kay[1], to efficiently generate constraints for structure determination. We have taken such an approach to determine the structure of small molecule fragments bound to the N-terminal ATPase domain of HSP90. The 27 kDa protein was labeled such that only the backbone (complete protein) and sidechain resonances (I, L & V residues) were NMR visible. The sequential assignment was determined in about 2 weeks. The structure of the complex was determined by docking using 15 intermolecular NOE constraints between the ligand and backbone and sidechain methyl ^{1}Hs of the protein. The NMR and crystal structures will be compared and the usefulness of the method for guiding medicinal chemistry efforts will be presented.

TUESDAY, May 15 2012

Special lecture

Annette Beck-Sickinger (Univ. Leipzig, Germany)

Peptide Drugs – State of the Art and Innovative Applications

Peptides have become most interesting therapeutic molecules. Major indications for use of peptide based therapeutics include endocrine functions, especially diabetes and obesity, infectious diseases, and cancer. Here, peptide pharmaceuticals are currently used either as agonists or antagonists to directly treat cancer, others, including peptide diagnostics and tumour targeting pharmaceuticals, use peptides to shuttle a chemotherapeutic agent or a tracer to the tumour and allow sensitive imaging or targeted therapy. In the past, proteolytic cleavage and low bioavailability have been the major limitations. Significant progress has been made in the last few years to overcome these disadvantages in peptide design such as short half-time, fast proteolytic cleavage and low oral bioavailability. The advances include peptide pegylation, lipidisation or multimerisation, the introduction of peptidomimetic elements into the sequences, and innovative uptake strategies such as liposomal, capsule or subcutaneous formulations. We recently could identify analogues of neuropeptide Y/peptide YY and ghrelin that show significant inhibition of food intake. Chemical modification like PEGylation or lipidisation significantly improved biodistribution and stability, dependent on the position of modification. In vitro and in vivo data will support the study.

Session 3: Structural Biology of membrane protiens

Fiona Marshall (Heptares, UK)

Structure and fragment based drug discovery applied to G-protein coupled receptors

Structure based drug discovery approaches, frequently used for soluble enzyme targets, are very challenging for G-protein-coupled receptors (GPCRs) due to their inherent instability outside of the cell membrane. Engineering receptors by limited mutagenesis to increase thermostability enables the production of StaRs which can be readily purified and are amenable to structural approaches including biophysical/fragment screening and X-ray crystallography. Here we will present examples of how such approaches have been used for a number of GPCRs including the adenosine A_{2A} receptor and the orexin OX_2 receptor. Fragment Screening results for a number of StaR proteins will be discussed. Chemical optimization of representative fragment hits to give a potent hit series will be outlined. An overview of a method called Biophysical Mapping developed to

identify the binding mode of hits without the need for a co-crystal structure will also be given, showing how fragments can be positioned within the receptor's binding site.

So Iwata (UCL, UK)

Structural studies on G-protein coupled receptors

We have recently successfully solved two G-protein coupled structures, namely histamine H1 receptor and the adenosine A2A receptor complex with an antibody Fab fragment. My close collaborator has also determined the structure of M2 muscarinic acetylcholine receptor, which is an aminergic receptor and closely related to histamine H1 receptor. In my talk, I will discuss the molecular basis of H1 receptor antagonist (antihistamine) specificity against the receptor in comparison with M2 muscarinic acetylcholine receptor and a new strategy to modulate the activity of G-protein-coupled receptors using antibody fragments.

Gebhard Schertler (Paul Scherrer Institute, Switserland)

Structures of constitutively active G Protein Coupled Receptors

Constitutively active mutants are ubiquitously found among GPCRs and increase the inherent basal activity of the receptor, which often correlates with a pathological outcome. Here, we have used the M257Y$^{6.40}$ constitutively active mutant of the photoreceptor rhodopsin in combination with the specific binding of a C-terminal fragment from the G protein alpha subunit (GαCT) to trap a light activated state for crystallization. The structure suggests a molecular basis for the constitutive activity of 6.40 substitutions and the strong effect of the introduced tyrosine based on specific interactions with Y223$^{5.58}$ in helix 5, Y306$^{7.53}$ of the NPxxY motif and R135$^{3.50}$ of the E(D)RY motif, highly conserved residues of the G protein binding site.
In addition have studied the structure of the β1 adrenergic receptor in several crystal forms and with several ligands. This provides important insights into ligand selectivity, and G protein activation. The third intracellular loop which is important for G protein activation is resolved in several of our structures. For the first time we have observed a conformation of the fully inactivated receptor. The comparison of different loop conformations is allowing us to give a possible molecular explanation for the phenomenon of basal activity of GPCRs. It also suggests a mechanism for a number of constitutively activating mutants in beta adrenergic receptors. We also for the have observed full and partial agonists in the beta 1 adrenergic receptor, this is important for understanding the concept of inverse agonists and partial agonists in a molecular way.

Deupi, X., Edwards, P., Singhal, A., Nickle, B., Oprian, D., Schertler, G. & Standfuss, J. **Stabilized G protein binding site in the structure of constitutively active metarhodopsin-II.** Proceedings of the National Academy of Sciences in press
Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, Oprian DD, Schertler GF: **Crystal structure of constitutively active rhodopsin: How an agonist can activate its GPCR.** Nature 2011, 471:656-660
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Chris de Graaf (VU University Amsterdam, NL)

Fragments, Fits, Fingerprints: Structure-based virtual screening for fragment-like GPCR ligands

The recent crystal structure determinations of druggable GPCRs has opened up excellent opportunities in structure-based ligand discovery for this pharmaceutically important protein family.[1,2] I will illustrate the problems and possibilities of *in silico* prediction of GPCR-ligand interactions[3] using recent challenging studies on histamine receptors, key players in inflammation:

i) The identification of molecular determinants that drive histamine receptor isoform and subtype selectivity by combining experimental in-house fragment screening data, ligand-based 3D-QSAR/pharmacophore models, protein-based histamine receptor modeling studies, and *in silico* guided site-directed mutagenesis experiments.[4-7]

ii) The development of customized ligand- and structure-based virtual fragment screening approaches to identify diverse sets of novel fragment-like histamine receptor ligands, using molecular interaction fingerprint scoring techniques.[8-9]

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Marijn Sanders (Radboud University Nijmegen, NL)

Snooker: Multiple sequence alignment derived pharmacophores and its application in drug design

High throughput screening (HTS) has been the leading technique in small molecule drug discovery for the past 2 decades resulting in lead compounds in ~50% of all screening campaigns[1,2]. Lately, it has become recognized that target classes with mechanistic and structural information can be better addressed with more rational approaches. Structure based design in combination with structure activity relationship (SAR) exploration around HTS hits can guide the hit to lead optimization process in a more informed manner than solely SAR. Recent advances in sequencing technologies have led to an increase in protein sequences made available in Molecular Class-Specific Information Systems (MCSIS) like the GPCRdb[3]. We present ss-TEA[4], a method to identify specific ligand binding residue positions for any G-protein coupled receptor (GPCR), predicated on high quality sequence information and Snooker[5], a low resolution structure based approach to generate pharmacophore hypotheses for class A GPCRs.
We show that Snooker pharmacophore hypotheses reproduce literature supported binding modes for and are able to retrieve enriched sets of active compound. For the community wide GPCR dock 2010 assessment Snooker was combined with Fleksy[6] to predict the eticlopride binding mode in the human DRD3 receptor. All our 5 submitted eticlopride poses were amongst the top 10 predictions out of ~120 submitted predictions in

this competition and our top pose ranked 2nd. Furthermore, we used Snooker in combination with a ligand-(substructure)-approach to select compounds for three different receptors, tested them in a all-against-all cross screen and identified novel chemical entities (NCEs) for all three receptors.

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Werner Sieghart (University of Vienna, Austria)

GABAA receptor subtypes – exciting targets for the development of clinically important drugs

$GABA_A$ receptors are the major inhibitory transmitter receptors in the central nervous system and the site of action of benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants. They are pentameric ligand-gated chloride channels and depending on their subunit composition exhibit a distinct cellular distribution, pharmacology, and function. Recently, several benzodiazepine site ligands have been identified that selectively modulate certain $GABA_A$ receptor subtypes and elicit quite specific behavioural effects.

Using multiple homology models of the extracellular $GABA_A$ receptor domain and an unbiased docking protocol, we identified a diazepam-bound structure of the benzodiazepine site that is consistent with vast experimental evidence. Two independent virtual screening approaches based on this structure predicted potential new ligands for this site. These predictions were then confirmed experimentally, thus supporting the validity of our diazepam-bound structure of the benzodiazepine binding pocket and demonstrating its suitability for drug discovery and structure-based drug design. Our models can now be used for the *in silico* development of receptor subtype-selective drugs interacting either with the benzodiazepine binding site or with other related binding sites at these receptors. This will dramatically increase the spectrum of $GABA_A$ receptor subtypes that can be addressed experimentally and clinically and will thus have a substantial therapeutic potential

Chris Ulens (University of Leuven, Belgium)

Structural principles of agonist and modulator recognition in a bacterial GABA-gated ion channel

γ-aminobutyric acid type A ($GABA_A$) receptors are pentameric ligand-gated ion channels involved in fast inhibitory neurotransmission. $GABA_A$ receptors are allosterically modulated by therapeutically active compounds such as benzodiazepines, which have hypnotic and anxiolytic effects. We show that the bacterial pentameric ligand-gated ion channel ELIC is activated by GABA and modulated by benzodiazepines, with effects comparable to those observed at $GABA_A$ receptors. We find that benzodiazepines occupy two possible sites in ELIC. A so far undescribed intrasubunit site is adjacent to the GABA recognition site, but faces the channel vestibule. A second intersubunit site partially overlaps with the GABA site and likely corresponds to a low affinity benzodiazepine binding sites in $GABA_A$ receptors, which mediates inhibitory effects of the benzodiazepine flurazepam. Our study offers the first structural view how GABA and benzodiazepines are recognized at multiple allosteric binding sites.

Rogier Smits (Griffin Discoveries, NL)

Long receptor residence time and H_4R antagonism

There is a growing interest in studying the dissociative half-life of drug-target complexes and understanding the relation of this metric with in vivo efficacy.[1] The human histamine H_4-receptor (H_4R) is postulated to play a role in a variety of conditions including allergic asthma, itch and atopic dermatitis.[2] These are diseases that typically require treatment over an extended period of time and we have therefore optimized our H_4R antagonists to have a long dissociative half-life in an attempt to improve their efficacy and tolerability. In the course of our lead optimization work we were able to identify a long residence time H_4R antagonist (GD48) with an excellent selectivity-, DMPK- and tolerability profile and was found to have anti-inflammatory activity in a murine model of contact dermatitis at a dose much lower than some of its close analogues. The parallel study of several reference H_4R antagonists reveals that residence time varies greatly between scaffolds and suggests that this parameter may offer an explanation for the observed *in vivo* potency of H_4R antagonist JNJ7777120.

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Fabio Del Bello (University of Camerino, Italy)

Might Allyphenyline and cyclomethyline become profitable multifunctional tools in the opioid addiction management?

Opioid addiction, termed a "chronic, relapsing disease", is associated with a myriad of health and social problems and its management is an extremely important area of research. $_2$-Adrenoreceptors ($_2$-ARs), belonging to the superfamily of G-protein coupled receptors, have been demonstrated to be extremely sensitive to opioid exposure and to play a key role in opiate withdrawal symptoms. In detoxification $_2$-AR agonists, such as clonidine and lofexidine, are often clinically used to reduce the intensity of withdrawal symptoms. However, the activation of the $_{2A}$-AR subtype, triggered by these compounds, is responsible for evoked sedation and hypotension side effects. Therefore, selective $_{2C}$-AR agonists, devoid of the side effects induced by the $_{2A}$-AR stimulation, alone or in combination with opioids might represent an improvement over current therapies with clonidine-like drugs. Interestingly, depending on their preferred *extended* molecular conformation, allyphenyline (**1**) and its analogue cyclomethyline (**2**) behave as $_{2C}$-AR agonists/ $_{2A}$-AR antagonists.[1] Due to such a biological profile, **1**[2] and **2** prove to be devoid of sedative effect as well as to prevent and contrast morphine dependence at very low dose (0.05 mg/Kg) in mice. Elevate comorbidity between opioid addiction and depression emerges from several clinical studies. It is noteworthy that, unlike the clinically used clonidine, **1** and **2** are able to provide alone and at the same low dose (0.05 mg/Kg) both improvement of morphine withdrawal symptoms and potent antidepressant-like effect. The study of the enantiomers of **1** and **2** highlights that the antidepressant response is associated with an additional 5-HT_{1A} receptor (5-HT_{1A}-R) activation. **1** and **2** display favourable in vitro ADME profiles and negligible activity on the hERG channel.

1, Allyphenyline **2**, Cyclomethyline

$_{2C}$-AR agonists/ $_{2A}$-AR antagonists/5-HT_{1A}-R agonists

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Maikel Wijtmans (VU University Amsterdam, NL)

Bisaryl-type Ligands: a Thin Line Between Antagonism and Agonism at Chemokine Receptor CXCR3

The chemokine receptor CXCR3 is a G protein-coupled receptor associated with various inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis. For this reason, small-molecule ligands for the CXCR3 receptor have received considerable attention as potential therapeutics. Previously, we have developed a class of small-molecule antagonists for CXCR3.[1] A medium-throughput screen revealed an adamantane-guanidine as a hit. Interestingly, the SAR study aimed at optimizing these ligands did not only lead to the identification of bisaryl-based antagonists but also several agonists, revealing the existence of a thin line between antagonism and full agonism at the CXCR3 receptor. That is, this agonist activity could be specifically modulated by very subtle changes in ligand structure. This compound series harbors the first non-peptidergic agonist class for the peptide receptor CXCR3. In conclusion, this series of CXCR3 ligands serves as a useful tool to study CXCR3 activation in higher molecular detail.

WEDNESDAY, May 16 2012

Special lecture

Martine Smit (VU University Amsterdam)

Therapeutic targeting of chemokine receptors with Nanobodies (VHH immunoglobulin-based single variable domains

G protein-coupled receptors (GPCRs) are mostly therapeutically targeted by small molecules and not commonly by antibodies. Nanobodies are single domains derived from naturally-occurring heavy chain-only *camelids* antibodies and represent new biological tools to efficiently tackle difficult drug targets such as GPCRs. Using chemokine receptors as templates, we have generated a panel of GPCR-targeting nanobodies. After selecting nanobodies in radiolabelled chemokine binding assays, we further characterized their ability to inhibit chemokine-induced signaling. Interestingly, besides monovalent antagonistic nanobodies, bivalent formats were generated against CXCR4 and CXCR7. In vivo models of chemokine receptor-mediated processes (stem cell mobilization, cancer) demonstrated that nanobodies were able to significantly inhibit the chemokine receptor-axis. As such, nanobodies can be used to efficiently and easily target a broad range of GPCRs and represent promising new therapeutics.

Session 4: Targeted tumor therapy via antibody-drug conjugates

Hans-Georg Lerchen (Bayer, Germany)

Antibody Drug Conjugates: Challenges in the interface between proteins and small molecules

Antibody Drug Conjugates represent a promising option for cancer treatment combining the specificity of mAbs with high potentcy of cytotoxic agents. A general introduction into the field with a particular focus on the technical challenges and progress with different toxophor linker chemistries including a case study will be provided.

Vincent de Groot (Synthon, NL)

Preclinical Development Progress of ADCs based on Novel Potent Payload Linker-Drug Technology

Antibody-Drug Conjugation (ADC) is gaining momentum as a next generation antibody therapeutics approach in oncology. Tumor specific delivery of the cell-killing agent maximizes the agent's potency while avoiding damage to healthy tissue resulting in a high therapeutic window.
Synthon's duocarmycin analogs and the CC-1065 derivatives represent a class of highly potent, cell-killing, DNA-alkylating, minor groove binding agents, suitable to target solid tumors.
Synthon SpaceLink technology is a unique releasable linker technology for ADC that can couple the duocarmycin via its hydroxyl group to the antibody. The free hydroxyl group is crucial for biological activity.

SpaceLink and duocarmycins form an excellent chemical match for ADC therapy, possessing an intrinsic complementarity.
Strong in vivo Proof of Concept with Potent Payload ADCs has been obtained against multiple targets. In human tumor xenograft models, substantial efficacies have been obtained based on low, single dose treatments, with minimal or absent side effects.
We discuss applicability and several other aspects of the technology. Latest results of preclinical development progress (in vivo efficacy, safety, etc.) will be presented, including in vitro drug and ADC potencies, plasma stabilities, cathepsin cleavage kinetics, linker cyclization kinetics and in vivo therapeutic window aspects for ADCs directed against HER2 and potentially other targets.

David Satijn (Genmab, Denmark)

Development of antibody drug conjugates against Tissue Factor for the treatment of solid tumors

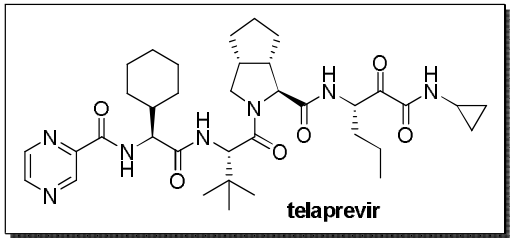
Tissue factor (TF) is a transmembrane glycoprotein that functions as the main physiologic trigger of coagulation. Under normal conditions, TF expression is limited to extravascular sites such as subendothelial layers of the vessel wall. Pathologically, however, oncogenic transformation may result in the constitutive expression of TF in cancer cells where TF has a role in tumor growth and metastasis. Therefore, TF may be an ideal target for immunotherapy in cancer.
A panel of human monoclonal antibodies has been generated and lead candidates have been characterized for TF binding, internalization, ADCC, inhibition of signaling and (limited) inhibition of blood coagulation. The internalization properties of TF make it a suitable target for an antibody-drug conjugate approach. Several anti-TF human monoclonal antibodies have been conjugated to auristatins, a class of toxins with tubulin inhibitor activity. In vitro and in vivo efficacy of these TF antibody-drug conjugates will be reviewed.

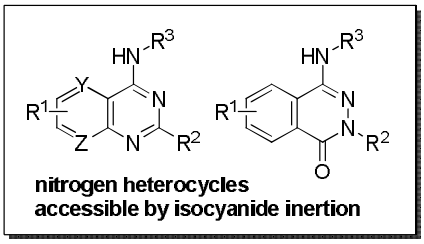
Late breaking topics

Eelco Ruijter (AIMMS, Bio-Organic Chemistry, VU University Amsterdam, NL)

Multicomponent reactions – opportunities for medicinal chemistry

The most important threats for the future of the pharmaceutical industry are the lack or R&D productivity[1] and the increasing pressure towards sustainable production.[2] Multicomponent reactions –reactions that combine three or more starting materials in one pot to give a single product that contains essentially all of the atoms of the reactants– offer many exciting opportunities for medicinal chemistry. Their most obvious application is in the drug discovery phase, where they can provide efficient access to large numbers of analogs for lead discovery or optimization. On the other hand, the inherent 'greenness' of multicomponent reactions makes them of increasing importance in the sustainable production of pharmaceuticals.
This presentation will focus on our recent efforts toward the discovery of new multicomponent reactions and other isocyanide-based reactions and their potential application in medicinal chemistry. As a showcase of what multicomponent reactions have to offer (combined with biocatalysis) in the production of complex pharmaceuticals, I will discuss our recent synthesis of telaprevir, an important new hepatitis C drug.[3] Also, our recent efforts in palladium-catalyzed isocyanide insertion reactions for the synthesis of diverse nitrogen heterocycles [4,5] will be dicussed as an indication of the potential of multicomponent (and related) reactions in lead discovery.





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Thiery Langer (Prestwick Chemical SAS, France)

3D Chemical Feature-based Pharmacophores: Efficient Tools In Lead Optimization and Profiling

Virtual screening (VS), as a complementary approach to high throughput screening (HTS) has gained considerable impact for time and cost efficient discovery of novel bioactive compounds in early stage pharmaceutical research. Several different VS approaches have been developed. Amongst them, the concept of chemical feature-based pharmacophore models has been established as one of the state-of-the-art technique for characterizing the interactions between a macromolecule and a ligand. The results of numerous case studies have been published, clearly indicating the merits of this approach for efficient hit discovery [1].
In the absence of a three-dimensional structure of the biomolecular target, feature-based pharmacophore creation from a set of bioactive molecules is a frequently chosen approach. However, structure-based pharmacophores are still lacking the reputation to be an alternative or at least a supplement to docking techniques, when the structure of the target is available. Nevertheless, screening using 3D chemical feature-based pharmacophore models as filters bears the advantage of being much faster than docking. Additionally, it transparently provides the user with relevant information that is used by the screening algorithms to characterize the ligand-macromolecule interaction.
At Inte:Ligand GmbH, the software package LigandScout [2] has been developed, as a user-friendly, rapid, and efficient tool for automatic interpretation of ligand-protein interactions and subsequent transformation of this information into 3D chemical feature-based pharmacophore models. As an extension of this approach, we have introduced parallel pharmacophore-based screening as an innovative *in silico* method to predict the potential biological activities of compounds by screening them with a multitude of pharmacophore models.[3] Using LigandScout, the entire Protein Databank has been processed, and a pharmacophore database of structure-based pharmacophore models for all targets of potential interest for drug development has been generated, in addition to ligand-based models for targets that lack information about their 3D structure.
We present an overview of this technology together with the results of an application example employing a set of antiviral compounds that were submitted to in silico activity profiling using a subset of the Inte:Ligand pharmacophore database. The results of the screening experiments show a clear trend towards correct prediction of activity profiles. In addition, using our approach one is able to obtain information about binding of the ligands under investigation also to 'anti'-targets, such as enzymes of the cytochrome P450 family[4], or to the hERG channel. Thus, off-target activity can be determined easily, giving support to the medicinal chemists in their hit-to-lead and lead optimization studies.

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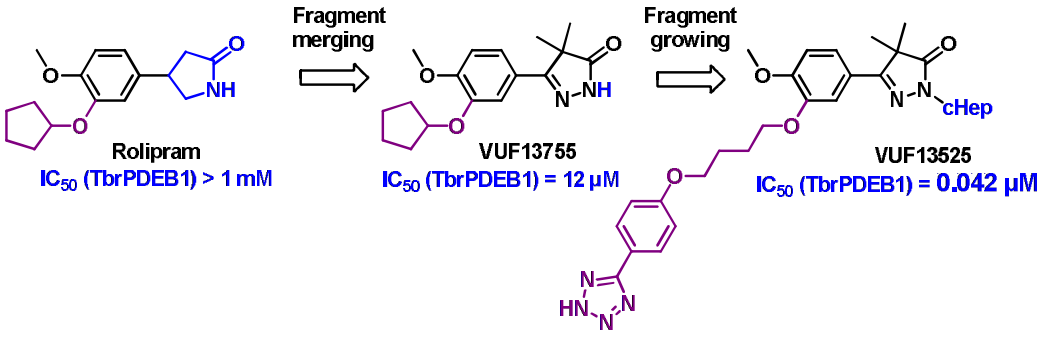
Kristina Orrling (TI Pharma consortium T4-302**,** NL**)**

From fragments to trypanocidals – PDE inhibitors as potential new treatment of African Sleeping Sickness

Human African trypanosomiasis (HAT), commonly known as African sleeping sickness, is caused by the protozoa *Trypanosoma brucei* (*Tbr*). Without treatment, all patients entering second stage HAT, spread to the CNS, will die.The available therapies are administered via intravenous or intramuscular injection, placing a heavy cost burden on production, delivery and the point of care. In addition, some are associated with life-threatening toxicity.[1]
The importance of phosphodiesterases B1 and B2 (TbrPDEB1 and TbrPDEB2) in the life cycle of *Tbr* has been validated both genetically and chemically.[2, 3] Just like the most investigated human PDE4 are TbrPDEB1 and TbrPDEB2 cAMP specific. The vast knowledge and generated expertise within the field of human PDEs provides a shortcut to inhibitors of parasitic PDEs. Scarcity in the drug research pipeline can thus be compensated with better pharmacological predictability and profound understanding of possible adverse effects. As an example, by modifying rolipram, a fragment-sized hPDE4 inhibitor, a low-affinity inhibitor was identified that led to the generation of a potent TbrPDE inhibitor (VUF13525) with trypanocidal activity.



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Session 5: Epigenetics: from chemical biology to new therapeutics

John Trzupek (Pfizer, USA)

Chemical Probes for Epigenetics

Epigenetics can be defined as heritable or acquired changes in gene expression that occur without a change in the underlying DNA sequence. In addition to its genome, each human cell also contains epigenetic information that is encoded through chemical modifications to both the DNA and histone components of chromatin. This epigenetic signature plays a key role in modulating chromatin structure and genome function, thus helping regulate the protein expression profile in the cell. Since many common diseases are driven by aberrant gene expression, it is believed that research into epigenetics has great promise to enable future drug discovery in many therapeutic areas including cancer, inflammation, diabetes, and neuroscience. This research could be significantly accelerated if suitable chemical probes for such targets were available for cell-based studies.

Pfizer is a member of a public-private partnership led by the Structural Genomics Consortium (SGC) to help identify a suite of high-quality chemical probes for epigenetic targets. This unique partnership brings the medicinal chemistry expertise within industry together with biological expertise in academia to drive basic research in an emerging area of important biology. This presentation will describe recent progress in this collaboration and highlight the discovery of novel chemical probes for epigenetic proteins that may have an important role in disease.

Jian Jin (University of North Carolina, USA)

Discovery of Chemical Probes for Methyl Writers of the Histone Code

Mounting evidence suggests that post-translational modifications (PTMs) of histones play a critical role in diverse biological processes including chromatin compaction, gene expression, transcriptional regulation, cell differentiation and organismal development. Among the 'writers' (the enzymes that produce PTMs) of the histone code, protein lysine methyltransferases (PKMTs) and protein arginine methyltransferases (PRMTs) have received great attention because of the essential function of histone methylation. The creation of a 'tool-kit' of chemical probes of PKMTs and PRMTs will permit disease hypotheses concerning these enzymes to be tested with high confidence. However, only a limited number of PKMT and PRMT inhibitors have been reported to date despite the fact that more than 50 human PKMTs and PRMTs have been identified since the first PKMT was characterized over a decade ago. In this talk, the discovery of substrate-competitive inhibitors of G9a and GLP with excellent potency and selectivity over a wide range of epigenetic and non-epigenetic targets, robust on-target activities in cells, and excellent separation of functional potency versus toxicity will be described. In addition, progress toward discovering allosteric inhibitors of PRMT3 and non-nucleoside-based cofactor-competitive inhibitors of PKMTs will also be presented.

Chun-wa Chung (GSK, USA)

Hedging your BETs: from phenotypic screening to fragment based drug discovery

Phenotypic assays are a rich source of novel targets and mechanisms for new NCEs, but improved compounds may be derived if their molecular mode of action can be determined. Using a combination of phenotypic screening, chemoproteomics, biophysical and structural studies we discovered potent small molecule inhibitors that disrupt the protein-protein interactions between the BET family of bromodomains (Brd2, 3, 4) and chromatin. These compounds have profound *in vivo* effects in inflammation and oncology models and bind within an acetyl-lysine recognition pocket that is highly conserved for ~80% of this epigenetic reader target class. Using fragment based approaches we have determined the essential binding features required for acetyl-lysine mimicry and identified chemotypes that may be used to target the wider bromodomain family. The ability to exploit both conserved and unique features of this pocket, to gain affinity and selectivity, is illustrated. This suggests that far from being an 'intractable protein-protein' module, bromodomains are amenable to fragment based as well as phenotypic drug discovery. Our experiences provide a unique opportunity to probe the benefits and disadvantages of phenotypic and fragment approaches for exploring this and other novel target classes.

THURSDAY, May 17 2012

Session 6: The drug resistance issue

Doriano Fabbro (Novartis, Switserland)

Drug resistance mechanisms for kinase inhibitors

Protein kinases act as molecular switches with remarkable plasticity and dynamics upon interaction with regulatory domains as well as modulators including kinase inhibitors (KIs). Conformation, therefore, provides a conceptual framework to understand many aspects of kinase biology.
Deregulation of protein kinase activity by mutation and/or amplification is associated with a variety of pathologies ranging from cancer to inflammatory diseases, diabetes, infectious diseases and cardiovascular and metabolic disorders. Pathologic kinase deregulation often shifts the conformation to an active “on” state, leading to constitutive signaling. Protein kinases with gain of function mutations appear to be more sensitive (and sometimes resistant) to kinase inhibtors (KIs) compared to the wt variant. Protein kinases escape resistance by either mutating key residues in their kinase domain or by pathway reactivation leading to a bypass of the targeted kinase.
Thus, one of the major challenges in kinase drug discovery today, among others, is to understand the mechanisms of resistance to kinase inhibition which will allow to develop appropriate strategies to override these various types of resistances including compounds capable of addressing the intrinsic target related drug resistance.

Ivana Scovassi (IGM-CNR, Italy)

Search for anticancer drugs: A look to apoptosis but also to autophagy!

Cancer still represents a major health problem worldwide, which urges the development of more effective strategies. Resistance toward chemotherapy remains one of the principal obstacles to the effective treatment and eradication of cancer, and is mainly related to cancer cell intrinsic failure to activate the apoptotic pathways. However, a protective effect of autophagy toward cancer cells has been recently observed, thus adding further complexity to the development of an effective approach counteracting cancer cell growth and improving the response to therapy. Basic apoptotic features, the molecular pathway of autophagy and its still controversial physiological functions will be described. Special attention will be devoted to the crucial factors governing the crosstalk between autophagy and apoptosis. Based on these considerations, the search for new anticancer drugs must imply the investigation of their pro-apoptotic properties coupled to the analysis of their impact on the autophagy process.

Anne Guogeon (University of Rennes, France)

Antibiotics and bacteria in biofilms: a daily fight

Biofilm growing bacteria can cause chronic infections, infections that induce immune response with persisting inflammation and tissue damage, increased tolerance displayed against antimicrobial agents and physical treatments, or development of biofilms on various medical devices. Some mechanisms can account for antibiotic tolerance, as failure of antibiotic to penetrate deeply in the mature biofilm, accumulation of high levels of antibiotic degrading enzymes, nutrient limitation in biofilm (bacteria are entering in a slow-growing or starved state, persisters or dormant cells), bacteria in biofilm can turn on stress response genes, or genetic changes can occur inside the biofilm (gene transfers and mutations, hypermutators).
With appropriate doses, some antimicrobial agents are able to inhibit biofilm formation, by inhibiting quorum sensing even in absence of growth inhibition as azithromycin.
Subminimal inhibitory concentrations of some antibiotics can also act as agonists of bacterial biofilm formation. Several mechanisms of action have been involved, depending on antimicrobial concentration and molecular mechanism of action. Gene expression of some efflux pumps are increased in biofilm. Metabolic stress is the key signal that could mediate biofilm induction.
A better understanding of biofilm inducibility and destruction might help us to find novel therapeutic agents synergistic with antibiotics.

Session 7: Kinetic and thermodynamic aspects of ligand binding

David Swinney (iRDN3, USA)

The *value of understanding and applying binding kinetics to drug discovery*

Binding kinetics define the rate of association (k_{on}) and dissociation (k_{off}) of a drug to its target. These rates determine a drug's affinity and are integral to a medicine's efficacy, safety, selectivity and duration of action. Consequently, binding kinetics will influence the therapeutic index and differentiate medicines. Greater understanding and application will also assist with experimental design and provide molecular descriptors for chemical optimization. The potential for mechanism-based toxicity differentiates between preferences for slow or irreversible, non-equilibrium kinetics versus fast reversible, equilibrium kinetics. Many best in class medicines with no mechanism-based toxicity evolved to have very slow binding kinetics. Some best in class medicines with the potential for mechanism-based toxicity have fast reversible kinetics that enabled physiological processes, such as competition, to minimize mechanism-based toxicity. In most cases, these best in class medicines were developed for reasons independent of their binding kinetics.

Methodologies to measure binding kinetics are improving. The awareness of the value of optimal binding kinetics to therapeutically useful drug action is increasing. However, realization of the maximum value will require a greater understanding of the molecular and chemical principles in order to design compounds with the desired kinetic properties. These principles will emerge as more binding kinetic data is generated and made available for analysis in the context of structure and function.

Glyn Williams (Astex, UK)

Mapping the Thermodynamic Landscape of Fragment-Based Drug Discovery

The emergence of experimentally-driven efforts to build potent inhibitors from weakly binding molecular fragments has had important consequences in several areas of drug design. For example, in many cases, the lack of suitable functional assays for detecting hits with millimolar affinities has led to the wider use of direct

binding methods, e.g. NMR, SPR, and ITC, some of which provide detailed thermodynamic information about molecular interactions. In addition, the simplifications imposed by fragment methods on molecular complexity have encouraged a greater focus on diversity and novelty in chemical library design, leading to the incorporation of new chemotypes in hits and leads. Finally, the drive towards generating leads with improved physical-chemical properties has encouraged the wider use of structural and computational methods to optimize and assess the value of new parameters such as ligand efficiency, lipophilic ligand efficiency and drug efficiency.
This talk will give an overview of the affinities, thermodynamic signatures and efficiencies of Astex hits and leads, generated in the course of 11 years of structure-led, fragment-based, drug discovery.

Ron Dror (DE Shaw Research, USA)

How drugs bind and control their targets: characterizing GPCR signaling through long-timescale simulation

Xavier Barril (University of Barcelona, Spain)

Water-shielded hydrogen bonds as structural determinants of binding kinetics

With growing awareness about the fundamental role of binding kinetics for drug efficacy, there is a pressing need to understand and predict structure-kinetic relationships. We have recently shown that water-shielded hydrogen bonds act as kinetic traps, a knowledge that creates opportunities to modulate the kinetic behavior of drug candidates.[1] An overview of the principle will be presented.

Peter Brandt (Beactica, Sweden)

Importance of binding kinetics and Quantitative Structure-kinetics modelling

Binding kinetics has emerged as a key parameter for understanding clinical efficacy and pharmacodynamics.[1,2,3,4] In addition, binding kinetics, as manifested by the residence time, can be of significance to target selectivity and off-target effects. However, there has been a lack of enthusiasm among medicinal chemists to add kinetic considerations to their already long list of parameters to optimize. One of the reasons is the lack of literature describing approaches for kinetics optimizations. Another reason is the lack of public datasets for method development. In fact, there are only a few datasets available describing kinetic data suitable for QSKR analysis. One of them, describing a series of compounds binding to p38 MAP kinase, was published by Regan *et al*. in 2003 (Boehringer Ingelheim Pharmaceuticals, Ridgefield).[5] Using this dataset, we are able to show that kinetic data is as amenable to QSKR modelling as affinity data are to QSAR modelling. Thus, as most of us agree that kinetics can play a role in the effect of a drug, it seems like the incorporation of binding kinetics into lead optimization should be as doable as ordinary multiobjective optimization of affinity and preclinical properties.

1. Holdgate, G. A.; Gill, A. L. *Drug Discovery Today* **2011**, *16*, 910.
2. Lu, H.; Tonge, P. J. *Current Opinion in Chemical Biology* **2010**, *14*, 467.
3. Swinney, D. C. Current *Opinion* Drug Discovery and Development **2009**, *12*, 31.
4. Tummino, P. J., Copeland, R. A. Biochemistry **2008**, 47, 5481.
5. Regan, J. *et al. Bioorganic & Medicinal Chemistry Letters* **2003***, 13*, 3101.

Iwan de Esch (VU University Amsterdam, NL)

Fragment screening and growing in acetylcholine binding protein, structural insights and thermodynamic considerations

Fragment-Based Drug Discovery (FBDD) approaches enable the efficient development of novel leads as these technologies are design intensive rather than resource intensive (as compared to high-throughput screening). Therefore, FBDD approaches are ideal for academic groups and small biotech companies. We have successfully screened our fragment library containing 1200 low molecular weight compounds on several well established FBDD targets, but this presentation will focus on a more unique target, namely Acetylcholine Binding Protein (AChBP). This water soluble protein is considered a functional and structural homolog of the ligand binding domain of trans-membrane Cys-loop receptors. AChBP is used as a bait to identify ligands for the nicotinic acetylcholine receptors (nAChRs). By combining orthogonal fragment screening, molecular modeling studies and structure-based hit optimization efforts, high affinity ligands have been obtained. The fragment optimization was guided by X-ray structures of ligand complexes and systematic thermodynamic analyses using surface plasmon resonance (SPR) biosensor analysis and isothermal titration calorimetry (ITC). Using site-directed mutagenesis and AChBP from different species, we find that specific changes in thermodynamic binding profiles, are indicative of interactions with the ligand-inducible subpocket of AChBP. This study illustrates that thermodynamic analysis can provide valuable information on ligand binding modes and is complementary to affinity data when guiding rational structure- and fragment-based discovery approaches. The studies also reveals the potential and also some limitations of using AChBP.

Anna Tigerström (AstraZeneca R&D, Sweden)

Competitive single injection ITC, a tool for thermodynamic profiling of fragments

Thermodynamic profiling of ligands with isothermal titration calorimetry (ITC) provides information about the parameters behind affinity. The driving forces for binding can be divided in enthalpic and entropic contributions, where the former parameter include contributions from specific interactions, e.g. van der Waals interactions and hydrogen bonds, whereas the latter reflects contributions which are seen as more ”unspecific”.

Data from thermodynamic profiling of fragments can be used to identify fragments with favourable enthalpy and good affinity. This information can be applied for prioritization of suitable staring points for further expansion. However, the low affinity of fragments presents a challenge in measuring the thermodynamic parameters. Low c-value titrations in ITC are commonly used as the experimental method for accessing the thermodynamics of fragments[1].
In this method the fragment is titrated with multiple injections until the protein target is fully saturated. This requires excellent fragment solubility, large amount of protein and is time consuming.

In order to address the experimental challenges an alternative method is presented. The method is based on a competition assay using a single injection of a reference ligand. Advantages as compared to low c-value titrations include rapid ranking of fragments with respect to a combination of favourable enthalpy and affinity, a higher throughput and lower protein consumption. Benchmarking towards data from multiple injection titration experiments demonstrate a good correlation of the ranking order.
The method presents an efficient tool for generation of enthalpic efficiency data for fragments. Evaluation of this information in the initial screening could potentially provide a guide as to which compounds should be selected and thereby impacting the decision-making process in drug discovery[2].

1. Turnbull, W B., *MicroCal Application Note*, **2005**.
2. Ladbury,J E et al. *Nature Reviews Drug Discovery* **2010,** 9, 23-27

ABSTRACT POSTERS NUMBERING

Poster	Presenting author	Title poster
1	Ameriks	Fragment-Based Identification of Diazinones as P2 Binding Elements in Noncovalent Cathepsin S Inhibitors
2	Andaloussi	H1/H4 dual-action antagonists: a new generation of antihistamines
3	Austin	Fragment Screening using Capillary Electrophoresis (CE)
4	Brandt	Importance of binding kinetics and Quantitative Structure-kinetics modeling
5	Brobeck Bach	Exploring the Orthosteric nACh Receptor Binding Site by Fragment Growing of the nACh Agonist DMABC
6	Custers	Bisaryl-type Ligands: from Screening Hit to CXCR3 Antagonists
7	Decamps	Synthesis of Molecules of Biological Interest. Contribution to the Synthesis of Bastadin
8	Edink	Fragment growing induces conformational changes in Acetylcholine-Binding Protein: a structural and thermodynamic analysis
9	Elinder	Better understanding of drug-target interactions through kinetic and thermodynamics analysis
10	Guo	Functional efficacy of adenosine A2a receptor agonists is positively linked to their receptor residence time
11	Hann	Finding the sweet spot. The role of nature and nurture in Medicinal Chemistry
12	Hasek	Role of protein crystallography and forced Molecular Dynamcs in inhibition of target enzymes
13	Jansen	Repositioning Drugs for use against African Sleeping Sickness
14	Kooistra	(Selective) structure-based virtual fragment screening for novel ligands of GPCRs
15	Kuhne	Fragment-Based Chemogenomics: Decorating GPCR binding surfaces with small chemical entities
16	van Linden	On the reaction of para-substituted 2-benzoyl cyclohexanones with cyanoacetamide
17	Mavromoustakos	Conformational Analysis of two novel cytotoxic C2-substituted pyrrolo[2,3-f]quinolines in organic and aqueous media, membrane bilayers and at the putative active site
18	Nascimento-Jr	Construction and validation of CCR3 and CCR4 homology models: Searching for new chemokine receptor antagonists with dual activity
19	Oakley	Novartis Horsham 2012 - A tast of new technologies adopted in a modern Medicinal Chemistry department

20	Roumen	Random Acceleration Dynamics simulations on the Histamine H1 Receptor explain experimentally measured ligand dissociation rates
21	Sanders	Snooker: Multiple sequence alignment derived pharmacophores and its application in drug design rates
22	Schultes	Mapping Histamine H4 Receptor - Ligand Binding Modes by Molecular Modeling and Site directed Mutagenesis
23	Siegal	Efficient Structure Determination of Protein - Fragment Complexes Using NMR and Site directed Mutagenesis
24	Sirci	Fragment-Based Virtual Screening: Hunting for Novel H3 Ligands via FLAP-GRID MIFs
25	Smits	Long receptor residence time and H4R antagonism
26	v d Stolpe	Knowledge recycling of PDE Inhibitors - the fast track to a new cure for African Sleeping Sickness?
27	Tigerström	New trends in drug research, competitive single injection ITC, a tool for thermodynamic profiling of fragments
28	Verheij	Fragment library screening reveals remarkable similarities between histamine H4 receptors and serotonin 5-HT_3 receptors
29	Vilums	Drug-target residence time - how to design better small molecule ligands: a case of CCR2 antagonists
30	Vlachou	Controlled release profile of new cytotoxic C2-subtituted pyrrolo[2,3-*f*] quinoline
31	Wijtmans	Bisaryl-type Ligands: a Thin Line Between Antagonism and Agonism at Chemokine Receptor CXCR3

Notes

ABSTRACT POSTERS

Fragment-Based Identification of Diazinones as P2 Binding Elements in Noncovalent Cathepsin S Inhibitors

Michael K. Ameriks[1]*, Scott D. Bembenek[1], Matthew T. Burdett[2], Ingrid C. Choong[2], James P. Edwards[1], Damara Gebauer[1], Yin Gu[1], Lars Karlsson[1], Hans E. Purkey[2], Siquan Sun[1], Robin L. Thurmond[1], Jian Zhu[1]

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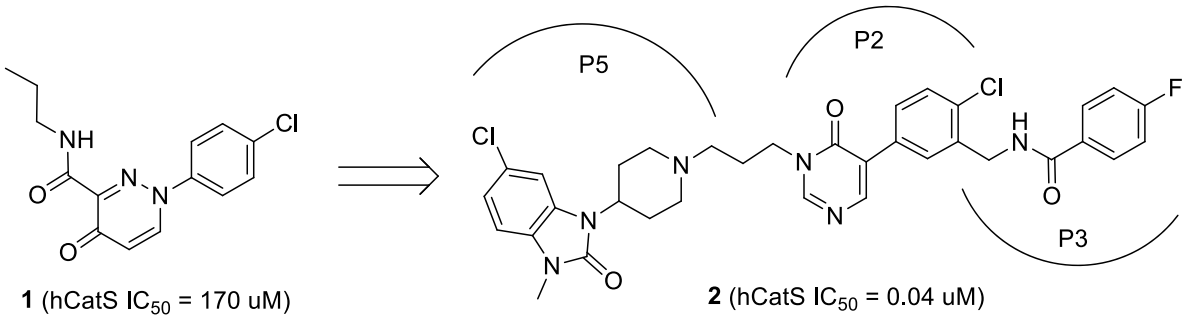
Cathepsin S (Cat S) is a cysteine protease that resides within the acidic lysosomes of hematopoietic cells and plays an important role in antigen presentation to $CD4^+$ T-cells. Thus, inhibition of CatS may help to attenuate hyperresponsiveness and provide relief from certain autoimmune disorders.[1] Recently, CatS has also been implicated in the pathophysiology of diseases related to other therapeutic areas, most notably neuropathic pain.[2] We originally identified nonpeptidic and noncovalent tetrahydropyrazolopyridine Cat S inhibitors from a virtual screen of the Janssen corporate compound collection.[3] As a complementary approach to lead generation, we also pursued a fragment-based technique known as Tethering[4], in which site-directed mutagenesis was used to introduce cysteine residues into various subsites of CatS. Each individual construct was then screened against a library of disulfide-containing compounds, and the resulting protein-ligand disulfide adducts were characterized by mass spectrometry. Herein we describe how a low affinity pyridazinone fragment **1** discovered through naïve Tethering was optimized for binding in S2 and linked to P3 and P5 groups previously identified for a tetrahydropyridinepyrazole P2 core to provide a new series of potent diazinone-based CatS inhibitors, such as **2**.[5]



1 (hCatS IC_{50} = 170 uM)

2 (hCatS IC_{50} = 0.04 uM)

(1) Gupta, S. et al., *Exp. Opin. Ther. Targets* **2008**, *12*, 291
(2) Irie, O. et al., *J. Med. Chem.* **2008**, *51*, 5502
(3) Thurmond, R.L. et al., *J. Med. Chem.* **2004**, *47*, 4799
(4) Erlanson, D.A. et al., *Proc. Nat. Acad. Sci.* **2000**, *97*, 9367
(5) Ameriks, M.K. et al., *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4060

H1/H4 dual-action antagonists: a new generation of antihistamines

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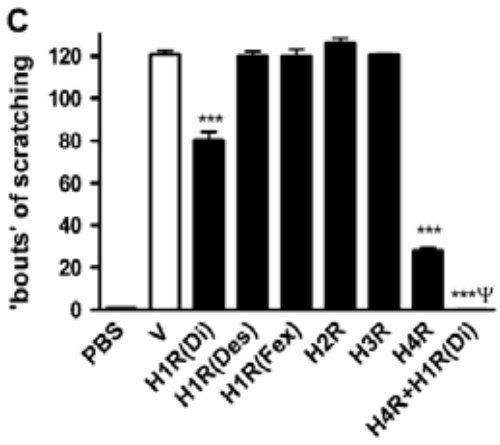
Antihistamines are histamine H_1 receptor (H_1R) antagonists currently used to treat a number of disorders. They typically block the allergic and inflammatory effects that histamine causes after it has been released from the mast cells and other sites of storage. However the histamine receptor family is larger than just the H_1R and consists of four distinct subtypes (H_1R-H_4R). Recently, evidence is accumulating in the literature that supports the concept of combination therapies consisting of a H_1R antagonist and a H_4R antagonist in a single preparation to treat histamine-induced pruritis (see figure).[1] A recent publication also described a H_1/H_3 single-ligand dual-action antagonist acting on two histamine subtypes for the treatment of allergic rhinitis.[2] Therefore, we became interested in exploring if novel H_1/H_4 dual-action antagonists could be used for the treatment of histamine induced itch. We started our lead discovery program by evaluating the H_1/H_4 affinity of a library of compounds designed to bind to both receptor subtypes. We found a set of 6 compounds based on different scaffolds that combined good affinity for both the H_1 and H_4 histamine receptors. One of these compounds (VUF11838) was evaluated in a histamine-induced itch model and demonstrated a significant additive effect compared to a selective H_4 antagonist.



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[2] Procopiou, A. *et al*, *Journal of Medicinal Chemistry*, 2011, 2183-95

Fragment Screening using Capillary Electrophoresis (CE)

Carol Austin

[1]Selcia Ltd

Fragment-based screening (FBS) is now a recognised and successful complementary approach to high throughput screening. FBS requires technqiues that can detect the weak affinity fragment/target interactions and, in addition, those fragment hits need to be confirmed by more than one technique to obtain validated fragment hits. Selcia have developed a capillary electrophoresis method (CEFragTM screen) to detect weak fragment binding interactions with soluble protein targets in solution, without the need to immobilise the target protein(s).

CEFragTM is a microscale, high resolution, separation technique that can be used either as a primary screen or as an orthogonal screening tool to confirm fragment hits obtained by other methods. The principle of the technique uses CE to detect a reduction in the interaction of a probe ligand with its target protein in the presence of a binding fragment. The competitive nature of this interaction ensures that fragment “hits” bind in a defined manner and avoids the problem of false positives caused by non-specific binding. The technique is broadly applicable to a wide range of targets with low consumption of protein and other reagents. Case studies of several different targets, including protein-protein interactions, screened against the Selcia fragment library, will be presented.

Exploring the Orthosteric nACh Receptor Binding Site by Fragment Growing of the nACh Agonist DMABC

Tinna Brøbech Bach, Christine Ussing, Anders A. Jensen, Jette G. Petersen, Stefania Ghio, Thomas Balle, and Bente Frølund

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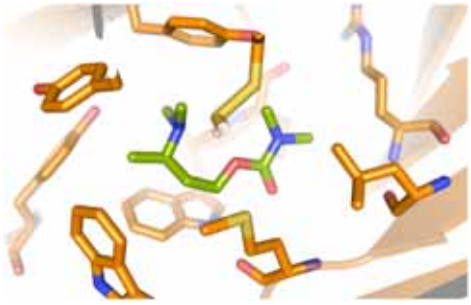
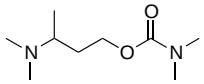
The nicotinic acetylcholine receptors (nAChRs) are expressed throughout the central and peripheral nervous systems, where they play essential roles in numerous physiological processes. Consequently therapeutic intervention in nAChR signalling has proven beneficial in many neurodegenerative and psychiatric disorders like Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia, pain and depression.

The nAChRs are one of the major receptor classes of the super family of ligand-gated ion channels (LGICs). The nAChRs are pentameric assemblies of subunits (α1-10, β1-4, γ, δ and ε), which form an ion channel either as homomeric or heteromeric receptors.

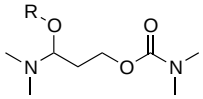
In recent years, highly valuable insight into the molecular architecture of the LGIC receptor complex has been obtained because several high-resolution x-ray crystal structures of ACh-binding proteins (AChBPs) from various snails and some bacterial ion channels have been solved. The AChBPs display significant amino acid sequence homologies with the ligand binding domain of the LGICs and are thereby excellent templates for homology models of these domains, especially the ionotropic nAChRs and $GABA_A$ receptors.

The compound, 3-(dimethylamino)butyl dimethylcarbamate (DMABC), is an acetylcholine related compound that exhibits high selectivity for neuronal over muscarinic nAChRs and significant selectivity towards the $\alpha_4\beta_2$ subtype over the $\alpha_3\beta_2$ and the α_7 subtypes. The design of previously synthesised DMABC analogues was based on docking into homology models of the nAChRs. Recently, a x-ray crystal structure of DMABC co-crystallised within AChBP was solved giving new information about the binding mode of the ligand. New DMABC analogues were designed based on the x-ray crystal structure and fragment growing was applied to give access to an additional identified cavity associated to the orthosteric binding pocket. Several analogues were chosen for synthesis based on docking scores. Pharmacological testing of the synthesized analogues in a [^{3}H]epibatidine binding assay at the $\alpha_4\beta_2$, $\alpha_3\beta_2$, and $\alpha_4\beta_4$ subtypes and a FLIPR Membrane Potential Blue assay at the $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes revealed new important information about the binding mode of the DMABC analogues.

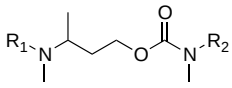




DMABC



R = alkylaryl, aryl or heteroaryl



R_1 = alkylaryl or hydroxyalkylaryl

R_2 = methyl or hydrogen

Christine Ussing et al., unpublished results

Importance of binding kinetics and Quantitative Structure-kinetics modeling

Peter Brandt [1]

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Abstract:

Binding kinetics has emerged as a key parameter for understanding clinical efficacy and pharmacodynamics.[1,2,3,4] In addition, binding kinetics, as manifested by the residence time, can be of significance to target selectivity and off-target effects. However, there has been a lack of enthusiasm among medicinal chemists to add kinetic considerations to their already long list of parameters to optimize. One of the reasons is the lack of literature describing approaches for kinetics optimizations. Another reason is the lack of public datasets for method development. In fact, there are only a few datasets available describing kinetic data suitable for QSKR analysis. One of them, describing a series of compounds binding to p38 MAP kinase, was published by Regan *et al*. in 2003 (Boehringer Ingelheim Pharmaceuticals, Ridgefield).[5] Using this dataset, we are able to show that kinetic data is as amenable to QSKR modelling as affinity data are to QSAR modelling. Thus, as most of us agree that kinetics can play a role in the effect of a drug, it seems like the incorporation of binding kinetics into lead optimization should be as doable as ordinary multiobjective optimization of affinity and preclinical properties.

(1) 1. Holdgate, G. A.; Gill, A. L. *Drug Discovery Today* **2011**, *16*, 910.

(2) 2. Lu, H.; Tonge, P. J. *Current Opinion in Chemical Biology* **2010**, *14*, 467.

(3) 3. Swinney, D. C. Current *Opinion* Drug Discovery and Development **2009**, *12*, 31.

(4) 4. Tummino, P. J., Copeland, R. A. Biochemistry **2008**, 47, 5481.

(5) 5. Regan, J. *et al. Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, 3101.

Bisaryl-type Ligands: from Screening Hit to CXCR3 Antagonists

Hans Custers, Maikel Wijtmans, Dennis Verzijl, Leontien Bosch, Martine Smit, Iwan de Esch, Rob Leurs

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Small-molecule ligands for the CXCR3 chemokine receptor receive considerable attention as a means to interrogate the roles of CXCR3 in vitro and in vivo and/or to potentially treat various conditions (inflammatory diseases, cancer). We have developed a novel class of small-molecule antagonists for CXCR3. A medium-throughput screen revealed an adamantane-guanidine (IPAG) as a hit. The guanidine unit was replaced by a small quaternary ammonium group, leading to ca. 0.7 log unit increase in affinity. Substitution of the adamantane group by a myrtenyl moiety further increased affinity, while the benzyl group was decorated with an additional (substituted) aryl ring. This led to the identification of several bisaryl-based antagonists with CXCR3 affinities of around 100 nM.[1]

(1) Wijtmans, M.; Verzijl, D.; Bergmans, S.; Lai, M.; Bosch, L.; Smit, M. J.; de Esch, I. J.; Leurs, R. *Bioorg Med Chem* **2011**, *19*, 3384.

SYNTHESIS OF MOLECULES OF BIOLOGICAL INTEREST. CONTRIBUTION TO THE SYNTHESIS OF BASTADIN.

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The metabolites are a biochemical area whose interest is in constant progression. Indeed, these molecules are biologically active and are used in therapeutic treatments. The bastadin are an ever-growing family of marine natural products isolatd from sponges of the order Verongida. This kind of molecule, are macromolecules with two amides bonds and two diphenyl ethers linkage and represent an important class of compounds recongized as potential anticancer drugs. Those compounds exhibit antifouling, antiviral, cytotoxicity, antifungal and antibacterial activities (1,2,3). It's involved in the mechanism governing the release of calcium in the cell. The latter property is very interesting and requires the industrial production of bastadin.

A complete synthesis scheme of bastadin was developed by C. Couladouros (4) in homogeneous phase, but il requires a lot of steps (20-30) with an overall yield of synthesis extremely low (3.5 %).

This work focused on the development and implementation of an original synthesis scheme of bastadin. This synthesis is made in heterogeneous phase from dopamine. Indeed, the left fragment of the bastadin can be achivied via a coupling reaction in basic medium between modified dopamine on solid support ans a diaryliodonium salt. Then, the macro cyclization of the molecule is obtained by condensation reaction of amines on the left side with acids from the right side.

(1) Fusetani, N. ; Masuda. Y; Nakato, Y.;Matsunaga, .S.; van Soest, R. W. M. Tetrahedron 2001, 57, 7507, and references therein.

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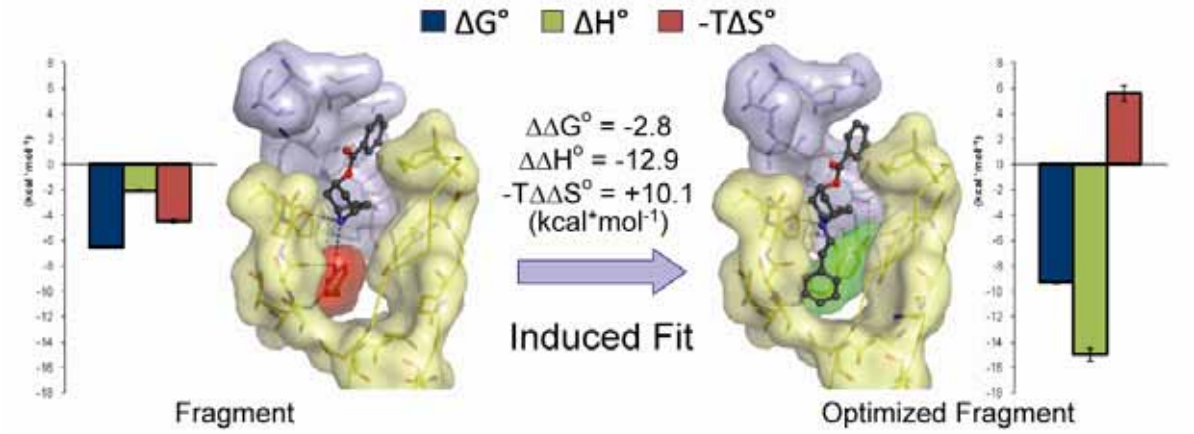
(3) Ryan C. Schoenfeld, Susan Conova, Daniel Rittschof and Bruce Ganem, Bioorganic and medicinal chemistry letters 12 (2002) 823-825

(4) Elias A. Couladouros and Vassilios I. Moustos, Tetrahedron letters 40 (1999) 7023-7030

Fragment growing induces conformational changes in acetylcholine-binding protein: A structural and thermodynamic analysis

Ewald Edink[1], Prakash Rucktooa[2], Kim Retra[1], Atilla Akdemir[1], Tariq Nahar[3], Obbe Zuiderveld[1], René van Elk[3] , Elwin Janssen[4], Pim van Nierop[3], Jacqueline van Muijlwijk-Koezen[1], August B. Smit[3], Titia K. Sixma[2], Rob Leurs[1] and Iwan J.P. de Esch[1]

[1]Leiden/Amsterdam Center of Drug Research (LACDR), Division of Medicinal Chemistry, Faculty of Sciences, VU University Amsterdam, The Netherlands, [2]Divison of Biochemistry, Netherlands Cancer Institute, The Netherlands, [3]Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, VU University Amsterdam, The Netherlands and [4]Department of Chemistry and Pharmaceutical Sciences, VU University Amsterdam, The Netherlands.
E-mail: i.de.esch@vu.nl



Optimization of fragment hits towards high affinity lead compounds is a crucial aspect of fragment-based drug discovery (FBDD). In the current study, we have successfully optimized a fragment by growing into a ligand-inducible subpocket of the binding site of acetylcholine-binding protein (AChBP). This protein is a soluble homolog of the ligand binding domain (LBD) of Cys-loop receptors. The fragment optimization was monitored with X-ray structures of ligand complexes and systematic thermodynamic analyses using surface plasmon resonance (SPR) biosensor analysis and isothermal titration calorimetry (ITC). Using site-directed mutagenesis and AChBP from different species, we find that specific changes in thermodynamic binding profiles, are indicative of interactions with the ligand-inducible subpocket of AChBP. This study illustrates that thermodynamic analysis provides valuable information on ligand binding modes and is complementary to affinity data when guiding rational structure- and fragment-based discovery approaches.[1]

(1) Edink *et al*. *J Am Chem Soc* **2011**, *133*, 5363-5371.

Better understanding of drug-target interactions through kinetic and thermodynamics analysis

Malin Elinder[1], Matthis Geitmann[1], Johan Winquist[2], U. Helena Danielson[1,2]

[1]Beactica AB, Husargatan 3, Box 567, 751 22 Uppsala, Sweden, [2]Uppsala University, Box 576, 751 23, Uppsala
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It is widely appreciated that interaction kinetics has a substantial impact on the pharmacodynamic and pharmacokinetic properties of a drug, and that kinetic parameters are a valuable source of information throughout the drug discovery process. In order to determine and evaluate the relevant kinetic parameters of a drug-target interaction, the interaction mechanism needs to be understood. Far from all interactions can be adequately described by a simple 1:1 Langmuir interaction model. When the interaction mechanism is more complex, the kinetics are, accordingly, also more complex. In a multiple-step mechanism, it is important to identify the kinetic constants that determine the kinetic characteristics of the interaction. A similar interest for thermodynamic data is emerging, although the experimental basis for interpretations of thermodynamic data and its connection to interaction characteristics is still very limited.

Our research has focused on generating kinetic data by use of surface plasmon resonance (SPR) biosensors and interpreting the ligand target interactions from a drug discovery perspective[1,2]. We have provided evidence for interaction complexities that have been overlooked by the methods conventionally used for identification of weak hits and characterization of high affinity leads. By understanding such complexities it is possible for experimentalists to set up experiments appropriately and for medicinal chemists to focus on the critical features of an interaction in order to design compounds with ideal properties for clinical efficacy.

Similarly, we have generated experimental datasets exploring the thermodynamic profiles of different ligand-target interactions[3,4,5]. They illustrate that the text book interpretations of enthalpic and entropic effects are too simplistic for rationalization of thermodynamic data, demonstrating the need for structural data and further exploration of these characteristics and their relevance for design of optimal drugs.

(1) Elinder, M. *et al*., Biochem. Pharmacol. **2010**, 80,1133-1140
(2) Geitmann, M *et al*., J. Med. Chem. **2006**, 49(8): 2375 – 2387.
(3) Geitmann, M. *et al*., Bioorg. Med. Chem. **2007**, 15; 7344-54
(4) Elinder, M. et al., Manuscript in preparation.
(5) Winquist, J. *et al*., Manuscript in preparation.

Functional efficacy of adenosine A_{2A} receptor agonists is positively linked to their receptor residence time

D. Guo, T. Mulder-Krieger, A.P. IJzerman, L. H. Heitman

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The adenosine A_{2A} receptor ($A_{2A}R$) belongs to the superfamily of G protein-coupled receptors (GPCRs) and is a promising therapeutic target. Traditionally, the discovery of novel $A_{2A}R$ agents started from assessments performed in equilibrium, largely ignoring the kinetic aspects of the ligand-receptor interaction. The aim of the study was to test the binding kinetics of $A_{2A}R$ agonists and explore a possible relationship with their functional efficacy.

In this study, we set up and validated a novel kinetic radioligand binding assay at $A_{2A}R$, namely the competition association assay, which allows an accurate measurement of the binding kinetics of unlabelled ligands. Functional efficacies of agonists were determined in a cAMP assay and, for the first time, in the novel label-free impedance-based assay. A correlation was observed between the dissociation rate of $A_{2A}R$ agonists (i.e. residence time) and their functional efficacy in both assays. Notably, the affinity of $A_{2A}R$ agonists was not correlated to their functional efficacy.

Hence, this study indicates that the molecular basis of different agonist efficacies at the $A_{2A}R$ lies within different residence times at this receptor.

Finding the sweet spot

the role of nature and nurture in Medicinal Chemistry

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Given its position at the heart of small-molecule drug discovery, medicinal chemistry has a key role in tackling the well-known productivity challenges in pharmaceutical research and development. In recent years, extensive analysis of successful and failed drug compounds has improved our understanding of the role of physicochemical properties in drug attrition, and clarified the difficulties in finding the 'sweet spot' in lead discovery and optimization. Here, we discuss scientific, strategic, organizational and cultural considerations that could help transform present medicinal chemistry practices by promoting the more effective use of what is already known and the wider appreciation of the risks of pursuing sub-optimal compounds.

Role of protein crystallography and forced molecular dynamics in inhibition of target enzymes

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Using protein crystallography, we solved 12 complexes of HIV protease and its mutants (A71V, V82T, I84V) and (L63P, A71V, V82T, I84V) with a series of chemically very similar inhibitors (K_i in ranges from 10 pM to 400 μM)[1-4]. To explain the huge differences in observed Ki by five orders of magnitude[4] induced by distant enzyme mutations and tiny inhibitor tuning, several new concepts analyzing the complex stability (entropy and hydrophobicity based) were introduced:

Enthalpy components can be easily deduced from diffraction experiment because the experimentally determined geometries of interactions are well correlated with the energy of protein-ligand interactions (i.e. ion-ion and ion-dipole interactions, strengths of H-bonds, π-π interactions, VdW interactions, etc.)

Entropy related components connected with dynamics of the system can be extracted from X-ray diffraction by several non-standard ways involving:

- New type of the "**water perturbated multisolution refinement of protein structure**" revealing **conformational flexibility and mobility** (disorder) of interacting function groups and solvent. This approach provides information on **redistribution of solvent molecules** in the first hydration shell, on **water removal** due to the ligand binding, on ligand freedom in the binding site and on changes of **water circulation near the protein surface.**
- **Conformational flexibility of function groups can stabilize or destabilize the complex** depending on their locations and orientations in the complex**.**
  - **Shock energy absorbing blocks** in a non-critical position of inhibitor molecule decrease chances of inhibitor escaping from its binding site.
  - **Energy dissipation blocks** transporting kinetic energy from active site to solvent increase generally stability of the system.
  - **Multi-step mechanism of ligand binding and release** (found e.g. in the case of HIV protease by molecular dynamics with forced withdrawal of ligand) **increases stability of the system.**
- Protein mutations near binding site influence stability of complex dominantly by direct interactions (enthalpy). **In the case of distant mutations the influence is realized namely by modulation of low frequency vibration modes.** Mutations decreasing **a number of low-energy metastable states** (mobility in the critical zone) decrease entropically stability of the protein-ligand complex and can change significantly dissipation of kinetic energy.

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REPOSITIONING DRUGS FOR USE AGAINST AFRICAN SLEEPING SICKNESs

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Drug repositioning has proven to be a valuable route to the discovery of novel treatments. Applying marketed drugs to new indications allows the costs of drug development to largely be side stepped. Clearly the binding sites of the known and novel targets must be highly similar in order to bind the same drug. This logic has encouraged the application of binding site comparison tools in the search for new opportunities for drug repositioning.

An example of drug repositioning from the field of neglected diseases is that of eflornithine. Developed as a potential cancer drug in 1977 by Merrell Toraude & Co, eflornithine found application as a topical hair growth inhibitor. The same target enzyme, ornithine decarboxylase, plays a vital role in polyamine production in *Trypanosoma brucei*.[1] This resulted in the eflornithine becoming one of the most successful treatments for African sleeping sickness to date.

Using the same drug target, or looking at the same protein family, in a different species is a way to anticipate drug site similarity. The trypanosomal Phosphodiesterases (PDEs) B1 and B2, were recently validated as new drug targets for the control of *Trypanosoma brucei* by knock down experiments.[2] This inspired the hope of repositioning drugs targeting human PDEs as trypanocidals.

A binding site comparison was performed to identify the human PDE which showed greatest overlap with the trypanosome PDEs. In this study a database of 126 published phosphodiesterase (PDE) crystal structures was compared with a homology model of a trypanosome PDEB1. The study identified human PDE4B as the most likely candidate from which to attempt to reposition drugs for use against trypanosomal PDEB1.

A new method of comparing protein binding sites was employed for this study. This involved the application of ROCs[3] to the search for similarity in protein binding sites. ROCs was designed for the comparison of small molecules. This novel implementation of ROCs proved successful at identifying pocket similarity.

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(SELECTIVE) STRUCTURE-BASED VIRTUAL FRAGMENT SCREENING FOR NOVEL LIGANDS OF GPCRS

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The recent crystal structure determinations of druggable class A GPCRs (1) have opened up excellent opportunities in structure-based ligand discovery (2) for this pharmaceutically important protein family. We have developed a customized structure-based virtual fragment screening method by combining molecular docking simulations (using PLANTS) with a protein-ligand interaction fingerprint (IFP) (3, 4) scoring method.

This method was initially validated for the histamine H1 receptor (H_1R) (5). Based on two sets of known H_1R ligands and a set of decoys, we retrospectively validated and optimized the model for a prospective screening. This optimized in silico screening approach was successfully applied (on a set of more than 100.000 fragment-like compounds) in order to identify a chemically diverse set of novel fragment-like (≤ 22 heavy atoms) H_1R ligands with an exceptionally high hit rate of 73%. Of the 26 tested fragments, 19 compounds had affinities ranging from 10 μM to 6 nM (5). The *combination* of binding mode similarity with the experimentally supported H_1R-doxepin pose (determined by IFP) and an energetically favourable H_1R-ligand configuration (assessed by the PLANTS docking score) was required to obtain this high *in vitro* hit rate of novel *in silico* predicted H_1R ligands.

Because of these principles, this method can therefore be applied to *other targets* as well in the search for (selective) novel and fragment-like ligands.

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Fragment-Based Chemogenomics: Decorating GPCR binding surfaces with small chemical entities

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The structure and function of G protein-coupled receptors are investigated using an integrative fragment-based chemogenomics platform, combining biochemical and in silico screening of an in-house library of small fragment-like molecules to more efficiently explore GPCR-ligand interaction space (when compared to drug-sized compounds). Experimental fragment screening data combined with protein modeling studies are used to map receptor binding pockets and elucidate receptor-ligand interactions. Fragment bioaffinity profiles across related and unrelated protein targets are explored to identify ligand-affinity cliffs and molecular selectivity switches, demonstrating that fragment screening can be used to gain new insights into the atomic details of (selective) protein-ligand interactions.

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ON THE REACTION OF PARA-SUBSTITUTED 2-BENZOYL CYCLOHEXANONES WITH CYANOACETAMIDE

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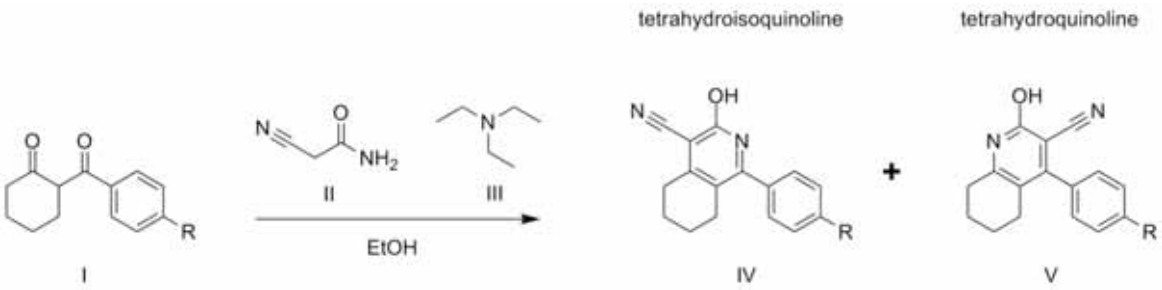
In the synthesis of a more complex heterocyclic system, we explored 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) as an important intermediate. The reaction of substituted 2-benzoyl cyclohexanone (I) with cyanoacetamide (II) and triethylamine (III) in ethanol at room temperature has been reported to lead to the formation of 3-hydroxy-1-phenyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitrile (IV) (1). However, depending on the nature of the para substituent on the phenyl ring, formation of regioisomeric 2-hydroxy-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile can compete (V).



No molecular determinants for the regiochemistry have been described. Therefore, we undertook efforts to gain more insights in the mechanism of this reaction. We investigated the effect of different para-substituents and their influence on the observed ratio between formed tetrahydroisoquinoline and tetrahydroquinoline products. For the characterization and quantification of precursors and products we used an array of techniques, including GC-MS and 2D NMR. We postulate that the electron-donating or -withdrawing properties of the para substituents directly determine the ratio of product formation.escription

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Conformational Analysis of two novel cytotoxic C2-substituted pyrrolo[2,3-f]quinolines in organic and aqueous media, membrane bilayers and at the putative active site

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We have explored the conformational analysis of two novel cytotoxic C2-substituted pyrrolo[2,3-*f*]quinolines **5e** and **5g** [1] in dimethylsulphoxide (DMSO) utilizing NOE results from NMR spectroscopy. The absence of long-range interactions suggests that both molecules adopt an extended conformation. Due to their differential cytotoxic effects, we expanded the scope of the conformational analysis by examining their conformational properties *in silico* in media that could reasonably simulate the biological environment. MD calculations in vacuum showed that the more cytotoxic molecule **5e** adopted more frequently the open conformation, while in water and DMSO **5e** solely preferred an extended conformation. Conversely, **5g** in water adopted a semi-closed conformation (V-conformation). Since these drug molecules have to pass the membrane barrier in order to exert their biological action, we have studied their ability to pass from the aqueous layer to the bilayer core. Both molecules were incorporated in the lipid bilayer core, but in a different manner. Compound **5e**, as it did in other environments, spanned in an extended conformation the entire hydrophobic bilayer core, with its long axis parallel to the alkyl bridge chains. By contrast, **5g** adopted a semi-closed V conformation with its long axis perpendicular to the alkyl chains. This finding may, in part, explain their different cytotoxic activity. By adopting this awkward orientation and anchoring in the interface, compound's **5g** permeation into the nucleus, where it is postulated to act with DNA, may be retarded. Conversely, compound **5e**, by adopting a favourable orientation and an extended conformation, spans more easily the hydrophobic core and thus readily penetrates the lipid bilayer. The docking and MD results on DNA nucleotide sequences again show that **5e** forms more favourable interactions with DNA than **5g** and causes a cross-link in its major groove. The above results may explain the difference in cytotoxicity of the two molecules. Compound **5e** on its journey to DNA finds no difficulty to pass the lipid bilayer barrier in contrast to **5g**, which anchors tightly to the membrane interface. In addition, **5e** not only reaches the target easier, but also in a more energetically favourable fashion.

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CONSTRUCTION AND VALIDATION OF CCR3 AND CCR4 HOMOLOGY MODELS: SEARCHING FOR NEW CHEMOKINE RECEPTOR ANTAGONISTS WITH DUAL ACTIVITY

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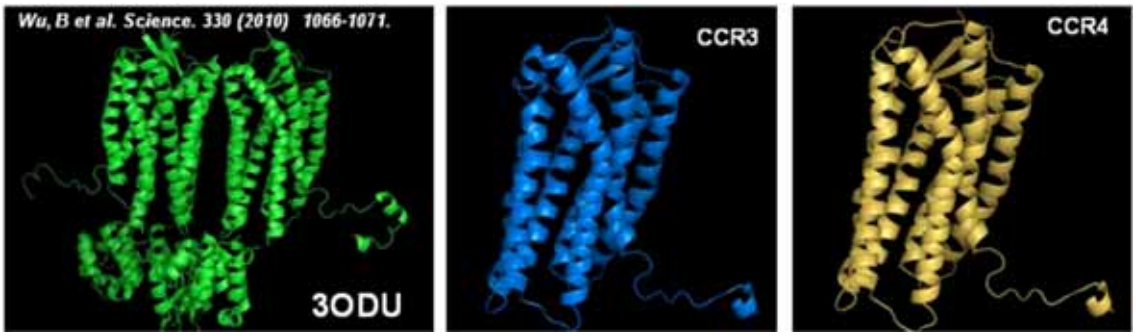
The development of crystallographic techniques has resulted in other GPCR crystallographic structures, for example: β_2-adrenergic, histamine H1 and CXCR4 receptors. Consequently, homology models based on crystallographic structures have accelerated the discovery of new ligands of interest. A good example of important results in crystalographical studies is based on the recently elucidated CXCR4 crystal structure (pdb code: 3ODU), solved with a small-molecule antagonist.[1] In this context, virtual screening has been used to filter a large number of compounds by using validated *in silico* models expected to be more reliable than earlier ones.

Among the known GPCRs, the chemokine receptors are described as being related to several important diseases and it is possible to identify around 20 of these receptors in the literature.[2] Chemokine receptors are responsible for guiding the migration of specific cells on a series of inflammatory processes, being directly involved in the following diseases: asthma, atopic dermatitis, multiple sclerosis, AIDS, COPD and cancer. Consequently, chemokine receptors are important targets for the development of new drug candidates.[3]

In this context, we aim to identify new CCR3 and CCR4 antagonists, which may be used for the treatment of asthma and atopic dermatitis, by using the respective validated homology models. For this purpose we have constructed the CCR3 and CCR4 homology models by using the CXCR4 crystalographic structure available in the Protein Data Bank.[1] The models were validated with several protein analysis tools and different scoring functions (ChemScore, GoldScore, ASP and ChemPLP) for small molecule antagonists retrieved from the literature.



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Novartis Horsham 2012 – A taste of new technologies adopted in a modern medicinal chemistry department.

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This poster describes the new technologies adopted within Global Discovery Chemistry at Novartis Horsham to attempt to accelerate the drug discovery process. Examples include the use of flow-chemistry, Lab2Lab – an automated method for submitting analytical samples and a photochemistry initiative.

Random Acceleration Dynamics simulations on the Histamine H1 Receptor explain experimentally measured ligand dissociation rates.

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The histamine H1 receptor is a G-protein coupled receptor that is targeted for the development of anti-allergic drugs (anti-histamines). Several generations of anti-histamines have been developed, each addressing problems observed for its previous generation (e.g. cognitive effects, adverse cardiac effects). Although most adverse clinical effects have been nullified, the current third generation anti-histamines possess unfavorable binding kinetics. Their fast dissociation rates imply a necessarily higher administration dose, which in turn could pose issues with pharmacodynamics and pharmacokinetics (adme) as well as toxicity.

Recently, a crystal structure was elucidated for the histamine H1 receptor (H1R) co-crystallized with a first generation anti-histamine doxepin [1]. In this study we have used this crystal structure to explain the dissociation rate of several third generation anti-histamines [2] by random acceleration molecular dynamics (RAMD) simulations in which a small acceleration is imposed on the ligand to probe ligand exit channels from the receptor. The third generation ligands investigated in this study feature a racemic center for which kinetics data indicate that the R-enantiomer dissociates slower than the S-enantiomer [2]. In addition, mutation data relates the importance of several residues to ligand dissociation rates.

The structurally stable behaviour of the H1R-doxepin complex embedded in a lipid membrane has been verified by molecular dynamics that indicate that the structural fluctuations during the molecular dynamics are well below the crystallographical uncertainty. In line with experimental kinetic measurements, the subsequent RAMD simulations of the anti-histamines show that the R-enantiomer dissociates slower from the H1R pocket than the corresponding S-enantiomer. In addition, different potential ligand exit channels have been delineated as well as several residues that influence ligand exit along these channels. Although RAMD simulations on various mutants are still ongoing, already these preliminary data prove valuable information for the optimization of third generation anti-histamines.

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Snooker: Multiple sequence alignment derived pharmacophores and its application in drug design

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High throughput screening (HTS) has been the leading technique in small molecule drug discovery for the past 2 decades resulting in lead compounds in ~50% of all screening campaigns[1,2]. Lately, it has become recognized that target classes with mechanistic and structural information can be better addressed with more rational approaches. Structure based design in combination with structure activity relationship (SAR) exploration around HTS hits can guide the hit to lead optimization process in a more informed manner than solely SAR.

Recent advances in sequencing technologies have led to an increase in protein sequences made available in Molecular Class-Specific Information Systems (MCSIS) like the GPCRdb[3]. We present ss-TEA[4], a method to identify specific ligand binding residue positions for any G-protein coupled receptor (GPCR), predicated on high quality sequence information and Snooker[5], a low resolution structure based approach to generate pharmacophore hypotheses for class A GPCRs.

We show that Snooker pharmacophore hypotheses reproduce literature supported binding modes for and are able to retrieve enriched sets of active compound. For the community wide GPCR dock 2010 assessment Snooker was combined with Fleksy[6] to predict the eticlopride binding mode in the human DRD3 receptor. All our 5 submitted eticlopride poses were amongst the top 10 predictions out of ~120 submitted predictions in this competition and our top pose ranked 2nd. Furthermore, we used Snooker in combination with a ligand-(substructure)-approach to select compounds for three different receptors, tested them in a all-against-all cross screen and identified novel chemical entities (NCEs) for all three receptors.

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Mapping Histamine H4 Receptor-Ligand Binding Modes by Molecular Modeling and Site directed Mutagenesis

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The histamine H4 receptor belongs to the G protein-coupled receptor (GPCR) family and plays an important role in immune and inflammatory responses. Several potent H4R compounds have been previously reported. In this study we focus on two H4R compound classes, the indolcarboxamides and 2-aminopyrimidines. Our aim was to reveal the binding mode of these compounds in the H4R. Based on homology models of H4R we could identify several possible binding modes. The combination with molecular dynamic (MD) simulations allowed us to analyse interaction patterns of the compounds with H4R residues in the different binding modes. In addition, we investigated the protonation state of the 2-aminopyrimidines upon H4R binding. The computational data were supported by site-directed mutagenesis (SDM) studies and indicated that both indolcarboxamides and aminopyrimidines share the same binding mode in the H4R. Finally, our data suggests double protonation of the investigated aminopyrimidines upon H4R binding as this model could best rationalize the combined SAR (structure-activity relationship) and SDM data.

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Efficient Structure Determination of Protein-Fragment Complexes Using NMR

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As Fragment-Based Drug Discovery has matured and been integrated into the pipelines of nearly every major pharmaceutical company, it has been applied to an ever greater array of targets. Being held under the microscope has exposed some of the weaker points in the process. One particularly challenging problem is the lack of structural information concerning protein-fragment complexes, especially during the early stages of a drug discovery project. While X-ray crystallography is most often used to obtain this information, the success rate is at best moderate with the low affinity hits generated in a fragment screen. In principle NMR, which is extremely sensitive to weak protein-fragment interactions, can be used to fill this gap. However, NMR projects based on complete determination of the protein structure are both slow and limited to proteins of moderate size. The key then is to develop a method to elucidate the NMR structure of a protein-fragment complex rapidly yet reliably.

Quite frequently the structure of a protein target (or closely related homolog) is available at the beginning of a drug discovery project. When this is the case, the available structural information can be combined with advanced isotopic labeling schemes such as that proposed by the group of Lew Kay[1], to efficiently generate constraints for structure determination. We have taken such an approach to determine the structure of small molecule fragments bound to the N-terminal ATPase domain of HSP90. The 27 kDa protein was labeled such that only the backbone (complete protein) and sidechain resonances (I, L & V residues) were NMR visible. The sequential assignment was determined in about 2 weeks. The structure of the complex was determined by docking using 15 intermolecular NOE constraints between the ligand and backbone and sidechain methyl ^{1}Hs of the protein. The NMR and crystal structures will be compared and the usefulness of the method for guiding medicinal chemistry efforts will be presented.

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Fragment-Based Virtual Screening:

Hunting for novel H3 ligands via FLAP-GRID MIFs[1]

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ABSTRACT

Challenges in virtual screening (VS) for fragment-like molecules are still unresolved. These challenges include sampling and scoring of small fragment docking poses in structure-based VS as well as the impact of size on ligand-based VS performance. In the current study the novel FLAP (Fingerprint of Ligands And Proteins) methodology, which is based on molecular interaction fields (MIFs), was evaluated for its ability to overcome these problems by identifying essential protein-ligand interaction features. We have compared FLAP with other ligand- and structure-based VS methods in retrospective studies to identify ligands for the histamine H_3 receptor (H_3R), a pharmaceutically relevant GPCR target. Ligand- and structure-based FLAP models were constructed, validated, and optimized using unique in-house fragment training and test sets. Optimized FLAP models were successfully used to identify novel fragment-like H_3R ligands by prospective virtual screening of 156.090 fragment-like compounds. Of the 29 tested fragments, 18 compounds had affinities for H_3R with K_i values ranging from 0.5 to 10 μM. Our H_3R studies demonstrate that customized virtual fragment screening strategies based on chemically diverse fragment training sets that include true actives *and* inactives are pivotal to overcome the challenges of *in silico* fragment screening against other protein targets in the future.

(1) **Sirci F, Istyastono EP, Vischer HF, Kooistra AJ, Nijmeijer S, Kuijer M, Wijtmans M, Mannhold R, Leurs R, de Esch IJP[2], Chris de Graaf C.** Optimizing Virtual Fragment Screening for GPCRs: Identification of Novel Ligands for the Histamine H_3 Receptor Using Ligand- and Structure-Based Molecular Fingerprints. *Manuscript submitted to the Journal of Medicinal Chemistry (April 2012)*

Long receptor residence time and H_4R antagonism.

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There is a growing interest in studying the dissociative half-life of drug-target complexes and understanding the relation of this metric with in vivo efficacy.[1] The human histamine H_4-receptor (H_4R) is postulated to play a role in a variety of conditions including allergic asthma, itch and atopic dermatitis.[2] These are diseases that typically require treatment over an extended period of time and we have therefore optimized our H_4R antagonists to have a long dissociative half-life in an attempt to improve their efficacy and tolerability. In the course of our lead optimization work we were able to identify a long residence time H_4R antagonist (GD48) with an excellent selectivity-, DMPK- and tolerability profile and was found to have anti-inflammatory activity in a murine model of contact dermatitis at a dose much lower than some of its close analogues. The parallel study of several reference H_4R antagonists reveals that residence time varies greatly between scaffolds and suggests that this parameter may offer an explanation for the observed *in vivo* potency of H_4R antagonist JNJ7777120.

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KNOWLEDGE RECYCLING OF PDE INHIBITORS – THE FAST TRACK TO A NEW CURE FOR AFRICAN SLEEPING SICKNESS?

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African sleeping sickness cause significant morbidity and mortality and seriously affects society in the poorest areas of the world. The current therapies suffer from severe side effects, inconvenient administration and high costs.[1]

In the search for an urgently needed new treatment, cyclic nucleotide phosphodiesterases (PDEs) have emerged as attractive molecular targets. For example, both genetic knock-down and chemical inhibition of PDE activity resulted in halted proliferation and eventually elimination of *Trypanosoma brucei* (Tbr), the causative agent of African sleeping sickness.[2]

The vast knowledge and generated expertise within the field of human PDEs has provided a shortcut to high-affinity inhibitors of parasitic PDEs. Scarcity in the drug research pipeline can thus be compensated with better pharmacological predictability and profound understanding of possible adverse effects. Interestingly, TbrPDEB1 and TbrPDEB2 are cAMP specific, like one of the most investigated human PDEs, hPDE4. The catalytic domains of the hPDE4 and parasitic PDEs show a high degree of homology, as well as parasite-specific features (Figure 1).[3] This has allowed fast optimization of hit compounds and generated nanomolar TbrPDE inhibitors with trypanocidal activity.

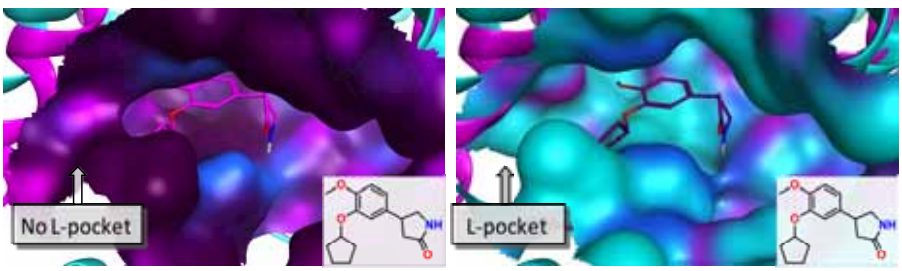

Figure 1 left) The vdW surface of the active site of hPDE4B (magenta) co-crystallized with rolipram and ***right)*** the homology model TbrPDEB1 (cyan) with rolipram superimposed and the arrow pointing at the parasite-specific P-pocket.

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New trends in drug research, Competitive single injection ITC, a tool for thermodynamic profiling of fragments

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Thermodynamic profiling of ligands with isothermal titration calorimetry (ITC) provides information about the parameters behind affinity. The driving forces for binding can be divided in enthalpic and entropic contributions, where the former parameter include contributions from specific interactions, e.g. van der Waals interactions and hydrogen bonds, whereas the latter reflects contributions which are seen as more ”unspecific”.

Data from thermodynamic profiling of fragments can be used to identify fragments with favourable enthalpy and good affinity. This information can be applied for prioritization of suitable staring points for further expansion. However, the low affinity of fragments presents a challenge in measuring the thermodynamic parameters. Low c-value titrations in ITC are commonly used as the experimental method for accessing the thermodynamics of fragments[1]. In this method the fragment is titrated with multiple injections until the protein target is fully saturated. This requires excellent fragment solubility, large amount of protein and is time consuming.

In order to address the experimental challenges an alternative method is presented. The method is based on a competition assay using a single injection of a reference ligand. Advantages as compared to low c-value titrations include rapid ranking of fragments with respect to a combination of favourable enthalpy and affinity, a higher throughput and lower protein consumption. Benchmarking towards data from multiple injection titration experiments demonstrate a good correlation of the ranking order.
The method presents an efficient tool for generation of enthalpic efficiency data for fragments. Evaluation of this information in the initial screening could potentially provide a guide as to which compounds should be selected and thereby impacting the decision-making process in drug discovery[2].

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Fragment library screening reveals remarkable similarities between histamine H4 receptors and serotonin 5-HT_3 receptors.

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Chemical genomics brings together diversity-oriented chemical libraries and information rich cellular assays with the intention of discovering novel hits for further optimization. A fragment library containing 1040 diverse compounds was screened against a wide variety of protein targets; these include an Ion channel (5-HT_3), a GPCR (Histamine H4), Acetyl Choline Binding Protein (AChBP) and a kinase (PKA). It was shown that the fragment library does not contain promiscuous binders and, in general, different targets bind different fragments. However, the fragment hit set of two receptors, namely 5-HT_3 and H4, show remarkable overlap, illustrating similarities in ligand binding and indicating selectivity issues for 5-HT3 and H4 drug development

Drug-target residence time – how to design better small molecule ligands: a case of CCR2 antagonists

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Pre-clinical models of inflammatory diseases (e.g. neuropathic pain, rheumatoid arthritis and multiple sclerosis) have pointed to a critical role of CCR2 and its ligand, CCL2. Therefore, it was suggested that CCR2 might be an attractive target for treatment of these diseases. However, one of the biggest problems of high affinity inhibitors of CCR2 is their lack of efficacy in clinical trials. In order to improve the *in vivo* efficacy, we aim to develop antagonists that have a longer residence time on the CCR2 receptor. Hence, in this study we report a new approach for the design of potent and efficacious CCR2 antagonists.

A project was started to investigate the relation of ligand's structure and its receptor residence time (i.e. SRTR), next to traditional SAR. A library of CCR2 antagonists was synthesized based on a *cis*-aminocyclopentane carboxamide scaffold. On the "left side" of the molecule we introduced substituents with different spacer lengths and different aliphatic ring systems. On the "right side" of the molecule we looked at different positions of substituents and their nature (EWG; EDG). All compounds were tested for their affinity at the CCR2 receptor. If the K_i value was <100 nM, the residence time was determined by a novel kinetic competition association assay for which a small molecule CCR2 antagonist was radiolabeled. As expected, we found that affinity is not correlated to the residence time of an antagonist.

In conclusion, this project provides additional knowledge of a ligand's receptor residence time with the prospect of better clinical candidates with increased duration of receptor-blockade *in vivo* and consequently of drugs with higher efficacy for CCR2-related diseases.

CONTROLLED RELEASE PROFILE OF A NEW CYTOTOXIC C2-SUBSTITUTED PYRROLO[2,3-*f*]QUINOLINE

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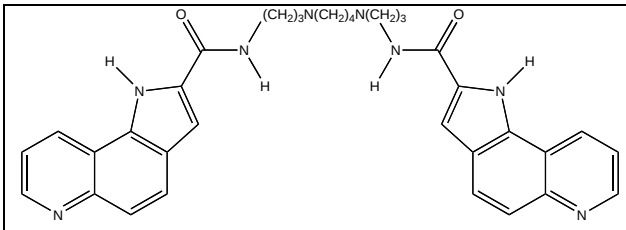
DNA cross-linking agents are known for their significant antitumor activity, which has been attributed to their ability to form irreparable base pair adducts at precisely defined genomic locations. Most of the reported cross-linking probes involve three or four aromatic chromophores in their skeleton and are of sufficient size to recognize two or three base pairs [1]. An extension of this limited sequence recognition, which is attained by the use of larger aromatic heterocycles, has been found to drastically enhance cytotoxicity due to the formation of more rigid irreversible interstrand bonds.

In the course of our program directed towards the development of new DNA-complexing agents, we have previously reported on the synthesis and cytotoxic profile of a series of C2-substituted pyrrolo[2,3-*f*]quinolones [2]. In an attempt to further explore the activity of these agents we designed and synthesized the symmetrical pyrrolo[2,3-*f*]quinoline **1**.



1: *N*2-{[3-(4-{3-[(1*H*-pyrrolo[2,3-*f*]quinoline-2-carbonylamino)] propylamino}butylamino)propyl]}-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxamide

Table 1. Formulations composition (w/w %) of cylindrical-shaped tablets

Formulation	8	10	15
Fumaric salt of **1**	1.025	1.180	0.875
HPMC K 15M	30.01	29.92	30.22
Carbopol 971P	-	-	15.90
Polyox 4x10^6	-	15.90	-
Sodium alginate	15.90	-	-
Avicel PH102	52.00	52.00	52.00
MgStr	1.00	1.00	1.00

The overall size of the skeleton of **1** has been increased, allowing the new probe to span a larger number of base pairs and possibly act as a cross-linker. Indeed, the new compound exhibited a highly selective cytotoxic action against the non-small lung cancer cell line (NSCLC-N16-L16). However, *in lieu* of its poor water solubility, its exploitation as bioactive agent in *in vivo* tests and clinical trials, is seriously hampered. It was thus intriguing to develop cylindrical-shaped solid pharmaceutical formulations of this analog and probe its controlled release in simulated aqueous gastric and intestinal media. The % release figures obtained for the fumarate salt of **1**, in both media, suggest that the new compound has a satisfactory dissolution profile, which in the cases of formulations 8, 10 and 15 (Table 1) follow zero or near zero order release kinetics.

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Bisaryl-type Ligands: a Thin Line Between Antagonism and Agonism at Chemokine Receptor CXCR3

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The chemokine receptor CXCR3 is a G protein-coupled receptor associated with various inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis. For this reason, small-molecule ligands for the CXCR3 receptor have received considerable attention as potential therapeutics. Previously, we have developed a class of small-molecule antagonists for CXCR3.[1] A medium-throughput screen revealed an adamantane-guanidine as a hit. Interestingly, the SAR study aimed at optimizing these ligands did not only lead to the identification of bisaryl-based antagonists but also several agonists, revealing the existence of a thin line between antagonism and full agonism at the CXCR3 receptor. That is, this agonist activity could be specifically modulated by very subtle changes in ligand structure. This compound series harbors the first non-peptidergic agonist class for the peptide receptor CXCR3. In conclusion, this series of CXCR3 ligands serves as a useful tool to study CXCR3 activation in higher molecular detail..

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