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Medicinal Chemistry, Dr. Max Luethi Award Lecture **67**

Immunosuppressive Drugs and their Interaction with the Efflux Transporter P-Glycoprotein (ABCB1) (Dr. Max Luethi Prize Lecture)

Simon Lang‡ and Anna Seelig*

‡Fachhochschule Nordwestschweiz, *Biophysikalische Chemie, Biozentrum, Universität Basel

The ATP binding cassette (ABC) transporter P-glycoprotein (ABCB1, MDR1) strongly contributes to multidrug resistance (MDR). In contrast to other proteins it does not recognize specific atoms or chemical structures, but specific hydrogen bond acceptor patterns which could interfere with the genetic information of the cell. The hydrogen bond acceptors are likely to be recognized by the numerous hydrogen donor groups in the TMDs of Pgp. Immunosuppressive drugs are assumed to interact with P-glycoprotein because they can alter the intestinal absorption of other drugs taken in parallel (which leads to so-called drug-drug interactions), and because over-expression of P-glycoprotein in T-lymphocytes is implicated in the failure of immunosuppressant therapy. The aim of our work was to characterize the interaction of twelve structurally most diverse, commonly used immunosuppressive drugs with P-glycoprotein. We show that substrate binding occurs in two steps, a partitioning step from water to the lipid membrane and a binding step from the lipid membrane to the transporter. We derived the free energy of transporter-water binding from ATPase activity measurements, and assessed the free energy of lipid-water partitioning by means of surface activity measurements. The free energy of transporter-lipid binding of the substrate (which reflects the direct substrate-transporter affinity) could be determined as the difference of the latter two. We showed that the substrate affinity to the transporter increased with the number of hydrogen bond acceptor patterns per compound and that the rate of transport decreased with increasing affinity to the transporter. The present results allow predicting the behavior of immunosuppressive drugs with P-glycoprotein at the intestinal barrier as well as in T-lymphocytes.

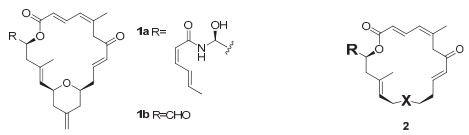
Medicinal Chemistry, Lecture **68**

Synthesis and Biological Evaluation of (–)-Zampanolide and (–)-Dactylolide Analogs

Didier Zurwerra, Florian Glaus, Jürg Gertsch, Karl-Heinz Altmann

Institute of Pharmaceutical Sciences/ETH Zürich, Wolfgang-Pauli-Str. 10, CH-8093 Zürich, Switzerland

(–)-Zampanolide (**1a**) [1] and (–)-dactylolide (**1b**) [2] are structurally related polyketide-based macrolides with a highly unsaturated 20-membered macrolactone core structure, including a *cis* 2,6-disubstituted tetrahydropyran (THP) ring. While **1a** is a marine natural product with nM antiproliferative activity, **1b** is the antipode of the natural product (+)-dactylolide with antiproliferative activity in the μM range. **1a** has been shown to be a microtubule stabilizer [3], whereas the mode of action of **1b** has not been studied.



We have recently completed the total synthesis of **1a** and **1b** and based on the chemistry developed in the course of this work we have started to investigate the SAR of these lead structures. In this contribution we will report on the chemical synthesis of a series of side chain- and THP ring-modified analogs of **1a**/**1b** (i. e. **2**) and the evaluation of their tubulin-polymerizing and cell growth inhibitory activity. These studies have led to the identification of simplified analogs of **1a** and **1b** that retain potent antitumor activity *in vitro*.

[1] Uenishi, J. *et al. Org. Lett.* **2009**, *11*, 3262 (and citations).
[2] Cutignano, A. *et al. Eur. J. Org. Chem.* **2001**, 775.
[3] Field, J. J. *et al. J. Med. Chem.* **2009**, *52*, 7328.
[4] Zurwerra, D. *et al*., submitted.

Medicinal Chemistry, Lecture **69**

Inhibitors of MK2 for the treatment of inflammatory diseases

Achim Schlapbach[1], Laszlo Revesz[1], Juraj Velcicky[1], Guido Koch[1], Henrik Moebitz[1], Clemens Scheufler[2], Markus Kroemer[2], Roland Feifel[1], Stuart Hawtin[3], Christine Huppertz[3]

[1]Novartis Institutes for BioMedical Research, Global Discovery Chemistry, CH-4002 Basel
[2]Novartis Institutes for BioMedical Research, Center for Proteomic Chemistry, CH-4002 Basel
[3]Novartis Institutes for BioMedical Research, Autoimmunity, Transplantation & Inflammation, CH-4002 Basel

The p38 pathway has been in the center of interest for anti-inflammatory drug discovery for many years, as it is crucial for the biosynthesis of TNFα, IL-1β and other mediators. Many of the anti-inflammatory effects of p38 inhibition are mediated through MK2 (MAPKAP-K2, MAPK-activated protein kinase 2), a direct downstream target of p38, making MK2 a very interesting drug target for the treatment of inflammatory diseases. Despite this prominent role no MK2 inhibitor advanced into clinical trials so far. In this communication we will discuss our efforts in identifying low-molecular weight MK2 inhibitors using scaffold morphing and pharmacophore modeling approaches. Further optimization led to MK2 inhibitors which potently suppress inflammation in acute rodent models upon oral administration.

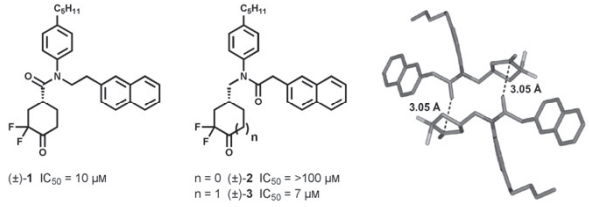
Medicinal Chemistry, Lecture **70**

Difluoroketones: Building Blocks in Inhibitors of the Malarial Protease Plasmepsin and Dimers based on Multipolar C=O···C=O-Interactions

Christoph Fäh[1], L. A. Hardegger[1], S. Meyer[2], D. Bur[2], F. Diederich[1]

[1] ETH Zurich, Laboratory of Organic Chemistry, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich
[2]Actelion Pharmaceuticals, Hegenheimermattweg 91, CH-4123 Allschwil

The development of novel fluorinated building blocks for medicinal chemistry is a challenging target, since new binding modes and pharmacokinetic behavior could prove to be useful in pharmaceuticals.[1] To contribute to this field of research, cyclic α,α-difluoroketones such as (±)-**1** to (±)-**3** have been synthesized and tested against the malarial aspartic protease plasmepsin II, with activities down to the single digit micromolar range.[2]



It was possible to solve an X-ray crystal structure of the inactive derivative (±)-**2**, in which a network of multipolar orthogonal C=O•••C=O-interactions was observed. This lead to an investigation in solution, revealing the first homodimer based on this weak dipole–dipole interaction, with association constants of $K_a = 0.68\ M^{-1}$ in C_6D_6 and $K_a = 17.54\ M^{-1}$ in C_6D_{12}. [3]

[1] K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881.
[2] C. Fäh, L. A. Hardegger, L. Baitsch, W. B. Schweizer, S. Meyer, D. Bur, F. Diederich, *Org. Biomol. Chem.* **2009**, *7*, 3947.
[3] C. Fäh, L. A. Hardegger, M.-O. Ebert, W. B. Schweizer, F. Diederich, *Chem. Commun.* **2010**, 67.

Medicinal Chemistry, Lecture **71**

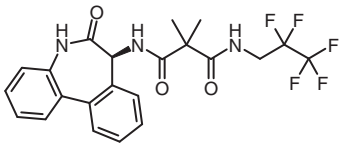
Gamma-Secretase Inhibitors: From Alzheimers Disease to Cancer

R. Jakob-Røtne[1], J. Boylan[2], A. Flohr[1], G. Galley[1], D. Heimbrook[2], H. Jacobsen[1], L. Luistro[2], J.-U. Peters[1], J. Roberts[2], W. Wostl[1].

[1]F. Hoffmann-La Roche Ltd., Basel, Switzerland
[2]F. Hoffmann-La Roche Ltd., Nutley, USA

Gamma-Secretase is an intra-membrane protease complex interfering with a number of substrates, the most prominent being Amyloid Precursor Protein (APP) and Notch. Cleavage of APP to ß-Amyloid is a key element in the pathology of Alzheimer's Disease whereas Notch signaling regulates cell fate by controlling the balance between cell proliferation and differentiation.

RO4929097 is a potent and orally active inhibitor of Gamma-Secretase. *In vivo,* RO4929097 shows excellent antitumor activity and this effect is sustained for many days after administration has been stopped, permitting intermittent dosing. Initially developed in a program targeting Alzheimer's disease, RO4929097 is now evaluated in clinical studies for oncology.



Medicinal Chemistry, Lecture **72**

E- and P-selectin: differences, similarities and implications for the design of P-selectin antagonists

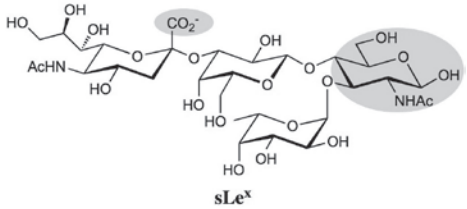
Florian Binder[1], Katrin Lemme[1], Martin Smiesko[1], Brian Cutting[1], John Patton[2], John L. Magnani[2], Beat Ernst[1]

[1]Institute of Molecular Pharmacy, University of Basel, CH-4056 Basel
[2]GlycoMimetics Inc., Gaithersburg, MD, USA

E-, P-, and L-selectin form a family of Ca^{2+}-dependent carbohydrate binding proteins (lectins). They are key players in the inflammatory cascade, as they initiate the migration of leukocytes to sites of inflammation. Since excessive invasion of leukocytes can cause acute and chronic inflammatory diseases, blocking of selectins is regarded as a potential therapeutic approach [1].

The tetrasaccharide sialyl Lewis^x (sLe^x), which is recognized by all three selectins, was the starting point of most lead optimization programs in the selectin area. To learn more about differences and similarities between P- and E-selectin sLe^x was modified in a stepwise approach.

Whereas mimics of the *N*-acetyl glucosamine moiety of sLe^x had the same effect on E- and P-selectin binding, mimics of the *N*-acetyl neuraminic acid (Neu5Ac) moiety had a pronounced affect on specificity. In contrast to numerous reports on P-selectin antagonists, we could show that the negative charge of the carboxylate of the Neu5Ac moiety is no prerequisite for P-selectin affinity.



[1] D. Vestweber, J.E. Blanks, *Physiol. Rev.* **1999**, *79*, 181-213.

Medicinal Chemistry, Lecture **73**

A combined *in silico-in vitro* fishing for intracellular targets of phenazopyridine

Y. Westermaier[1], D. Suter[2], K.-H. Krause[2], L. Scapozza[1]

[1]Pharmaceutical Biochemistry, School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland
[2]Department of Pathology and Immunology, Centre Médical Universitaire / Clinical Pathology, Rue Michel-Servet 1, CH-1211 Geneva 4, Switzerland

We applied *in silico* inverse screening to find intracellular targets of phenazopyridine that induce the homogeneous differentiation of embryonic CGR8 stem cells into neural ones [1]. Two different *in silico* protocol validations were performed using GOLD 4.0 [2]: (i) 12 ligands were chosen randomly and their corresponding targets seeded into a database of 188 decoy proteins. Of the ligands docked into their known targets, 58% displayed root mean square deviations (RMSDs) ≤ 2 Å compared to the experimentally measured binding orientation and 75% were found in the first 3.5% of the interaction energy-based ranking, discriminating between "true" and supposedly "false" targets. (ii) Using well-studied targets with ligands of different specificity degrees, we focused on docking/scoring accuracy and enrichment. A ranking threshold of 10% was defined to eliminate promiscuous binders. Subsequently, phenazopyridine was docked into over 5000 protein crystal structures. Docking the corresponding co-crystallized ligand into each protein served as a control. Selection criteria included the interaction energy-based ranking of phenazopyridine, the control ligand RMSD, shared key target interactions between phenazopyridine and the control as well as publications fitting into the biological context. Complementary functional inhibition tests, competitive binding assays and phosphoproteome analyses served as experimental validation. When applying 3 of the 4 selection criteria to *in silico* screening, 80% of the experimentally identified targets were retrieved retrospectively, providing us with a rough selectivity profile.

[1] D.M. Suter et al., *J. Cell. Mol. Med.* **2009**, *13*, 3517.
[2] G. Jones et al., *J. Mol. Biol.* **1997**, 267, 727.

Medicinal Chemistry, Lecture **74**

High Performance Separations:

Enabling Innovation in Discovery Chemistry

Daniel Zimmerli*, David Wechsler

Discovery Chemistry Technologies and Innovation,
F. Hoffmann-La Roche, 124 Grenzacher Str., CH-4070, Basel, Switzerland.

In the last 5 years there has been a revolutionary advance in both the hardware and column-ware technology associated with HPLC. This has resulted in benefits to chemistry projects that can consider chiral hits or leads as viable start points without the need to immediately develop an asymmetric synthesis.

The presentation will illustrate using selected key examples the impact of integrated preparative HPLC systems in conjunction with new column technology leading to rapid reversed phase LC separations of challenging molecules. Recently new chiral stationary phase columns have also emerged that extend far beyond classical amylose and cellulose-phase columns, such that now direct separation of acids and bases without derivitization is possible. Finally, the use of supercritical fluid separations delivers fast, efficient and a green alternative for the larger scale preparative separation of chiral molecules.

Taken together, the impact of these transformations in high performance separations enables chemistry teams to select from a wider choice of starting points including racemates that can be rapidly and routinely separated that offers distinct benefits, in the early phases of lead generation, thus we consider the role of an embedded separations group as an innovation enabler to chemistry project teams in Roche discovery chemistry.

Medicinal Chemistry, Invited Lecture **75**

Design and preparation of Potent, Nonpeptidic, Bioavailable Renin Inhibitors

Olivier Bezençon, Olivier Corminboeuf, Corinna Grisostomi, Lubos Remen, Sylvia Richard-Bildstein, Daniel Bur, Lars Prade, Patrick Hess, Panja Strickner, Walter Fischli, Beat Steiner, and Alexander Treiber [1]

[1] Drug Discovery Chemistry & Biochemistry, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland.

The Renin-Angiotensin-Aldosterone System (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.

After developing diazabicyclononene,[1] tetrahydropyridine, and other bicyclic derivatives,[2] we focused our efforts on the development of 3,4-disubstituted piperidines. Starting from the optimized substituents that were identified with **ACT-077825**,[1] we developed new, more polar benzyl amides (position 3) and phenyl substituents (position 4), leading to bioavailable renin inhibitors with sub-nanomolar potencies in human plasma. These compounds led to a sustained blood pressure decrease in the double transgenic rats (TGRs) at a dose of 1 mg/kg po.

[1] Bezençon et al. *J. Med. Chem.* **2009**, *52*, 3689.
[2] Remeň et al. *Bioorg. Med. Chem. Letters*, **2009**, *19*, 6762.

Medicinal Chemistry, Lecture **76**

Development and synthesis of a new generation of high-affinity selectin antagonists with improved binding kinetics.

Jonas Egger, Céline Weckerle, Katrin Lemme, Matthias Wittwer, Daniel Schwizer, Brian Cutting, Said Rabbani, Oliver Schwardt, and Beat Ernst*

Institute of Molecular Pharmacy, Pharmacenter, University of Basel, CH-4056 Basel, Switzerland

Selectins, a family of carbohydrate-binding proteins, play a key role in a number of deseases such as cancer or chronic inflammatory disorders (e.g. asthma or arthritis). Therefore, selectins represent a valuable drug target.

Due to the shallow and highly solvent-accessible binding site, selectin ligands typically have low (μM) affinities and dissociate quickly (within seconds) from the protein. This, as well as the lack of knowledge about the pharmacokinetic behavior of carbohydrate mimics, has been a major challenge in the development of selectin antagonists [1].

Using a fragment-based combinatorial approach [2], we identified a series of small (second-site) ligands that bind in the vicinity of a known first-site ligand. Suitable linking of first- and second-site ligands yielded a new generation of selectin antagonists with nM affinites and improved dissociation half-life time. Furthermore, the pharmacokinetic profiling of the most potent ligands (PAMPA, log D, plasma protein binding, CMC) revealed a selectin antagonist with drug-like properties.

[1] B. Ernst and J. L. Magnani, *Nat. Rev. Drug Discovery*. **2009**, *8*, 661.
[2] S. Shelke, B. Cutting, X. Jiang, H. Koliwer-Brandl, D. S. Strasser, O. Schwardt, S. Kelm, and B. Ernst, *Angew. Chem. Int. Ed*. **2010**, in press.

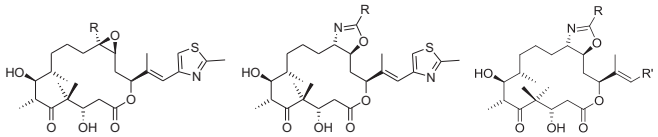
Medicinal Chemistry, Lecture **77**

Structure-Activity Relationships (SAR) of C12-C13-Oxazoline Derivatives of Epothilone A

Bernhard Pfeiffer, Kurt Hauenstein, Philipp Merz, Chiara Rickenbacher, Jürg Gertsch, Karl-Heinz Altmann*

ETH Zürich,Institute of Pharmaceutical Sciences, HCI, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland

Epothilones (Epo's) (**1**) are naturally occurring microtubule-stabilizing macrolides and potent inhibitors of human cancer cell growth *in vitro* and *in vivo*. The interest in these agents resulted in an advanced understanding of their SAR and provided the basis for epothilones as successful lead structures for the development of new anticancer drugs [1]. Our own SAR work on epothilones has shown that some epothilone derivatives (**2**), that are characterized by a 2-substituted *trans*-fused C12-C13-oxazoline ring, show antiproliferative activities that are comparable with those of the parent compound **1a** [2]. In this presentation we will discuss the SAR of this new structural class of epothilone derivatives with respect to the nature of the 2-substituent on the oxazoline ring as well as additional modifications of the side chain (**3**). Possible binding modes of these structures to tubulin have been investigated by molecular-modeling and also for derivatives specifically designed for affinity labeling of tubulin, in order to gain a better understanding of their interactions with the tubulin/microtubule system.



(**1a**) R=H EpoA
(**1b**) R=Me EpoB

(**2**) R = aryl, alkyl

(**3**) R,R' = aryl

[1] K.-H. Altmann, B. Pfeiffer *et al.*, *ChemMedChem* **2007**, *2*, 396-423.
[2] B. Pfeiffer *et al.*, *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 3760-3763.

Medicinal Chemistry, Lecture **78**

Scoring of protein structures and ligand-induced fit: A new look at the Ramachandran plot

Sampath Koppole, Michael Schaefer*

Novartis Institutes for Biomedical Research, Klybeckstrasse 141, CH-4057 Basel, Switzerland

Since its conception, the Ramachandran plot [1] has been a cornerstone of protein structure evaluation [2][3]. To be able to use the Ramachandran plot early in the refinement of protein structures and homology modeling, we introduce a Potential of Mean Force (PMF) for the backbone dihedrals of individual amino acids derived from statistics on non-homologous protein structures in the Protein Data Bank. The statistical data are converted to a continuous, periodic free energy function by two-dimensional Fourier series expansion. Both the absolute PMF and the ΔPMF relative to the free energy of all amino acids in the parameterization set were evaluated by scoring 350 datasets of protein decoys in comparison with their respective experimental structures. Of all decoy sets, 81% can be solved using the PMF alone, meaning that the native structure scores better than all decoys. On average, 92% of decoys have a higher PMF energy than the native conformation. These success rates exceed or parallel those for other statistical energy functions reported in the literature [4]. Examples are given for the use of the PMF to score sets of models from NMR structure determination, the effect of ligand-induced fit on target proteins, sets of homology models, and sequence alignments.

[1] G.N. Ramachandran, V. Sasisekharan, *J. Mol. Biol.* **1963**, *7*, 95.
[2] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, *J. Appl. Cryst.* **1993**, *26*, 283.
[3] R.W.W. Hooft, G. Vriend, C. Sander, E.E. Abola, *Nature* **1996**, *381*, 272.
[4] J. Skolnick, *Curr. Opin. Struct. Biol.* **2006**, *16*, 166.

Medicinal Chemistry, Lecture **79**

11β-HSD1 Inhibitors for Type II Diabetes and Beyond - The Metabolic Syndrome

W. Neidhart, A. Mayweg, Bernd Kuhn, J. Ackermann, D. Hunziker, A. Ruf, M. Otteneder, A. Luehe, E. Tozzo, P. Verry & K. Amrein

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

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an endoplasmic reticulum-associated enzyme that acts as an NADPH-dependent reductase and converts inactive cortisone to the active glucocorticoid (GC), cortisol.

As such, it is a regulator of intracellular cortisol concentration and has been implicated in a number of metabolic disorders of increased glucocorticoid tone: visceral adiposity, high blood pressure, glucose intolerance and dyslipidemia, a cluster of cardiovascular risk factors defined as metabolic syndrome. Features of metabolic syndrome resemble the clinical picture of patients with increased plasma GC levels or Cushing's syndrome. The enzyme is expressed in most tissue, particularly in adipose tissue and liver. There is evidence from studies with transgenic animals that metabolic syndrome may result from increased intracellular glucocorticoid levels in liver & adipose tissues and that pharmacological inhibition of 11β-HSD1 may normalize intracellular GC levels and be of therapeutic value for the treatment of type II diabetes & metabolic syndrome.

In the effort towards novel, potent and selective 11β-HSD1 inhibitors, a new class of functionalized N-heterocycles was identified and optimized through molecular design and X-ray structural data. Key compounds showing high enzyme and cell potencies with IC50 and EC5O values in the low nanomolar range were evaluated in depth. In PK/PD studies in mice they inhibited enzyme activity after oral dosing and in relevant mouse models they were shown to ameliorate insulin resistance and dyslipidemia.

Conclusion: It was demonstrated that pharmacological inhibition of 11β-HSD1 can improve features of the metabolic syndrome.

Medicinal Chemistry, Lecture **80**

BGG492, A COMPETITIVE AMPA/KAINATE ANTAGONIST IN CLINICAL DEVELOPMENT FOR EPILEPSY AND MIGRAINE

Henri Mattes, David Orain, Manuel Koller, Kurt Lingenhoehl, Jörg Kallen, Mario F. Pozza, Markus Schmutz, Sandrine Desrayaud, Stephan Urwyler, Johanne Renaud, Christian Trendelenburg, Armin Brülisauer and Yves P. Auberson, on behalf of the discovery and preclinical development team.

Novartis Institutes for BioMedical Research, 4002 Basel, Switzerland

With few exceptions, earlier attempts to develop competitive AMPA antagonists resulted from an extensive SAR exploration of substituted quinoxalinediones. Several clinical development compounds were identified (e.g. YM-90K, MPQX, or AMP397), but none of them made it to the market. In contrast to the previous candidates, BGG492, an orally active AMPA antagonist, results from the optimization of quinazolinedione sulfonamides, which have a related SAR but improved overall properties.
BGG492 shows anticonvulsant activity in several animal models of epilepsy, including electroshock and chemically-induced seizures in rodents, WAG/Rij rats (a genetic model of absence epilepsy), the rat amygdala kindling model (indicating a potential anti-epileptogenic effect), and in fully kindled rats (a model of therapy-resistant partial seizures in human). It is well understood that properties required for high affinity at AMPA receptors are contrary to those required for oral bioavailability. As a consequence, BGG492 has moderate binding affinity for rat and human AMPA receptors (IC_{50} = 0.19 and 0.2 μM), but >100-fold selectivity with regards to the glycine-binding site of NMDA receptors and no significant affinity in a 150-target safety panel. In rats, BGG492 is hardly metabolized, and does not inhibit CYP450 enzymes. Its favorable safety profile is evidenced by a lack of cardiovascular, phototoxic, genotoxic or teratogenic potential, as well as by results of toxicology studies in rats, dogs and monkeys, where only minor and reversible effects were observed. In animals, dose-limiting adverse effects were related to the classical signs of exaggerated pharmacology for AMPA receptor antagonism, mostly ataxia and decreased locomotor activity. BGG492 is in clinical evaluation in epileptic and migraine patients.

Medicinal Chemistry **81**

Piperidines as potent, orally active renin inhibitors: optimisation of the amide substituent

Olivier Bezençon, Daniel Bur, Thomas Weller, Sylvia Richard-Bildstein, Ľuboš Remeň, Thierry Sifferlen, Olivier Corminboeuf, Corinna Grisostomi, Christoph Boss, Lars Prade, Stéphane Delahaye, Alexander Treiber, Panja Strickner, Christoph Binkert, Patrick Hess, Beat Steiner, and Walter Fischli[1]

[1] Drug Discovery Chemistry & Biochemistry, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland.

The Renin-Angiotensin-Aldosterone System (RAAS) is one of the major and most intensively studied regulating systems of the arterial blood pressure in humans. It plays a pivotal role not only in cardiovascular diseases, but also in renal diseases, and other metabolic diseases as well. The RAAS consists of a two-step cascade. First, the aspartic proteinase renin cleaves its only known substrate, angiotensinogen, in the rate-limiting step to the decapeptide angiotensin I. In a second step, angiotensin I is cleaved either by the metalloproteinase angiotensin-converting enzyme (ACE) or by the serine proteinase chymase to the tensoactive octapeptide angiotensin II. The exclusive and rate-determining function in the RAAS makes renin an ideal pharmacological drug target.

Recently, we have described rational design and the preparation of a new series of 3,9-diazabicyclo[3.3.1]nonene derivatives as potent, non-peptidic and bioavailable renin inhibitors (J.Med. Chem. 2009, 52, 3689).

Based on this and previous knowledge (Farmaco 2001, 56, 21-27), we have designed a new series of potent, orally active renin inhibitors with a piperidine core structure. After optimization of the amide substituent and with the help of modeling based on X-ray structural analysis, we have prepared compounds with excellent potency against renin (in buffer and in plasma) and with very good selectivity profiles. These compounds were well absorbed and efficacious orally at 1 mg/kg in double transgenic rats (TGRs).

Medicinal Chemistry **82**

New piperidine-based renin inhibitors

Olivier Corminboeuf, Olivier Bezençon,* Corinna Grisostomi, Ľuboš Remeň, Sylvia Richard-Bildstein, Daniel Bur, Lars Prade, Patrick Hess, Panja Strickner, Walter Fischli, Beat Steiner, and Alexander Treiber

Drug Discovery Chemistry & Biochemistry, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland

The Renin-Angiotensin-Aldosterone System (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.

Starting from known diazabicyclononene[1] and tetrahydropyridine[2] derivatives, the development of a new class of piperidines as renin inhibitors is reported. Using substituents previously optimized to fill the renin S3 subpocket in a different series,[3] highly potent piperidine based inhibitors were obtained. Further introduction of polar groups at the 4-position of the piperidine moiety allowed to discover new inhibitors with good pharmacological activities in a double transgenic rat model.

[1] Bezençon et al. *J. Med. Chem.* **2009**, *52*, 3689.
[2] Remeň et al. *Bioorg. Med. Chem. Letters*, **2009**, *19*, 6762.
[3] Chen et al. *Bioorg. Med. Chem. Letters*, in press.

Medicinal Chemistry **83**

New Class of Potent, Non-peptidic, Orally Active Renin Inhibitors

Ľuboš Remeň, Olivier Bezençon, Christoph Boss, Daniel Bur, Olivier Corminboeuf, Corinna Grisostomi, Sylvia Richard-Bildstein, Thierry Sifferlen, Thomas Weller, Christoph Binkert, Walter Fischli, Patrick Hess, Lars Prade, Beat Steiner, and Panja Strickner

Drug Discovery and Preclinical Research, Actelion Pharmaceuticals Ltd, Hegenheimermattweg 91, CH – 4123 Allschwil, Switzerland

The Renin-Angiotensin-Aldosterone system is widely accepted as an important regulator of cardiovascular, renal and adrenal function playing a major role in water and salt homeostasis, as well as blood pressure control.

As a part of our renin inhibition program we recently published a new class of bicyclic 3,9-diaza-bicyclononenes[1]. The most potent compounds in this series inhibited renin with $IC_{50} < 1$ nM (in buffer) and showed *in vivo* efficacy at 10 mg/kg in double transgenic rats (TGRs) expressing both the human angiotensinogen and the human renin genes.

In order to explore the role of the CH_2-N-CH_2 bridge of the 3,9-diazabicyclononene moiety a series of other related bicyclic compounds with identical substituents at the key positions were prepared, i. e. 9-aza-3-oxabicyclononenes, 9-azabicyclononenes, 3,3-dioxo-3λ6-thia-9-aza-bicyclononenes, 3-oxo-3λ4-thia-9-aza-bicyclo[3.3.1]non-6-enes, and 8-azabicyclononenes. For comparison also the corresponding tetrahydropyridine analogs were designed and prepared. Among others, we identified the *first achiral, potent renin inhibitors*. Some of these compounds are efficacious after oral administration (10 mg/kg) in TGRs.

[1] Olivier Bezençon, Daniel Bur, Thomas Weller, Sylvia Richard-Bildstein, Ľuboš Remeň, Thierry Sifferlen, Olivier Corminboeuf, Corinna Grisostomi, Christoph Boss, Lars Prade, Stéphane Delahaye, Alexander Treiber, Panja Strickner, Christoph Binkert, Patrick Hess, Beat Steiner, and Walter Fischli *J. Med. Chem.* ***2009****, 52, 3689.*

Medicinal Chemistry **84**

Optimization of the first generation renin inhibitors

Corinna Grisostomi, Sylvia Richard-Bildstein, Ľuboš Remeň, Olivier Bezençon, Olivier Corminboeuf, Daniel Bur, Lars Prade, Thierry Sifferlen, Christoph Boss, Thomas Weller, Stéphane Delahaye, Alexander Treiber, Panja Strickner, Christoph Binkert, Patrick Hess, Beat Steiner and Walter Fischli

Drug Discovery & Preclinical Development, Actelion Pharmaceuticals Ltd, Gewerbestrasse16, CH – 4123 Allschwil

The renin-angiotensin system (RAS) has been known for a long time as being a pivotal player in the regulation of blood pressure as well as in the maintenance of sodium and electrolyte balance. RAS blockers have demonstrated efficacy in hypertension, cardiac failure, and in renal protection.

Recently we have disclosed a class of small molecule bicyclic inhibitors of renin, such as ACT-077825 (*IC*50 buffer = 0.2 nM, *IC*50 plasma: 19 nM), a highly potent renin inhibitor, but prone to high plasma protein binding.

In order to reduce plasma protein binding we have explored the introduction of polar functionalities at various positions of the bicyclic inhibitors. The introduction of a series of tertiary amines at the „linker" region and the replacement of the „central" phenyl mojety with a series of 5-membered heteroaryls and their activity *in vitro* and *in vivo* will be presented.

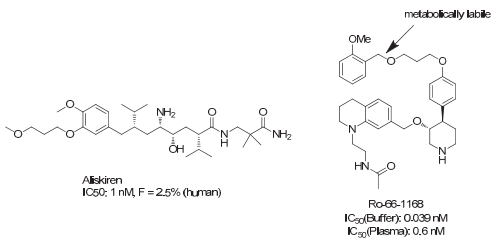
Medicinal Chemistry **85**

Novel Renin Inhibitors via Scaffold Hopping

Christoph Boss, Olivier Bezençon, Daniel Bur, Thomas Weller, Sylvia Richard-Bildstein, Ľuboš Remeň, Thierry Sifferlen, Olivier Corminboeuf, Corinna Grisostomi, Lars Prade, Panja Strickner, Christoph Binkert, Patrick Hess, Beat Steiner, and Walter Fischli[1]

[1]Drug Discovery and Preclinical Development, Actelion Pharmaceuticals Ltd, Hegenheimermattweg 91, CH-4123 Allschwil / BL; Switzerland

Renin is a key enzyme in the blood pressure regulation cascade, responsible for the production of angiotensin I via cleavage of its only known substrate angiotensinogen. The exclusive and rate determining function makes renin an ideal target to treat pathological conditions asking for blood pressure regulation / lowering. Renin inhibitors therefore are of broad clinical importance with diverse potential clinical indications. The first renin inhibitor successfully entering the market was Aliskiren. First efforts towards clearly non-peptidomimetic renin inhbitors resulted in piperidine based structures as e.g. Ro-66-1168. These compounds were highly potent but suffered from poor pharmacokinetic properties. By scaffold hopping and optimization of the side chains we obtained a clinical development candidate. Further efforts to identify follow-up compounds by again applying the scaffold-hopping concept led to azabicyclo-nonene-based inhibitors as well as to cis-azabicyclo-octene based compounds. The results obtained with these two scaffolds will be discussed in detail on the poster.



Medicinal Chemistry **86**

Bicyclic peptide ligands for the inhibition of tumor cell invasion

Alessandro Angelini[1] and Christian Heinis[1]

Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédéral de Lausanne (EPFL), Lausanne, Switzerland.

Bicyclic peptide ligands with antibody-like binding properties have recently been generated by Christian Heinis and Sir Gregory Winter at the Laboratory of Molecular Biology (LMB) in Cambridge, UK, using a novel phage display based approach [1]. Briefly, cysteine-rich peptides displayed on phage were reacted with a small organochemical scaffold to form bicyclic peptide structures (please see the figure below). Large combinatorial libraries (> a billion variants) of bicyclic peptides (bound to phage) were generated and binders with nanomolar affinities and high specificities to various human disease targets were isolated. I have used the phage-based methodology for the isolation of bicyclic peptide inhibitors of human urokinase-type Plasminogen Activator (uPA). uPA is a serine protease that plays an important role in pericellular proteolysis and its inhibition is a promising strategy to decrease the invasive and metastatic activity of tumour cells. In phage affinity selections, I had isolated inhibitors of human uPA with submicrormolar inhibitory activities and excellent target specificities. Furthermore, I had tested the therapeutic potential of the inhibitor in biological systems. The bicyclic peptide inhibitors of uPA are a promising therapeutic format that should combine key qualities of antibodies (high affinity and specificity) and advantages of small molecules (chemical synthesis, tissue penetration).

[1] C. Heinis, T. Rutherford, S. Freund and G. Winter. Phage-encoded combinatorial chemical libraries based on bicyclic peptides. *Nature Chemical Biology*, **2009**, 5(7), 502-507.

Medicinal Chemistry **87**

Macrocyclic BACE Inhibitors Acutely Reduce Abeta in Brain after p.o. Application

Lerchner A., Machauer R., Betschart C., Veenstra S., Tintelnot-Blomley M., McCarthy C., Jaton, AL., Rabe S., Desrayaud S., Enz A., Staufenbiel M., Paganetti P., Rondeau JM., and Neumann U.

Novartis Institute for BioMedical Research Basel, Switzerland

Deposition of amyloid plaques, mainly consisting of 40-42 amino acid β-amyloid peptides, is a pathological hallmark of Alzheimer's Disease. The consecutive action of Beta-site APP Cleaving Enzyme (BACE) and γ-secretase generates β-amyloid peptides from the Amyloid Precursor Protein (APP). They subsequently form oligomers, fibrils, and plaques, which are believed to induce neurodegeneration. Inhibition of BACE is therefore expected to provide a disease-modifying therapy for AD.

We were able to identify BACE inhibitors from the peptide starting structures by size-reduction and macro-cyclization.[1] It has been shown by us and by others that poor blood-brain barrier permeation and P-glycoprotein mediated efflux is a major hurdle for in vivo activity of BACE inhibitors. We present here our approach to identify brain-penetrable macrocyclic BACE inhibitors, via reduction of their pKa. Compounds of this series then reduced levels of C99 and Aβ in brain after oral single dose administration.[2]

[1] R. Machauer, K. Laumen, S. Veenstra, J. Rondeau, M. Tintelnot-Blomley, C. Betschart, A. Jaton, S. Desrayaud, M. Staufenbiel, S. Rabe, P. Paganetti, U. Neumann *BMCL* **2009**, *19*, 1366.

[2] A. Lerchner, R. Machauer, C. Betschart, S. Veenstra, H. Rueeger, C. McCarthy, M. Tintelnot-Blomley, A. Jaton, S. Rabe, S. Desrayaud, A. Enz, M. Staufenbiel, P. Paganetti, J. Rondeau, U. Neumann *BMCL* **2010**, *20*, 603.

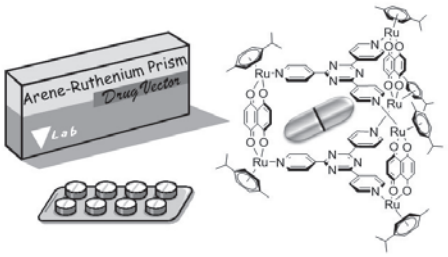
Medicinal Chemistry **88**

Arene-Ruthenium Metalla-Prisms: New Drug Vectors

Nicolas P. E. Barry, Dr. B. Therrien.

University of Neuchatel, 51 Ave de Bellevaux, 2000 Neuchatel, Switzerland

Based on "enhanced permeability and retention" (EPR) effect, passive targeting of tumours appears as a promising solution against the general toxicity and side effects of classical chemotherapeutics.[1] We proposed some years ago a new type of large drug delivery systems built from arene-ruthenium units which allowed the encapsulation of palladium or platinum complexes inside their cavities.[2] Moreover, the uptake of encapsulated molecule into cancer cells *via* fluorescence microscopy was also studied.[3]



Host-guest properties have been recently observed with a slightly different arene-ruthenium metalla-prism, and fluorescence microscopy assays seem to show a faster release of the guest molecule inside cancer cells, thus opening new perspectives for these metalla-cages.

[1] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387.

[2] B. Therrien, G. Süss-Fink, P. Govindaswamy, A. K. Renfrew, P. J. Dyson, *Angew. Chem. Int. Ed.* **2008**, *47*, 3773.

[3] O. Zava, J. Mattsson, B. Therrien, P. J. Dyson, *Chem. Eur. J.* **2010***, 16,* 1428.

Medicinal Chemistry **89**

Structure-Based Design and Inhibition of the Antimalarial Target Falcipain-2

Veronika Ehmke[1], François Diederich[1]

[1]ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

Research efforts in novel malaria chemotherapies are currently focused on the design of potent inhibitors for malarial proteases, among which falcipain-2 (FP2) plays a key role [1]. FP2 is required for hemoglobin degradation to provide free amino acids for the parasite's energy supply (Figure 1).

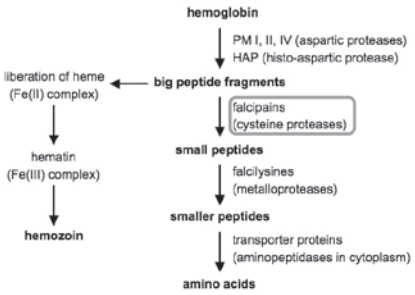


Figure 1. Overview of enzymes involved in parasitic hemoglobin degradation during a malaria infection.

Through the detailed exploration of the active site binding properties, further insights into this degradation process should be obtained. With first X-ray crystal structures available, it is possible to apply our molecular recognition-based approach for rational FP2 inhibitor design and mapping of the active site binding properties of this cysteine protease.

[1] D. E. Goldberg, A. F. Slater, R. Beavis, B. Chait, A. Cerami, G. B. Henderson, *J. Exp. Med.* **1991**, *173*, 961.

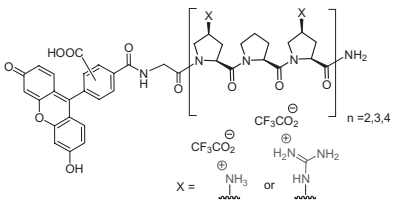
Medicinal Chemistry **90**

Cell Penetrating Peptides based on Cationic Amphiphilic Polyproline Structures

Yvonne A. Nagel and Helma Wennemers*

Department of Chemistry, University of Basel,
St. Johanns-Ring 19, 4056 Basel, Switzerland

Herein we present recently developed amphiphilic oligoproline-derivatives, which cross cell membranes of human cervical cancer cells. These cell penetrating agents were designed and synthesized to bear cationic and hydrophobic moieties along the backbone of a polyproline II (PP II) helix as well-defined scaffold.[1] The efficacy of the cellular uptake was evaluated using fluorescence activated cell sorting (FACS) and localisation was visualised by confocal microscopy. Cell penetrating properties of the tested compounds strongly depend on concentration, chain length and therefore cationic moieties. All examined peptides showed generally low cytotoxicity. In order to investigate the mechanism of internalisation, experiments at lower temperature (4 °C) and under ATP depletion were performed.



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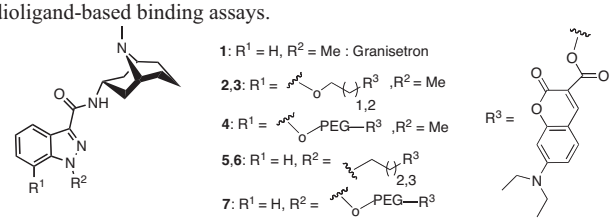
[2] For other related CPPs see: a) I. Geisler, J. Chmielewski, *Chem. Biol. Drug. Des.* **2009**, *73*, 39-45; b) B. A. Smith, D. S. Daniels, A. E. Coplin, G. E. Jordan, L. M. McGregor, A. Schepartz, *J. Am. Chem. Soc.* **2008**, *130*, 2948-2949.

Medicinal Chemistry **91**

Synthesis of fluorescent ligands for the 5-HT$_3$ receptor and application to develop a fluorescent polarisation assay

Jonathan Simonin[1] and Martin Lochner[1]
[1]*Universität Bern, Department of Chemistry and Biochemistry, CH-3012 Bern, Switzerland*

The 5-HT$_3$ receptor is a ligand-gated ion channel (LGIC), it is a member of the Cys-loop family of receptors, which also includes nicotinic acetylcholine, glycine and GABA$_A$ receptors. Antagonists of the 5-HT$_3$ receptor are widely used clinically for the treatement of emesis and irritable bowel syndrome.[1] In this work we use the granisetron core, which is a well-known 5-HT$_3$ receptor antagonist to attach coumarin type fluorescent molecules at different positions with different linkers.[2] Once synthesized, the fluorescent ligands will be characterised by radioligand binding and fluorescence spectroscopy and used to develop a fluorescence polarisation assay. The aim of this work is to synthesize fluorescent ligands of type **2-7** that bind with high affinity to the 5-HT$_3$ receptor and show a high fluorescence polarisation over background when bound. A fluorescence polarisation assay will then be developed as an alternative for the radioligand-based binding assays.



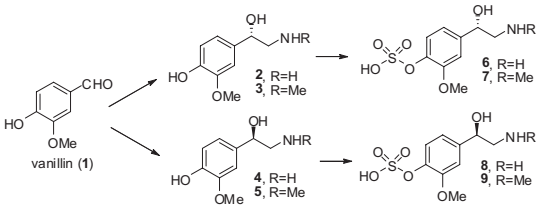
[1] A.J. Thompson; S.C.R. Lummis. *Expert Opin. Ther. Targets* **2007** ; *11*, 527.
[2] S.K.V.Vernekar; H.Y. Hallaq; G.Clarkson; A.J.Thompson; L.Silvestri; S.C.R.Lummis; M.Lochner, *J. Med. Chem*., **2010**, *53*, 2324.

Medicinal Chemistry **92**

Asymmetric synthesis of sulfoconjugated metanephrines for applications in clinical chemistry

Jean-Baptiste Gualtierotti,[1] Olivier Sallin,[1] Jonathan Simonin,[1] Eric Grouzmann[2] and Sandrine Gerber-Lemaire[1]
[1] *ISIC, BCH, EPFL, CH-1015 Lausanne, Switzerland*
[2] *Division de Pharmacologie et Toxicologie Cliniques, CHUV, CH-1011 Lausanne, Switzerland*

The diagnosis of pheochromocytoma, a rare neuroendocrinoma tumor deriving from the neural crest relies on measurement of free metanephrines in plasma and sulfoconjugated metanephrines in urine and plasma.[1] Urinary and plasma metanephrines are usually measured after an acid-hydrolysis step or by enzyme treatment with arylsulfatase that liberates the free from the sulfate-conjugated metabolites. These methods are widely used for 40 years in clinical chemistry laboratories but they are indirect since they use free metanephrines as calibrators instead of sulfated calibrators to assess their quantification. We report herein the development of synthetic pathways toward both enantiomers of sulfoconjugated metanephrines (**6-9**) for the preparation of calibration curves needed to measure these molecules in routine clinical chemistry.



[1] a) Mornex, R.; Peyrin, L.; Pagliari, R.; Cottet-Emard, J.- M. *Horm. Res*. **1991**, *36*, 220-226. b) Eisenhofer, G.; Siegert, G.; Kotzerke, J.; Bornstein, S. R.; Pacak, K. *Horm. Met. Res*. **2008**, *40*, 329-337. c) d'Herbomez, M.; Rouaix, N.; Bauters, C.; Wémeau, J.- L. *Presse Med*. **2009**, doi: 10.1016/j.lpm.2008.08.011.

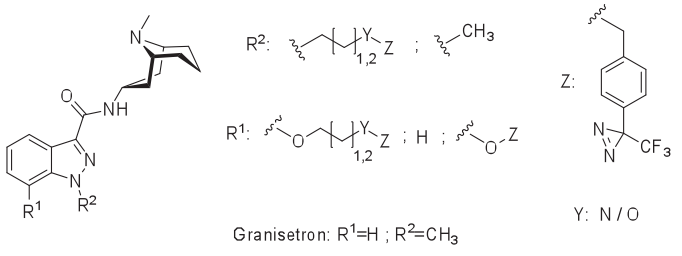
Medicinal Chemistry **93**

Synthesis of photoaffinity probes for the site-selective chemical modifications of the 5-HT$_3$ receptor

Thomas Jack and Martin Lochner

University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, CH-3012 Bern

It is very difficult to investigate the structure of ligand-gated ion channels like the 5-HT$_3$ receptor which is important for the transmission of nerve impulses in the central nervous system.[1] Classic approaches like crystallization and x-ray have difficulties to characterize membrane bound receptors. As an alternative way to find out more about structure and function of the 5-HT$_3$ receptor and the binding of small molecules to it, we synthesized photoaffinity probes based on the structure of granisetron, a well known antagonist of the receptor. Trifluoromethyl-diazirin as photo-labile building block was attached to different positions with different linkers to the granisetron core in order to develop a probe with high binding affinity for the receptor. The possibility to activate and covalently attach the molecule by irradiation will be used in further studies to modify the protein.



[1] Reeves D.C.; Lummis S. C. R., *Mol. Membr. Biol*. **2002**, *19*, 11.

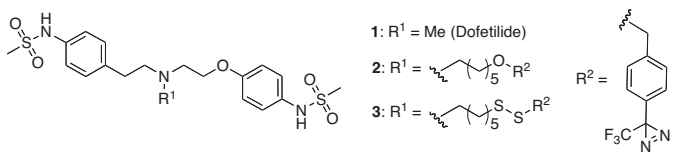
Medicinal Chemistry **94**

Towards the site-specific chemical modification of the hERG channel

Suradech Singhanat[1] and Martin Lochner[1]

[1] University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, 3012 Bern, Switzerland

The human ether-a-go-go related gene (hERG) encodes for a potassium channel that is expressed in the heart muscle and is critical for repolarization of cardiac tissue during the heart beat cycle[1]. It has been shown that a number of market therapeutic drugs have off-target affinity for the hERG channel and cause QT prolongation (LQT) which can lead to potentially lethal cardiac arrhythmia[2]. In consequence, every drug candidate has to be tested in order to avoid LQT side effects. The existing assays used in pharmaceutical industry, including electrophysical patch clamp assay and radioligand-based competition binding assays, have some disadvantages and are not suitable for high-throughput screening[1]. The aim of this project is to covalently attach a small fluorophore to the hERG channel using photoaffinity probes **2-3** based on dofetilide **1**, the ligand which is known to bind to the channel with high affinity[3]. Modifiable linkers will allow further post-photoaffinity labeling modification of the hERG channel in order to introduce a small fluorophore near the channel for which would provide a means for sensitive and rapid detection of potential channel blockers.



[1] D. H. Singleton et al., *J. Med. Chem*. **2007**, *50*, 2931.
[2] M. E. Curran, *Cell* **1995**, *80*, 795.
[3] G.J. Diaz et al., *J. Pharmacol. Toxicol. Methods*. **2004**, *50*, 187.

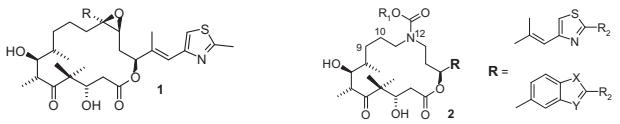
Medicinal Chemistry **95**

SAR Studies on 12-Aza-Epothilones (Azathilones) – A New Structural Class of Microtubule Stabilizers

Andrea Jantsch[1], Oliver Horlacher[1], Fabian Feyen[1], Jürg Gertsch[2], Karl-Heinz Altmann[1]

[1]ETH Zürich/Institute of Pharmaceutical Sciences, HCI H492, Wolfgang-Pauli-Str. 10
[2]University of Bern, Institute of Biochemistry and Molecular Medicine

Epothilones (Epo's)[1] are bacterial natural products with potent antitumor activity *in vitro* and *in vivo*[2] and at least seven epothilone-derived agents have entered clinical trials in humans. While all of these compounds are closely related structurally to the natural product leads Epo A (1, R = H) and B (1, R = CH_3), our own work involves the design, synthesis, and biological evaluation of analogs with significantly altered structural features, which would represent new lead structures for anticancer drug discovery.[3] This paper will discuss the synthesis and biological properties of epothilone analogs of the general structure **2** (R_1 = alkyl; X, Y = N(CH_3), CH=CH, N, R_2 = H, CH_3).



In spite of significant architectural differences between these analogs and natural epothilones, the biological activity of individual compounds from both structural series is within the same potency range as that of Epo A or B. At the same time, some of the typical SAR features of natural epothilones are not reproduced in compounds of type **2**, which suggests that their tubulin-bound conformation may differ from that of natural epothilones.

[1] Gerth, K.; *et al.. J. Antibiotics* **1996**, *49*, 560.
[2] Review: Altmann, K.-H.; *et al. ChemMedChem* **2007**, *2*, 396.
[3] Feyen, F.; *et al. Acc. Chem. Res.* **2008,** *41*, 21.

Medicinal Chemistry **96**

Sweet Previsions: Modeling the Permeation of Carbohydrate Antagonists

Matthias Wittwer, Martin Smiesko, Beat Ernst

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

The prediction of permeation remains a challenging task within pharmacokinetic modeling [1]. Whereas active transport processes are difficult to predict due to their complexity and specificity, considerable success has been achieved in the field of passive transport [2]. Recently, various approaches have been developed to identify the underlying molecular descriptors.

In our study we focus on the development of an *in-silico* tool for the prediction of permeabilities of carbohydrate mimetics determined by PAMPA (parallel artificial membrane permeation assay) [3]. To our knowledge, it is the first time that such an approach is applied within this class of compounds. The methodology elucidates the contribution of various molecular descriptors to passive permeation. Boltzmann-weighted averaging of molecular descriptor values is used in order to account for conformer flexibility. A Monte Carlo search algorithm is employed to a set of carbohydrate mimetics to identify the combination of descriptors with the highest information content regarding quantitative description of structure-property relationships.

The poster will present an overview of the studied parameters and their usefulness for the prediction of permeation. Furthermore, the final algorithm and its reliability will be discussed.

[1] P. Burton, J. Goodwin, T. Vidmar, B. Amore, *Pharm. Exp. Therap.* **2002**, *303*, 889.
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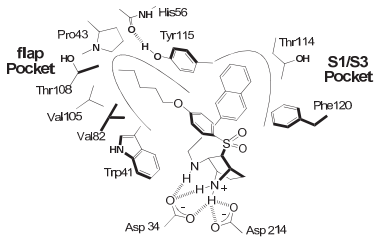
Medicinal Chemistry **97**

Structure-Based Design and Synthesis of Novel Functionalized 7-Azabicyclo[2.2.1]heptanes as Malarial Plasmepsin Inhibitors

Valentina Aureggi[1], Jörg Wieland[2], Martina Zürcher[2], Solange Meyer[3], Daniel Bur[3], Bernhard Breit[2], François Diederich[1]*

[1]ETH Zurich, Wolfgang-Pauli-Str. 10, CH-8093 Zurich, Switzerland
[2]University of Freiburg, Albertstr. 21, 79104 Freiburg, Germany
[3]Actelion Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123, Allschwil, Switzerland

The development of new therapeutic agents against malaria has become urgent, due to the increased prevalence of multi-drug-resistant strains of *Plasmodium falciparum*, the parasite that causes the deadliest form of malaria.[1]



Functionalized 7-azabicyclo[2.2.1]heptanes have been designed as potent and selective inhibitors against malarial plasmepsin proteases PMs which are involved in hemoglobin degradation.[2]

[1] World Health Organization. World Malaria Report 2008 (Geneva).
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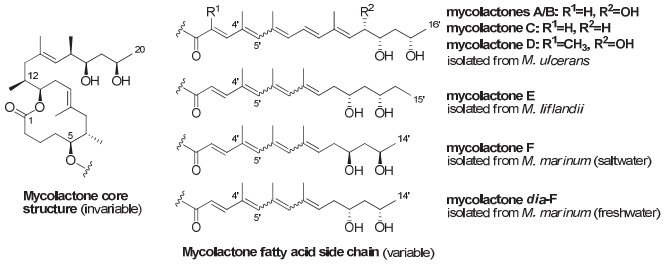
Medicinal Chemistry **98**

Mycolactones: Syntheses and Structure-Toxicity Relationships

Philipp Gersbach, Jun Li, Karl-Heinz Altmann*

ETH Zürich, Institute of Pharmaceutical Sciences, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland

Mycolactones are 12-membered macrolides that have been isolated from aqueous mycobacteria. Due to their immunosuppressive and cytotoxic properties they play a decisive role in the pathogenesis of human and animal skin diseases, such as Buruli ulcer, although the underlying cellular mechanisms remain unknown [1,2].



In this contribution we present new total syntheses of mycolactones A/B and of mycolactone F; in addition, we have prepared a number of simplified mycolactone analogues. All compounds were tested for their *in vitro* cytotoxicity against human immune cells, in order to establish a first structure-toxicity relationship for these mycotoxins. The results of these studies will aid in the design of mycolactone conjugates that could serve as tools in the identification of the cellular target(s) of mycolactones.

[1] Simmonds, R. E.; Lali, F. V.; Smallie, T.; Small, P. L. C., Foxwell, B. M. *J. Immunol* **2009**, *182*, 2194.
[2] Kim, H. J.; Jackson, K. L.; Kishi, Y.; Williamson, H. R.; Mosi, L.; Small, P. L. *Chem Commun (Camb)* **2009**, 7402.

Medicinal Chemistry **99**

Synthesis and Biological Studies of FK866 Analogues as Potential Antitumor Agents

Annabelle Gillig, Pierre Vogel*

Laboratoire de Glycochimie et de Synthèse Asymétrique, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Nicotinamide phosphorybosyltransferase (NMPRTase) has a crucial role in the salvage pathway of NAD^+ biosynthesis; it salvages released nicotinamide and converts it to nicotinamide mononucleotide. This important role of NMPRTase in NAD^+ biosynthesis makes it an attractive target for the development of new anticancer agents. Tumor cells have a high rate of NAD^+ turnover owing to elevated ADP-ribosyl activity, and NMPRTase expression is up-regulated in cancers.[1] Most notably, the compound FK866 (Figure 1) inhibits the human NMPRTase , reduces intracellular NAD^+ levels in tumors and thus, induces apoptosis in these cells while having little toxicity to normal cells.

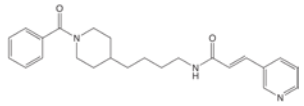


Figure 1 : Structure of the FK866

The crystal structures of human and murine NMPRTase alone and in complex with the reaction product nicotinamide mononucleotide or the inhibitor FK866 have been determined.[2] Based on these crystal structures, new analogues of FK866 have been designed. Here we report the synthesis and biological studies of these new potential antitumor agents.

1) Van Beijnum, J. R.; Moerkerk P. T. M.; Gerbers, A.; De Bruïne, A. P.; Arends, J.-W.; Hoogenboom, H. R.; Hufton, S. E.; *Int. J. Cancer* **2002**, *101*, 118-127.
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Medicinal Chemistry **100**

Design, Synthesis, and Evaluation of Trypanothione Reductase Inhibitors as Potential Antiprotozoal Agents

C. Eberle[1], J. A. Burkhard[1], B. Stump[1], R. Brun[2], M. Kaiser[2], R. L. Krauth-Siegel[3], and F. Diederich[1]

[1]Laboratorium für Organische Chemie, ETH Zürich, Hönggerberg, HCI, 8093 Zürich (Switzerland)
[2]Swiss Tropical Institute; University of Basel, 4002 Basel (Switzerland)
[3]Biochemie-Zentrum der Universität Heidelberg (BZH), Im Neuenheimer Feld 504, 69120 Heidelberg (Germany)

Trypanothione reductase (TR) is a flavoenzyme unique to trypanosomatid parasites and a target for lead discovery programs. Different inhibitor scaffolds have emerged in the past, exhibiting moderate affinity for the parasite enzyme. We showed that the combination of two structural motifs of known TR inhibitors – diaryl sulfides[1] and mepacrine[2] – enables simultaneous addressing of two hydrophobic patches in the active site. The binding efficacy of these conjugates is enhanced compared to that of the respective parent inhibitors. They show K_{ic} values for the parasite enzyme down to 0.9 ± 0.1 μM and exhibit high selectivity for TR over human glutathione reductase (GR).
In vitro studies revealed IC_{50} values in the low micromolar to submicromolar range against *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* as well as the malaria parasite *Plasmodium falciparum* which does not possess a trypanothione metabolism. The inhibitors exhibit strong fluorescence which allowed visualization of the drugs in the parasite where high accumulation was observed by fluorescence microscopy.[3]

[1] B. Stump, C. Eberle, M. Kaiser, R. Brun, R. L. Krauth-Siegel, F. Diederich, *Org. Biomol. Chem.* **2008**, *6*, 3935.
[2] E. M. Jacoby, I. Schlichting, C. B. Lantwin, W. Kabsch, R. L. Krauth-Siegel, *Proteins* **1996**, *24*, 73.
[3] C. Eberle, J. A. Burkhard, B. Stump, M. Kaiser, R. Brun, R. L. Krauth-Siegel, F. Diederich, *ChemMedChem* **2009**, *4*, 2034.

Medicinal Chemistry **101**

Phytochemicals and Bioactivation of Cytotoxic Anticancer Drugs

Melanie M. Erzinger, Katrin M. Hecht, Shana J. Sturla

ETH Zürich, Schmelzbergstr. 9, CH-8092 Zürich, Switzerland

Acylfulvenes (AFs) are semisynthetic derivatives of mushroom-derived illudin S with potent antitumor activities. Enzyme-mediated bioactivation is needed to generate chemical intermediates with the capacity to alkylate cellular targets, e.g. DNA or proteins. The diet can act as an important modulator of this process. For example, dietary constituents could either have an indirect effect via transcriptional regulation of drug-metabolizing enzyme levels, or a direct effect by interacting with the metabolic enzyme at the protein level. Alkenal/one oxidoreductase (AOR), heretofore known as dithiolethione-inducible gene-1, is an antioxidative enzyme that catalyzes the reduction of the carbon-carbon double bond of α,β-unsaturated aldehydes and ketones and contributes to the cellular bioactivation of AFs. Illudin S and irofulven (hydroxymethylacylfulvene, HMAF) are complementary test probes of molecular mechanisms because of their differences in cytotoxic selectivity profiles but similarities in chemical structures. In this study, their reductase-mediated transformation was characterized by measuring, via UV absorption, the disappearance of substrate and/or the rate of consumption of NADPH by the enzyme. Kinetic parameters were determined for AOR-mediated transformation of cytotoxic drugs (illudin S, HMAF). Finally, data will be presented regarding the influence of common dietary constituents, specifically phytochemicals with an established potential to modulate xenobiotic-metabolizing enzymes, on the bioactivation efficiency of AOR.

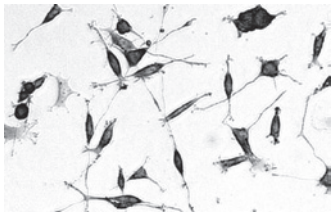
Medicinal Chemistry **102**

Induction of Neurite Outgrowth by Farinosone C Derivatives: Investigation of the Mode of Action and Target Identification

Patrick Burch, Henning J. Jessen and Karl Gademann

University of Basel, Department of Chemistry, St. Johanns-Ring 19 CH-4056 Basel

Due to the aging of our society certain diseases become more and more accentuated. Among of these sufferings, neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington's are of particular importance.[1] One strategy to face these medical challenges is based on the regeneration of neuronal networks by stimulation of neurite outgrowth via small organic molecules capable of mimicking neurotrophin actions.[2,3] Our group recently accomplished the total synthesis of farinosone C, which has been shown to induce neurite outgrowth in the PC-12 cell line (pheochromocytoma).[4]



Farinosone C

This project is aiming at the development of simplified farinosone C derivates with retained or increased activity. Structure-reactivity-relationship studies as well as investigations concerning the mode of action will be presented.

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Medicinal Chemistry **103**

Blood Purification using Functionalized Core/Shell Nanomagnets

I.K. Herrmann[1]; M. Urner[2]; F. M. Koehler[1]; B. Beck-Schimmer[2] and W.J. Stark[1,*]

[1] ETH Zurich, Institute for Chemical and Bioengineering, Wolfgang-Pauli-Strasse 10, Zurich, Switzerland

[2] University Hospital Zurich, Rämistrasse 100, Zurich, Switzerland

The direct removal of disease causing factors from blood would be most attractive in a number of clinical situations (e.g. intoxications, severe inflammatory response syndrome and sepsis)[1]. At present, such direct removal is routinely performed for small molecules (urea, potassium, creatinine) by (hemo-) dialysis, filtration and adsorption. These processes depend on diffusion through membrane barriers where the pore size and structure currently limit hemodialysis to low-molecular compounds (filter cut off at ~50 kDa)[2]. The majority of harmful substances, e.g. endo-/exotoxins in sepsis, antibodies and immune-complexes in immunologic disorders are large biomolecules and are therefore poorly accessible to dialysis[2]. In this work, the direct injection of stable nanomagnets into whole blood for the efficient, magnetic extraction-based extracorporeal removal of low and high molecular compounds is investigated. We demonstrate the successful extraction of metal ions (Pb^{2+}), steroid drugs (digoxin, cardiac drug) and proteins (Interleukin-6, inflammatory mediator) whilst monitoring blood integrity using a series of clinically important parameters[3].

[1] C. Ronco, R. Bellomo, G. Lonneman et al., *N. Engl. J. Med.* **2003**, *348*, 1600.
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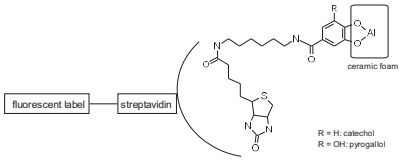
Medicinal Chemistry **104**

Functionalisation of Ceramic Foams for Bone Tissue Engineering.

Horacio Comas,[1] Catherine Chapuis-Bernasconi,[2] Françoise Borcard,[1] Franziska Krauss-Juillerat,[3] Lucienne Juillerat-Jeanneret,[2] Urs Gonzenbach,[3] Sandrine Gerber-Lemaire.[1]

[1] Ecole Polytechnique Fédérale de Lausanne, Switzerland.
[2] University Institute of Pathology, CHUV, Lausanne, Switzerland.
[3] Department of Materials, ETH-Zürich, Zürich, Switzerland.

The development of functionalised and vascularised bio-scaffolds for bone tissue engineering is a promising approach for reconstructing bone defects. In this project we proposed to conjugate, using an organic linker, ceramic foams to living cells [1]. The former – an insoluble inorganic matrix – can provide mechanical resistance to the graft, while the cells can proliferate to regenerate the bone tissue. One of our first challenges was to find the proper moiety to bind the linker to the ceramic foam in a stable and efficient manner. Two moieties were considered – catechol and pyrogallol – since they can complex the Al present in the inorganic matrix [2] and function as an anchor for the linker. The attachment of linkers containing these functional groups was investigated by UV spectroscopy and fluorescent microscopy. A qualitative and quantitative comparison is presented and discussed.



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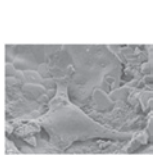
Medicinal Chemistry **105**

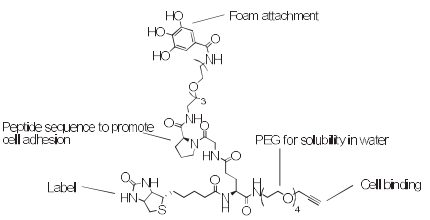
Functionalization of particle-stabilized foams for bio-applications

Françoise Borcard,[1] Horacio Comas,[1], Olivier Wagnière,[1] Franziska Krauss Juillerat,[2] Sandrine Gerber-Lemaire,[1] Lucienne Juillerat-Jeanneret[3] and Urs Gonzenbach[2]

[1] ISIC, BCH, EPFL, CH-1015 Lausanne, Switzerland,[2] Department of Materials, ETH-Zürich, Zürich, Switzerland,[3] University Institute of Pathology, CHUV, Lausanne, Switzerland

Tissue engineering represents a promising approach for the treatment of large bone defects, in which biomaterials are seeded with cells that are expected to promote bone reconstruction. This project aims at the synthesis of multifunctional linkers designed to covalently link both ceramic foams and cells. Following the recent development of new particle-stabilized foams [1] that allow the proliferation and survival of human endothelial (HCEC) cells within the porous material [2], we present here the synthesis of a chemical linker containing specific entities for cell surface functionalization, attachment to biomaterial and cell adhesion promotion. Biocompatibility evaluation of the biomaterial and the results of preliminary biological assays of the cell functionalization by click reaction [3] are also presented.





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[2] Krauss Juillerat, F.; Gauckler, L. J.; Scaletta, C.; Gerber-Lemaire, S; Applegate. L. A.; Juillerat-Jeanneret, L.; Gonzenbach, U., submitted.
[3] Bertozzi, C. R. *et al*., *J. Am. Chem. Soc*. **2006**, 128, 12078-12079

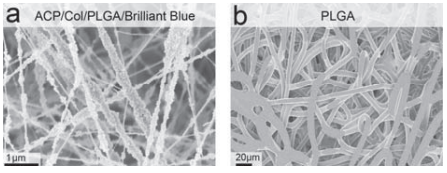
Medicinal Chemistry **106**

Electrospinning of Bioactive Collagen Fibres

Nora Hild, O.D. Schneider, D. Mohn, W.J. Stark*

* Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, ETH Zurich, CH-8093 Zurich, Switzerland

Guided bone regeneration is one of the challenges in orthopedic and dental surgery. Implants need to be osteoinductive and it would be advantageous to have an implant material that presents a chemical composition and a structure as close as possible to bone tissue. Amorphous calcium phosphate (ACP) nanoparticles synthesized by flame spray synthesis were combined with collagen (Col) and poly(lactide-co-glycolide) (PLGA) via electrospinning. To obtain a bifunctional double membrane, pure PLGA was electrospun as described by Schneider et al. [1]. On top of the pure polymer layer dyed ACP/Col/PLGA fibres were electrospun. Fibres were stable enough to undergo crosslinking and *in vitro* biomineralisation experiments. The anisotropic bilayer was investigated in a cell culture study with human mesenchymal stem cells. As an application the bilayer could be used as bone wrapping material with its osteoinductive side on the defect and its non-osteoinductive side being a barrier for cells other than osteoblasts [2].



[1] O.D. Schneider, S. Loher, T.J. Brunner, L. Uebersax, M. Simonet, R.N. Grass, H.P. Merkle, W.J. Stark, *J. Biomed. Mater. Res., Part B*, **2008**, *84B(2)* 350.
[2] N. Hild, O.D. Schneider, N.A. Luechinger, F.M. Koehler, D. Mohn, B.W. Thimm, S. Hofmann, R. Müller, W.J. Stark, in preparation.

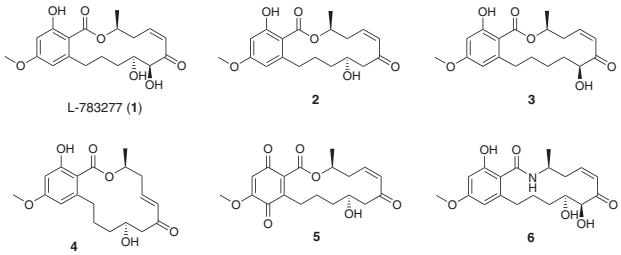
Medicinal Chemistry **107**

Analogues of Resorcylic Acid Lactone L-783277: Syntheses and SAR Studies

Marc Liniger, Christian M. Neuhaus, Michel R. Jordi, Karl-Heinz Altmann*

ETH Zürich, Institute of Pharmaceutical Sciences, HCI, Wolfgang-Pauli-Str. 10, CH-8093 Zürich, Switzerland

Resorcylic acid lactones (RALs) are mycotoxins produced by a variety of different fungal strains, of which those containing a *cis*-enone moiety have been reported to be potent, irreversible kinase inhibitors.[1] L-783277 (**1**) was first isolated from *Phoma* sp. (ATCO 74403) by Zhao *et al.* at Merck Research Laboratories in 1999 and shown to inhibit MEK1 *in vitro* with IC_{50} values of 4 nM. The *cis*-enone moiety is essential for its activity due to Michael addition of a cysteine residue present in the ATP-binding pocket of a subset of kinases.[2] The first total synthesis of L-783277 (**1**) has recently been published by our group.[3]



As part of our ongoing SAR studies around L-783277 (**1**), this contribution reports the syntheses and biological evaluation of the deoxy analogues **2**, **3** and **4**, the benzoquinone analogue **5** as well as the progress in the synthesis of lactam analogue **6**.

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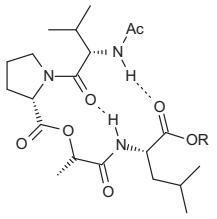
Medicinal Chemistry **108**

Profiling H-Bond Properties of Fragments

Stefan Höck, Rainer Riedl*

Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Campus Reidbach, CH-8820 Wädenswil, Switzerland

Understanding the H-Bond profiles of fragments is a key component for the design of druglike organic molecules with respect to their ability to form specific interactions with their biomolecular targets. In order to rationally design fragments for the efficient and selective binding to target proteins, β-turn peptides and –mimics are synthesized and their interactions with fragments are analyzed by NMR-spectroscopy.



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Molecules such as depsipeptide **1** show well defined NOEs between the two strands of the β-turn [1]. Conclusions about the strength of their interactions with H-bonding fragments as well as the alignment of the fragments in the β-turn are to be drawn from intra- and intermolecular NOEs.

[1] T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 6975.

Medicinal Chemistry **109**

Preparation of FimH Adhesin and Development of Competitive Solid Phase Binding Assay for the Evaluation of Antagonists

Said Rabbani, Xiaohua Jiang, Oliver Schwardt, Beat Ernst

Institute of Molecular Pharmacy, University of Basel, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland

Uropathogenic *Escherichia coli* (UPEC) are the primary cause of urinary tract infections (UTIs) [1]. Most UPEC strains express on their outer membrane multisubunit surface organelles called type 1 pili. The subunit FimH, which is located on the top of the pili, recognizes and binds specifically to mannosides on the surface of target cells [2]. Because of UPEC resistance to antibiotics several carbohydrate-based anti-adhesins are currently developed [3].

Taking advantage of our expertise in carbohydrate synthesis, several FimH antagonists were synthesized. To evaluate these antagonists, we expressed the carbohydrate recognition domain of FimH (FimH-CRD) and developed a competitive binding-assay. The cell-free assay is based on the interaction of a polyacrylamide glycopolymer containing mannoside residues with FimH-CRD. For the quantification, the biotinylated polymer is complexed with streptavidin coupled to horseradish peroxidase followed by a peroxidase reaction. The validated assay is sensitive, reproducible and is suitable for the evaluation of FimH ligands.

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[2] J.J. Martinez, M.A. Mulvey, J.D. Schilling, J.S. Pinkner, S.J. Hultgren, *EMBO. J.* **2000**, 19, 2803-2812.
[3] N. Sharon, *Biochim. Biophys. Acta.* **2006**,1760, 527-537.

Medicinal Chemistry **110**

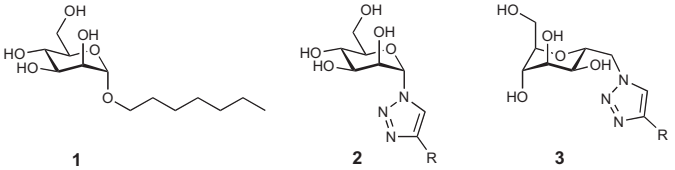
Biological Evaluation of α-D-Mannosyl Triazoles as Ligands for FimH

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

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

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

In our search for metabolically stable and pharmacokinetically improved FimH antagonists, a library of mannosyl triazoles of types **2** and **3** was synthesized and tested in target-based and cell-based binding assays. Compared to the reference compound **1**, the affinity of the triazoles was decreased about 2- to 10-fold. A thorough analysis by NMR spectroscopy (NOESY, COSY, HSQC) revealed that the *C*-mannosides **3** adopt an unusual $^1C_4$ conformation.



[1] T. M. Hooton, W. E. Stamm, *Infect Dis. Clin. North. Am.* **1997**, *11*, 551.
[2] J. Bouckaert *et al.*, *Mol. Microbiol.* **2005**, *55*, 441.

Medicinal Chemistry **111**

Crystallization and Crystal Structure Analysis of the Novel Bridged Monobactam, BAL29880

F. Richalet[1], H. P. Weber[2], M. Neuburger[2], E. Desarbre[1]

[1]Basilea Pharmaceutica International AG. Grenzarcherstrasse 487, CH-4005 Basel, Switzerland
[2]Laboratory for Chemical Crystallography, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Gram-negative bacteria (GNB) are becoming increasingly resistant towards β-lactam antibiotics. The main factor leading to this resistance is the production of β-lactamases, enzymes that hydrolyze the antibiotics and render them inactive. A successful approach to protect β-lactam antibiotics from attack by β-lactamases is to combine the antibiotic with an inhibitor of these enzymes. BAL29880 (Fig.1) is a novel bridged monobactam that potently inhibits class C β-lactamases and that is able to protect β-lactam antibiotics *in vitro* and *in vivo,* as exemplified by the investigational agent BAL30376[1]. The crystalline form is often desirable for isolation, purification and pharmaceutical development. Modifications of the initial synthesis have been identified that allow facile crystallization of BAL29880[2]. Analysis revealed that there are no solvent molecules in the crystals of BAL29880 and that the crystal structure consists of dimers of zwitterionic molecules.

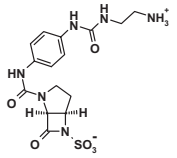


Fig. 1: BAL29880

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[2] E. Desarbre, F. Richalet, *Patent Application WO2009/071638*

Medicinal Chemistry **112**

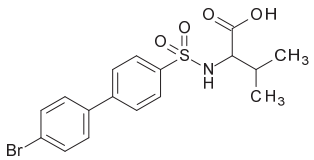
Structure-based Design and Synthesis of Selective MMP Inhibitors

Thomas Fischer, Rainer Riedl*

Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Campus Reidbach, CH-8820 Wädenswil, Switzerland

Matrix metalloproteinases (MMPs) are considered as promising targets for the treatment of cancer due to their strong involvement in malignant pathologies. Despite massive research and development efforts, the success rate of MMP inhibitors in the clinic has been very low. Possible reasons for the low success rate of MMP inhibitors in the clinic include unwanted side effects caused by their lack of selectivity, poor oral bioavailability and decreased potency in vivo [1].

In order to improve the selectivity of MMP inhibitors, we rationally design and synthesize libraries of S1'-binders based on X-ray data of MMP-inhibitor co-crystals.



1

Based on PDB deposited X-ray data [2], inhibitors such as **1** are ideal tool compounds for expanding into the selectivity affecting S1'-pocket by employing a wide range of synthetic organic transformations on the aryl bromide.

[1] J. Fisher, S. Mobashery, *Cancer Metastasis Rev.* **2006**, *25*, 115.
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Medicinal Chemistry **113**

A new class of non benzodiazepine $GABA_A$ α5 inverse agonists

A. Koblet, T. Ballard, B. Büttelmann, H. Fischer, R. Gasser, B. Han, M.-C. Hernandez, F. Knoflach, H. Knust, M. Nettekoven, G. Trube A.W. Thomas

Pharmaceutical Division, Discovery Research CNS & Medicinal Chemistry,

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

Extensive detailed pharmacological exists in both rodent and humans suggesting non selective inverse agonists at the benzodiazepine site of the $GABA_A$ receptor (BZR) enhance cognitive functions.[1] However, non selective inverse agonists induce anxiogenic and pro-convulsive effects. Through the greater understanding of the complex pharmacology of the $GABA_A$ α receptor sub-types it is now strongly believed that the cognitive effects are mediated through inverse agonism of the $GABA_A$ α 5 receptor sub-type which has been supported by recent results in the clinic.[2]

Within our research programme we have identified several novel series of potent and selective $GABA_A$ α5 receptor inverse agonists. The poster will describe the discovery and lead optimisation of the isoxazole based chemical classes [3,4,5] culminating in the full profile of key compounds.

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[5] Büttelmann, B.; Han, B.; Knust, H.; Thomas, A. W., WO2005040345; WO2007074078; US20070161654

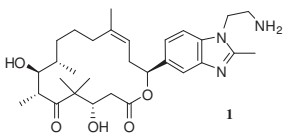
Medicinal Chemistry **114**

Total Synthesis of New Functionalized Epothilone Analogs for Prodrug Design and Tumor Targeting

S. Anthoine Dietrich[1], F. Gaugaz[1], J. Gertsch[2], K.-H. Altmann[1]

[1]ETH Zürich, Institute of Pharmaceutical Sciences, HCI, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland
[2]University of Bern, Institute of Biochemistry and Molecular Medicine, Bühlstrasse 28, CH-3012 Bern, Switzerland

Epothilones are microtubule-stabilizing natural products which exhibit strong antiproliferative effects *in vitro* and potent antitumor activity *in vivo*, including in multi-drug resistant human tumor models.[1] Despite their promising profile, their therapeutic utility would benefit greatly from an increase in their selectivity for tumor cells, reducing side effects and widening their therapeutic window. In this context we have devised and synthesized novel epothilone analog **1**,[2] with the goal of using the newly introduced primary amino group as an attachment site for various tumor-targeting moieties. To this end, folic acid[3] has been selected as a targeting agent towards folate receptor (FR) overexpressing malignancies.



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Various conjugates of **1** with folic acid have been synthesized, using different linker moieties designed either to be stable in cell culture conditions or to be cleaved upon cellular uptake. The *in vitro* biological activity of these new epothilone conjugates will be discussed with respect to their interactions with the tubulin/microtubule system and the inhibition of the proliferation of FR-positive and FR-negative human cancer cell lines.

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Medicinal Chemistry **115**

Structural studies of the GPCR-fragment TM1-TM2-TM3 of Ste2p

Martin Poms[1], Alexey Neumoin[1], Leah Cohen[2], Jan Becker[3], Fred Naider[2], Oliver Zerbe[1]

[1]University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich Switzerland
[2]College of Staten Island, CUNY, NY 10314, USA
[3]University of Tennesee, Knoxville, TN 37996

G protein-coupled receptors (GPCRs) are membrane receptors involved in numerous important physiological processes and therefore have become an interesting target for structure-based drug research. However, GPCR expression and purification, as well as their hydrophobic nature present a considerable challenge, explaining the low number of high-resolution structures published to date[1]. In order to bypass problems associated with the structure determination of full-length GPCRs we started to elucidate structures of large fragments using solution NMR [2,3].

Here we report on a fragment containing three transmembrane domains of Ste2p, a receptor involved in the mating process of *Saccharomyces cerevisiae.* [^{15}N, ^{13}C, ^{2}H]-labeled TM1-TM2-TM3 in LPPG/DPC micelles has been studied using 3-dimensional solution NMR techniques. Nearly complete backbone assignment was achieved. Preliminary analysis using backbone chemical shifts as well as ^{15}N relaxation data indicates correct folding of the three putative helices in accordance with a model.

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Medicinal Chemistry **116**

Binding affinities of 2'-*O*-methyl oligoribonucleotides complementary to the loop of pre-miR-122: A Surface Plasmon Resonance study.

Mario A. Rebhan[1], Luca Gebert[1], Harry Towbin[1], Jonathan Hall[1]

[1]ETH Zürich, IPW, Wolfgang-Pauli-Strasse 10, 8093 Zürich

Post-transcriptional regulation by microRNAs (miRNAs) has been identified as an important component of eukaryotic gene expression [1, 2]. It has been shown that this novel class of short (21–23 nt) non-coding RNAs plays an important role not only in natural development and function of organisms [3], but also in many disease mechanisms [4].

MiR-122 is a very abundant microRNA with a selective expression in adult liver cells (up to 70 % of all miRNA in these cells) [5] and has been shown to play an important role in fatty acid metabolism [6] and the replication of hepatitis C virus [7]. Its validation as a drug target has been performed by several pharmaceutical companies [8, 9]. We measured the affinities of short antisense oligonucleotides to the biotinylated hairpin-precursor (pre-miR-122) bound to the surface of a streptavidin-coated SPR-chip. Using this methodology, we identified ligands that interfered with processing of pre-miR-122 by the RNase III type enzyme dicer.

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Medicinal Chemistry **117**

Inhibition of pre-miR-122 processing by 2'-*O*-methyl antisense oligoribonucleotides targeting the loop region

Luca Gebert[1], Mario Rebhan[1], Jonathan Hall[1]

[1]ETH Zürich, IPW, Wolfgang-Pauli-Strasse 10, 8093 Zürich

MicroRNAs (miRNAs) are a recently-discovered [1] class of short non-coding RNAs (ncRNAs) which play important roles in the development and function of organisms [2] and in disease mechanisms [3]. The final step of their biogenesis in mammals is the cleavage of the precursor microRNA (pre-miRNA) by the RNase III type enzyme Dicer (Fig. 1) [4][5]. Antisense oligoribonucleotides with a 2'-*O*-Me modification have been used to inhibit this maturation step for the human miR-122, a miRNA higly expressed in liver, which plays a key role in replication of hepatitis C virus (HCV) [6] and in fatty acid metabolism [7].

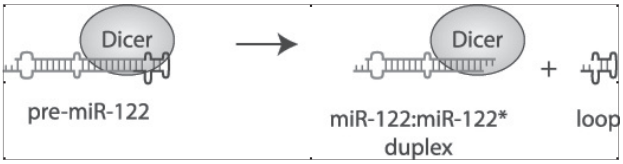

Figure 1: Dicer cleavage of pre-miR-122

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Medicinal Chemistry **118**

Structural studies of double TM fragments of the Y4 receptor, a human GPCR, using solution NMR techniques

Harsha Kocherla, Xuan Shao and Oliver Zerbe

University of Zürich, Winterthurerstr. 190, CH-8057, Zürich Switzerland

The structural studies of intact GPCRs by NMR remains a challenging problem because of the daunting technical difficulties in expression, purification, and the problems associated with their structural analysis. According to Popot and Engelman the transmembrane domains can be thought of as independent folding units and can be studied separately[1]. Our group has demonstrated previously, that double-transmembrane (2-TM) fragments of the yeast Ste2p receptor possess tertiary structure, and that such structures can be successfully solved in a membrane-mimetic, using solution NMR [2]. Moreover, we could demonstrate that a double-TM fragment of the Y4 receptor that contains the N-terminal domain as well as the first two transmembrane helices can be directly expressed, purified and refolded [3].

Our present efforts are directed towards the expression of various other transmembrane fragments of the Y4 receptor in *E.coli*. For sufficient expression, we varied the *E. coli* expression host and tested a number of fusion partners. Furthermore, the influence of codon usage was investigated as well as a purification strategy developed in different detergents, suitable for structure determination by NMR. Here, we present our results from double-TM fragments of the Y4 recpetor, that complement our previous studies on TM1-TM2, in order to derive a more complete picture of the entire receptor.

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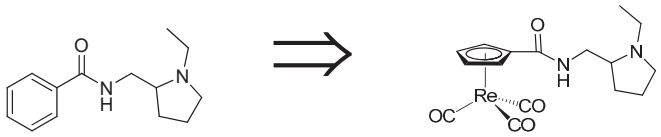
Medicinal Chemistry **119**

The [CpM(CO)$_3$] - Moiety (M = Mn, Tc, Re) as Phenyl Ring analog – a Promising Strategy Towards New Drugs and Radiopharmaceuticals

D. Can, H.P. N'Dongo, P. Schmutz and R. Alberto*

University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

As early as 1979 Hanzlik et al. studied the interaction of β-Ferrocenylalanine with phenylalanine hydroxylase and phenylalanine decarboxylase and showed that the ferrocene derivative behaved like phenylalanine analogues[1]. Ongoing investigations by Jaouen et al. showed years later, that substitution of a phenyl ring in tamoxifen by ferrocene similarly kept the biological activity of the lead compound intact[2].
As ^{99m}Tc is nowadays in the focus of the development of radiotracers, introducing group 7 transition metals as [CpM(CO)$_3$] into this analogy opens new directions not only towards new drugs but also towards very promising radiopharmaceuticals.



Following this strategy we will present the analogy of different classes of bioactive compounds containing [CpRe(CO)$_3$]: sulphonamides acting as carbonic anhydrase inhibitors with high binding affinities[3], histone deacetylase inhibitors and melanoma imaging agents with melanin afiinity.

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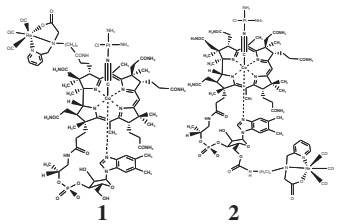
Medicinal Chemistry **120**

Targeted transport of anticancer agents with B12 derivatives

Mai Thanh Quynh Tran, Pilar Ruiz-Sanchez, Roger Alberto

ACI – University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Based on the high demand of vitamin B_{12} by fast proliferating cells, different possibilities to utilize vitamin B_{12} as a targeted transporter of anticancer drugs have been reported.[1] The vitamin B_{12} transport protein, transcobalamin II (TCII), mediates the transport of cobalamins into the cell by receptor-mediated endocytosis. Vitamin B_{12} derivatives bound to TCII have shown high B_{12} accumulation in the liver and kidney. Previous researches in our group aimed to interfere these interactions by derivatization of B_{12} at the b-side chain.[2] We propose b-[Re(CO)$_3$]-PAMA-B_{12} (**1**), which abolishes the interaction with TCII and reduces the kidney and liver accumulation, as a model for therapeutic drug delivery. As a reference, we investigate a TCII-binder molecule in which B12 is derivatized analogously at the ribose position (**2**). Furthermore, an anticancer agent like cisplatin is attached to the cyanide at the β-axial position for both compounds.



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Medicinal Chemistry **121**

CHEMO-GENETIC OPTIMIZATION OF DNA RECOGNITION BY METALLODRUGS USING A PRESENTER PROTEIN STRATEGY

J.M. Zimbron,[a] A. Sardo,[a] T. Heinisch,[a,b] T. Wohlschlager,[c] J. Gradinaru,[c] C.Massa,[b] T. Schirmer,[*b] M. Creus,[*a] T. R. Ward[*a]

[a]University of Basel, Department of Chemistry, Spitalstrasse 51, Basel 4056, Switzerland, [b]University of Basel, Biozentrum, Klingelbergstrasse 50/70, Basel 4056, Switzerland, [c]University of Neuchâtel, Institute of Chemistry, Avenue de Bellevaux 51, Neuchâtel 2009, Switzerland

DNA is a privileged target of anticancer metallodrugs like cisplatin. However, such drugs suffer from high toxicity and drug-resistance due to non-selective binding to other than oncogenic DNA.[1] To increase selectivity of small molecule drugs for macromolecular targets, "surface borrowing" can be used to provide additional surface contacts via a presenter protein, which modulates the specificity and affinity of ligand–macromolecule interaction.[2, 3] Inspired by presenter protein strategies and our previous experience of enantioselective artificial metalloenzymes,[4] we synthesized a biotinylated metallodrug based on promising anticancer Ru(II) piano-stool complexes,[5] for incorporation into streptavidin. Here we show that a supramolecular assembly of a drug with a presenter protein can modulate selectivity through provision of additional non-covalent interactions with the target, allowing selectivity *in vitro* toward macromolecules such as DNA telomeres.

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Medicinal Chemistry **122**

Urate repairs Tryptophan and Tyrosine Radicals in Proteins

Anastasia S. Domazou, Hongping Zhu and Willem H. Koppenol

Institute of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, CH-8093 Zürich, Switzerland

In vivo, PROS may react preferentially with proteins, which results in formation of radicals. These radicals may be repaired by antioxidants or may engage in reactions that ultimately result in denaturation. Urate is an important antioxidant, present at high concentrations in plasma and saliva.

We generated tryptophan and tyrosine radicals (Trp$^{\bullet}$ and TyrO$^{\bullet}$) in α-chymotrypsin, pepsin, and *N*-acetyl-tryptophan amide by pulse radiolysis.

Urate repaired Trp$^{\bullet}$ in chymotrypsin and *N*-acetyl-tryptophan amide with rate constants of 2.1×10^8 $M^{-1}s^{-1}$ and 1.9×10^7 $M^{-1}s^{-1}$, respectively, but not in pepsin ($k < 10^8$ $M^{-1}s^{-1}$). In contrast, urate repaired TyrO$^{\bullet}$ in pepsin with a rate constant of 4.9×10^8 $M^{-1}s^{-1}$ but not in chymotrypsin ($k < 2\times10^6$ $M^{-1}s^{-1}$). Electron transfer from Tyr residues to Trp$^{\bullet}$ – observed in both proteins – was inhibited efficiently by urate only in chymotrypsin but not in pepsin. Thus, TyrO$^{\bullet}$ in chymotrypsin and Trp$^{\bullet}$ in pepsin are not accessible to urate.

Our results show that urate can repair protein radicals and prevent damage *in vivo*. Recently, we reported rate constants in the range 10^5–10^8 $M^{-1}s^{-1}$ for the repair of Trp$^{\bullet}$ and TyrO$^{\bullet}$ by ascorbate in a number of proteins [1]. Urate may prove to be the more important repair agent in those tissue compartments where the concentration of urate is higher than that of ascorbate. The urate radical produced upon repair of protein radicals is, in turn, reduced by ascorbate. Loss of ascorbate is then the net result, while urate is conserved.

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Acknowledgement This work was supported by ETH-Zurich

Medicinal Chemistry **123**

Phosphorylation and Nitration of Tyrosine: Effect on Radical Decay and Electron Transfer in Peptides

Weiqun Shi, Anastasia Domazou, Patricia L. Bounds, Willem H. Koppenol*

Laboratory of Inorganic Chemistry, Department of Chemistry and Applied Biosciences,
ETH-Zurich, CH-8093 Zurich, Switzerland

Radical reactions play a productive role in a wide range of biological processes [1], but also contribute to oxidative damage to macromolecules. Oxidative reactions can initiate radical formation at various residues on the surface of a protein, which can then undergo long-range electron transfer (LRET) to relocate the unpaired electron to other residues. Intramolecular LRET in proteins often results in the radical being resident on a tyrosine (Tyr) sidechain. Reversible phosphorylation of Tyr residues [2] is a feature of cellular signaling pathways that can be modulated by the redox status of the cell [3] and impacted by LRET. Further, Tyr nitration, a recognized tissue biomarker for exposure to oxidative stress [4], is likely to be affected by LRET.

We have studied effects of Tyr phosphorylation and nitration on LRET and radical decay in model peptides (e.g., YPPW) and in fragments from the proteins β-tubulin and α-synuclein. Radical formation on tryptophan (Trp) was initiated by pulse radiolysis, and electron transfer to relocate the radical from Trp to Tyr was observed by UV/VIS spectrophotometry. Intramolecular LRET reactions were clearly observed in YPPW and β-tubulin, and rate constants were evaluated; phosphorylation of the Tyr residue blocks LRET in these peptides. We suggest that phosphorylation and nitration of Tyr residues contributes to the response to oxidative stress.

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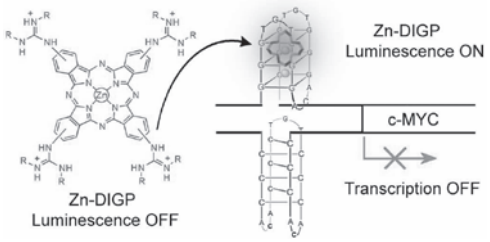
Medicinal Chemistry **124**

Multi-functional Probes for G-Quadruplexes

Balayeshwanth R. Vummidi, Jawad Alzeer, Philippe J.C. Roth and Prof. Nathan W. Luedtke

University of Zurich, Winterthurstrasse 190, 8057 Zurich, Switzerland.

Nucleic acids can form a number of complex structures aside from the well-known Watson-Crick double helix. Under certain physiological conditions, G rich sequences can fold into four stranded "G-quadruplex" structures that are stabilized by Hoogsten hydrogen bonding. It is speculated that G rich promoter regions of oncogenes such as c-myc, c-kit and PDGF can fold into G-quadruplexes that can suppress transcription. Small molecules that selectively bind and stabilize the G quadruplex structures are therefore potentially important to cancer research. Here, studies with tetra-(diisopropylguanidine) zinc phthalocyanine (Zn-DIGP) is shown to specifically bind to known G quadruplex structures with high affinity ($K_d \leq 2$ nM). Zn-DIGP is nontoxic up to 100 μM and shows significant increase in fluorescence upon binding nucleic acids, and upon entry into living and fixed cells. Low concentrations of Zn-DIGP (1 μM) reduced c-Myc mRNA expression in living cells by a factor of 3-fold in 2 hours. These results show how Zn-DIGP is multi-functional fluorescent probe specific for G-quadruplex DNA.



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Medicinal Chemistry **125**

Bulky DNA Lesion Processing Using Synthetic Nucleoside Triphosphates

Hailey L. Gahlon, Shana J. Sturla*

ETH Zürich, Schmelzbergstrasse 9, CH-8092 Zürich, Switzerland

Nitrosamines, derived from both dietary and environmental chemicals, can become bioactivated in vivo resulting in DNA adducts. These adducts, if not repaired, can lead to mutations in the DNA and possibly cancer initiation. For example, O6-Benzylguanine (O6BnG) is a bulky alkyl guanine adduct that can be generated from metabolic activation of N-methylbenzyl nitrosamine, and can also be an informative model for understanding the occurrence and potential negative biological impacts of bulky O6-alkylguanine adducts. Adduct paired synthetic DNA is a proposed approach for probing the occurrence and fate of O6BnG adducts. In this study, we examine the process of enzymatic incorporation of synthetic nucleoside triphosphates templated by O6BnG adducts vs. unmodified DNA. Incorporation efficiencies and steady state kinetic parameters for lesion-bypass synthesis are evaluated. It is expected that this mechanistic information regarding the selectivity of nucleotide incorporation, and how it is influenced by base structure, will lead to the realization of nucleoside analogues as molecular probes for the detection and study of bulky DNA adducts.

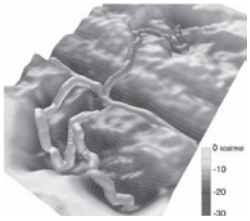
Medicinal Chemistry **126**

Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition

Vittorio Limongelli[1,2], Massimiliano Bonomi[1], Luciana Marinelli[2], Ettore Novellino[2], and Michele Parrinello[1]

[1]Computational Science, Department of Chemistry and Applied Biosciences, ETH Zurich, USI Campus, Via Giuseppe Buffi 13, CH-6900 Lugano, Switzerland
[2]Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", Via D. Montesano, 49, I-80131 Napoli, Italy

We have simulated the dynamic binding mechanism of the potent ligand SC-558 (IC50=9.3 nM) into the cyclooxygenase-2 using metadynamics. A key role in this process is played by the alpha-helices that guard the entrance to the cyclooxygenase site, whose breathing facilitates the passage of the ligand. We found that SC-558 can bind a site different from the X-rays one. Interestingly, this previously unreported binding mode is similar to that of non-selective inhibitors like ibuprofen. Several experimental data support our results. For instance, Arg120 and Tyr355, reported to play a crucial role in enzyme inhibition, despite being placed at the entrance of the enzyme, interact directly with the ligand on its way out of the enzyme. Our discovery sheds new light on the mechanism of COX inhibition also offering a new chance of developing new COX inhibitors with tuned selectivity.



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Medicinal Chemistry **127**

Understanding the inactivation mechanism of p38αMAP kinase.

Anna Berteotti[1], Vittorio Limongelli[1] and Michele Parrinello[1]

[1]Computational Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, USI Campus, Via Giuseppe Buffi 13, CH-6900 Lugano, Switzerland

Kinases play pivotal roles in the cell cycle regulation and in several pathological conditions.
Thus in the very recent years, a selective kinase inhibition has become an important goal for both the academia and pharmaceutical industry.
Here, using an advanced computational technique we have simulated the inactivation mechanism of p38αMAP kinase [1] regulated by the movement of about 10 Å of three well conserved amino acids.
In this study the data coming from the X-ray/NMR experiments are assessed and complemented shedding light on the dynamical character of this movement. Based on our results, the structural information such as the creation of additional and specific binding pocket, that might be specifically targeted, are of precious help for the future design of novel and selective kinase inhibitor.

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Medicinal Chemistry **128**

Interactions of metal-based anticancer drugs with biomolecules - implications for the mode of action

Michael Groessl[1], Paul J. Dyson[1]

[1] Institute of Chemical Sciences and Engineering, EPFL, 1015 Lausanne, Switzerland

In the last few years, two ruthenium-based drugs have entered clinic trials generating considerable interest in the medicinal properties of ruthenium compounds. These drugs are effective against primary tumours and metastasis, for which cisplatin and other related drugs are ineffective. The ruthenium-based drugs also show remarkably low toxicity which contrasts with other metal-based drugs.

The biomolecular interactions of most metal-based drugs, from intravenous application to delivery to the cell and entry into specific organelles such as the nucleus, are largely unknown. We attempt to clarify the ambiguity surrounding these compounds (KP1019 and NAMI-A, the two ruthenium drugs currently under clinical investigation, as well as RAPTA-T, a highly promising ruthenium(II)-arene anticancer compound which shows selectivity in solid metastatic cancers) by mapping the protein-drug interactions that occur inside the cell. In addition, the interactions with DNA in comparison with established platinum-based drugs are also characterized. Combining the information obtained from inductively coupled plasma (ICP) and electrospray ionization (ESI) mass spectrometry (MS) hyphenated to liquid separation techniques provides a deeper insight into the mode of action of the drugs and should aid the rational design of future pharmaceuticals.

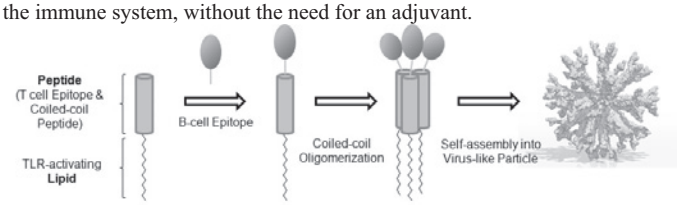
Medicinal Chemistry **129**

Synthetic Virus-like Particles in Rational Vaccine Design

Tina Riedel, Arin Ghasparian and John A. Robinson

Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Virus-like particles (VLPs) consist of viral capsid proteins or lipoproteins expressed *in vitro* through recombinant technologies. They represent a specific class of subunit vaccine that mimic the structure of authentic viruses and they are recognized readily by the immune system [1].
We explored an approach to a new class of virus-like particles composed of synthetically derived lipopeptide building blocks. The self-assembling properties of a coiled coil peptide and the aggregation effect of lipid chains lead to the formation of a synthetic-virus like particle (SVLP) which adopts a stable nanosize structure of around 20-30 nm. Biophysical studies revealed that each lipopeptide building block associates into parallel trimeric helical bundles which form micelle-like particles with the lipid chains buried in the core of the peptide [2]. We show that our SVLPs are amenable to engineering to allow the incorporation of B cell epitopes, T helper epitopes as well as ligands for Toll-like receptors which ensures the efficient stimulation of the immune system, without the need for an adjuvant.



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Medicinal Chemistry **130**

Billions of GDB-15 molecules for drug discovery

Lars Ruddigkeit[1], Ruud van Deursen[2], Lorenz C. Blum[2] and Jean-Louis Reymond[2]

[1]University of Berne, Freiestrasse 3, 3012 Berne, Switzerland
[2] University of Berne, Freiestrasse 3, 3012 Berne, Switzerland

As a rather small number of drugs have entered the market in the last 20 years, the need for new chemical entities is apparent.[1] *In silico* methods like molecular scaffolds analysis and exhaustive enumeration of chemical space[2]can assist the search for novel molecules. Recently our group published GDB-13, the largest publicly available small organic molecule database.[3]

Here we report the extension of the approach up to 15 atoms. We introduced an entirely new and faster algorithm. Furthermore a set of restricted filters for topology and functional groups was defined, based on the statistical occurrence of these structural elements in ZINC.[4] The total number of molecules was thus reduced substantially while focussing on a more relevant chemical space but expand still billions of molecules.

GDB-15 contains a vast quantity of structures not present in known databases such as ACX, Zinc or PubChem. As a result of this it can certainly contribute to the search for new bioactive molecules in chemical biology and medicinal chemistry.[5]

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Medicinal Chemistry **131**

Synthetic DNA-adduct analogs as probes for testing acylfulvene cytotoxicity mechanism

Marina Tanasova[1], Shana J. Sturla[2]

[1]ETH Zurich, IFNH, LFO D26, Schmelzbergstrasse 9, Zurich
[2]ETH Zurich, IFNH, LFO D15.1, Schmelzbergstrasse 9, Zurich

Acylfulvenes (AF) are a class of tumor-specific cytotoxic agents derived from naturally occurring fungal metabolites of the illudin family. Cytotoxic activities of acylfulvenes are linked with initiation of DNA damage, and recent data suggests that acylfulvene-derived adducts may inhibit transcription by stalling RNA polymerase II (RNAPII). However, AF-derived DNA adducts, which we have characterized in previous studies, are chemically unstable and prone to depurination. This property raises the question whether the initially formed adducts, or associated increases in abasic site formation, trigger biochemical responses. Furthermore, from a practical perspective, lack of stability makes it difficult to utilize acylfulvene-modified DNA as a molecular probe of RNAPII interaction specificity. Therefore, as a step toward understanding chemical and biochemical factors dictating the interactions between acylfulvene-induced DNA damage and RNAPII, we have designed and synthesized chemically stable analogs of drug-induced DNA damage products. The mechanistic probes prepared in this study include a 3-deazaadenine analog of the major 3-acylfulvene-adenine adduct, and related analogs as control substrates. The modified nucleobases were incorporated into DNA oligonucleotides and the influence of the modifications on to the thermal stability of DNA was investigated. Mechanistic investigations regarding AF-DNA lesion-RNAPII interaction employing transcription arrest assays are in progress and will provide an insight into the role of DNA lesion formation in acylfulvene cytotoxicity.

Medicinal Chemistry **132**

Computational Study of the Enzymatic Reaction Mechanism in Dengue Methyltransferase

Tobias Schmidt[1,2], Torsten Schwede[1], Markus Meuwly[2]

[1]Swiss Institute of Bioinformatics, Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland
[2]Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland

Dengue fever is a mosquito-borne viral infectious disease predominantly prevalent in tropical regions with annually 50−100 million cases and around 25000 death worldwide. Nowadays, it is one of the most important emerging infectious diseases in many areas of the world. Currently, neither vaccines nor specific drugs treatments are available.[1]
The dengue virus is an enveloped, single-stranded, positive sense RNA virus. The 5' end of the dengue genome contains a type 1 cap structure which is essential for viral replication by enhancing RNA stability and increasing translation efficiency.[2] The viral NS5 RNA methyltransferase is critical for the formation of the RNA cap structure and is thus an attractive target for drug discovery.[3,4,5]
The detailed enzymatic reaction mechanism of the methyl transfer from S-adenosyl-L-methionine to the viral RNA cap structure, catalyzed by the NS5 RNA methyltransferase, has not been elucidated so far. Thus, in this work, we investigate possible reaction pathways at an atomistic level, using high level ab initio calculations as well as reactive molecular dynamics simulations.[6] We aim at a better understanding of the molecular basis of this disease related enzymatic function, which significantly benefits rational drug discovery.

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Medicinal Chemistry **133**

New antibacterial coated surfaces for self-protecting implants

Priscilla. S. Brunetto, Katharina. M. Fromm*

University of Fribourg, Department of Chemistry, Chemin du Musée 9, 1700 Fribourg, Switzerland

The development of new bioactive surfaces represents a solution to a medical problem: bacterial adhesion on surfaces, mainly by S*taphylococci*. The resultant biofilm is resistant to aggressive pharmacological agents as well as host defences [1]. One way to prevent the establishment of infection is to render the implant surface bactericidal, creating a stable environment with a spectrum similar to the soluble antibiotics [2].
We chose the well-studied antibiotic vancomycin, the drug of last resort for treating Gram-positive bacterial infections, as targets to be bonded to the surface of implant materials [3]. The so-coated surfaces were characterized and exposed to bacteria in different assays and tested for their biocompatibility for soft- tissue integration. These different *in vitro* tests have turned out to be very promising for further investigations. This may be an efficient solution conquering bacterial adhesion and biofilm.

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Medicinal Chemistry **134**

Structure validation of NMR data using 10 ppm HMBC and "Logic for Structure Determination".
Application to a flavonoid of *Ouratea calantha*

Gaétan Bayiha Ba Njock[1,2,3], Trixie Ann Bartholomeusz[3], Philippe Christen[3], Jean-Marc Nuzillard[4], Damien Jeannerat*[1]

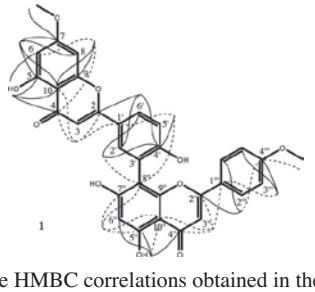
[1]Département de Chimie Organique, Université de Genève, 30 quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland

[2]Département de Chimie Organique, Faculté des Sciences, Université de Yaoundé I, B. P. 812, Yaoundé, Cameroun

[3]Section des Sciences pharmaceutiques Université de Genève, Université de Lausanne, Quai E. Ansermet 30, CH-1211 Geneva 4, Switzerland

[4]Institut de Chimie Moléculaire de Reims, CNRS, UMR 6229, Université de Reims, F-51687 Reims Cedex 2, France

Plants from the Ochnaceae family are used in traditional medicine and have been reported to be rich in flavonoids with important biological activities. The biflavonoids found in *Ouratea calantha* Gilg. represented a challenge because of signal clustering causing assignment problems in HMBC spectra. We used 10 ppm HMBC spectra to resolve signal overlap while the computational method called "Logic for Stucture Determination" validated that the structure is the only one compatible with NMR data.



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Dotted lines indicate HMBC correlations obtained in the 10 ppm spectrum.

Medicinal Chemistry **135**

Reactivity of Antitumor Acylfulvenes with Selenocysteine-Containing Redox-Regulating Enzymes in Cancer Cells

Kathryn E. Pietsch, Xiaodan Liu, Shana J. Sturla

ETH Zürich, Schmelzbergstrasse 9, CH-8092 Zürich, Switzerland

Acylfulvenes (AFs) are a family of alkylating agents derived from the natural product illudin S. AF analogues have antitumor properties and exhibit greater selectivity for tumor cells over normal cells compared to the parent compound. Alkylation of critical biomacromolecules like DNA contribute to the cytotoxicity of AFs. An unanswered question for AFs, as well as other alkylating agents, is the potential role of protein alkylation in cytotoxicity and whether there are chemical aspects of small molecule-protein interactions that influence toxic selectivity in tumor cells. To address this general problem we aim to understand how AFs react with proteins that contain nucleophilic active sites, including the critical redox-regulating proteins glutathione reductase and thioredoxin, which contain cysteine residues in their active sites. Another variant nucleophilic residue with potential reactivity toward electrophilic compounds is selenocysteine. Selenium is an important trace element nutrient required for the synthesis of selenocysteine, and selenium supplementation in the diet is implicated in the expression of selenium-containing proteins. Selenoproteins, like thioredoxin reductase (TrxR) and glutathione peroxidase (GPx), contain active site selenocysteine residues and are involved in regulating the cellular redox state to protect cells from harmful oxidants. Herein we present findings regarding AFs reactivity with selenoproteins and its influence on enzymatic activity. Enzyme inhibition by AFs was studied in cell and cell-free systems, and the covalent adduct arising from the reaction of AF with TrxR was identified by mass spectrometry. We also demonstrated that TrxR levels are elevated in HeLa cells cultured in selenite-supplemented media, and this increase in TrxR activity sensitizes cells toward AFs. Understanding chemical mechanisms of protein alkylation and its contributions to AFs' selectivity are expected to lead to better comprehension of selective chemical toxicity profiles, and how these may be controlled by essential nutrients or metabolic flux.

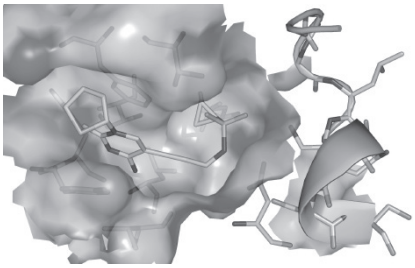
Medicinal Chemistry **136**

Structure-based Design and Synthesis of Inhibitors for IspE, an Enzyme in the Non-mevalonate Pathway of Isoprenoid Biosynthesis and Antimalarial Target

Paolo Mombelli[1], Anna K. H. Hirsch[1], Adelbert Bacher[2], Markus Fischer[3], François Diederich[1]

[1]ETH Zürich, HCI, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland, diederich@org.chem.ethz.ch
[2]TU München, Lichtenbergstrasse 4, D-85747 Garching, Germany
[3]Universität Hamburg, Grindelallee 117, D-20146 Hamburg, Germany

The enzymes of the non-mevalonate pathway of isoprenoid biosynthesis have been identified as promising therapeutic targets for the treatment of infectious diseases such as malaria or tuberculosis. The fourth enzyme of the pathway, the kinase IspE, was chosen for the *de novo* design of new inhibitors. Our research group recently published a series of inhibitors displaying *in vitro* activity against the enzyme from *E. coli* in the nanomolar range.[1] A new class of compounds was designed and synthesized as potential inhibitors of IspE.



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Medicinal Chemistry **137**

Novel ligands for the study of ligand-gated ion channels.

Ruud Hovius[1] Maurice Goeldner[2], Elias Sakr[2], Christoph Schreiter[1], Ana-Paula Tairi[1], Jörg Grandl[1], Andreas Peer[1], Ralf Schmauder[1], Davor Kosanic[1], Alexandra Patras[1], Horst Vogel[1]

[1] Laboratory of Physical Chemistry of Polymers and Membranes, Ecole Polytechnique Fédérale de Lausanne, Lausanne
[2] Laboratoire de Chimie Bioorganique UMR 7175 LC1 CNRS Université Louis Pasteur Strasbourg, Illkirch, France.

Ligand-gated ion channels of the Cys-loop family are hetero- or homo-oligomeric proteins, which upon the binding of agonist molecules open a transmembrane channel allowing ions to flow over the membrane according to their electro-chemical gradient. The binding of agonists to ligand-gated ion channels and subsequent channel opening are a model for allosteric activation, as the binding sites and the channel gate are distant by about 5 nm. Moreover, two or more agonist molecules have to bind to the oligo-pentameric receptors to evoke full activation, indicating that the agonist molecules act in a cooperative fashion.

Here, For both the muscle-type nicotinic acetylcholine receptor (nAChR) and the ionotropic serotonin receptor, we synthesized ligands by conjugation of a receptor-specific pharmacophore with a biochemical or spectroscopic probes. These compounds were used to the ligand-binding site, ligand-induced channel gating and receptor trafficking.

Medicinal Chemistry **138**

log *P* determination by UHPLC-TOF-MS in natural product analysis : issues and perspectives

Philippe J. Eugster, Sophie Martel, Davy Guillarme, Jean-Luc Wolfender, Pierre-Alain Carrupt

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland

Lipophilicity, described by log *P*, is a widely used key-parameter involved in many pharmacokinetic (PK) and pharmacodynamic (PD) processes. One of the most used techniques for log *P* determination is RP-LC (reversed phase liquid chromatography) [1]. The recent developments in chromatography and mass spectrometry (MS) were adapted to this technique: on one hand, the use of Ultra High Pressure Liquid Chromatography (UHPLC) systems working up to 1000 bars with sub-2 µm packing colums, and of columns stable at a large pH scale; on the other hand, MS instruments [2] such as time-of-flight (TOF) analyzers. All these improvements match the requirements of log *P* determination of natural products (NP) in a crude extract without isolation.

Yet the application of this technique to NP's presented some specific issues. Firstly, the standard method used for small and classical pharmacophores and based on a single correlation log k_w – log *P* has to be modified because of the more complex structures of NP's. Secondly, the lack of reliable experimental log *P* values for some whole classes of NP's is a problem when validating or establishing the correlation curve.

NP's log *P* determination is of great interest for several purposes. (a) The log *P* predicts PK/PD behavior and thus, its knowledge before isolation might save time. (b) Unlike the retention factor *k*, log *P* is not system-dependant and might be used in dereplication or isolation. (c) Such a chromatographic-related parameter might be useful in databases.

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Medicinal Chemistry **139**

Construction of a *Trypanosoma brucei* overexpression strain for the validation of adenosine kinase hyperactivation as novel strategy to develop trypanocides

Patricia Graven[1], Elena Facini[1], Marc Mosimann[2], Pascal Mäser[2], Leonardo Scapozza[1], Remo Perozzo[1]

[1]Pharmaceutical Biochemistry Group, University of Geneva & Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland
[2]Institute of Cell Biology, University of Bern, CH-3012 Bern, Switzerland

Human African trypanosomiasis (HAT), also known as sleeping sickness, is caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. The parasite is transmitted to humans through the bite of tsetse flies (*Glossina* spp). HAT is fatal if untreated.

There is urgent need for new antitrypanosomals, since the available drugs for treating HAT are considered dangerous and have limited efficacy. Our previous research reported that 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]-morpholine (CD12001) exhibits antitrypanosomal activity (IC_{50} of 1 μM). Adenosine kinase (TbAK) was identified as the intracellular target of this compound. CD12001 is a strong activator of the enzyme which is involved in the purine salvage pathway.

The aim of the present project is the genetic validation of TbAK as the intracellular target of CD12001 and the applicability of TbAK hyperactivation as novel strategy to develop trypanocides. To this end, we have developed a tetracycline inducible *ak* overexpression strain of *T. brucei*. We hypothesized that overexpressing TbAK would compare to hyperactivation of TbAK in presence of CD12001, thus overexpression of the enzyme would be toxic for the parasite. Moreover, the toxic effect of *ak* overexpression is expected to increase in presence of CD12001. In order to exclude a possible toxic effect of *ak* overexpression due to the presence of non-physiologically high levels of TbAK, a second strain expressing an inactive TbAK (D299V mutation) was constructed. The results regarding analysis of parasite viability (wild type and overexpression strains) and their sensitivity towards compound CD12001 will be presented.

Medicinal Chemistry **140**

Evaluation of Drug Release from a Metalla-Cage Delivery Vector *in vivo*

Olivier Zava[1],Nicolas Barry[2], Johan Mattsson[2], Bruno Therrien[2] and Paul J. Dyson[1]

[1]EPFL LCOM
[2]Université de Neuchâtel

Cancer chemotherapy is a successful method of cure, but it is weakened by its lack of specificty, which leads to general toxicity. One possible targeting method involves using large carrier compounds which release a drug once inside a cancer cell.[1] Recently, we have shown that the encapsulation of a hydrophobic metallodrug guest in a water-soluble hexacationic arene ruthenium cage delivery vector produces a synergistic effect.[2]

We have encapsulated in the cavity of different metalla cages a intrinsically fluorescent pyrenyl compounds, thus giving rise to hexanuclear metalla-prisms in which the pyrenyl derivative occupies the cavity of the cage. The fluorescence of pyrene is quenched inside the metalla-prism cavity. A previous study of this new system has provided direct evidence that once inside the cell, the hexaruthenium cage releases the fluorescent guest.[2, 3]

We have completed these experiments with measures of the Ruthenium uptake and the monitoring of the fluorescence of the cargo pyrene molecules.

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Medicinal Chemistry **141**

Expression, purification and structural characterization of a 3-TM fragment of the human Y4 neuropeptide receptor

Jacopo Marino and Oliver Zerbe

University of Zürich, Winterthurerstr. 190, CH-8057, Zürich Switzerland

Structure-based drug discovery is currently considered to be one of the most reliable and efficient methods for the design of novel drugs. Therefore, much effort has been undertaken to determine the structure of new potential drug targets. In this context, the G-protein coupled receptor protein family (GPCRs) represents one of the most important class of drug-targets. Unfortunately, only a limited number of GPCRs structures has been solved up to now, exclusively using X-ray crystallography (1). Besides crystallography, NMR spectroscopy is a powerful alternative (2,3). The study of GPCR fragments is justified mainly for two reasons: first, because a fragment can be considered as in independent folding unit (4) from which we can obtain structural informations (e.g. ligand binding); second, the work with the fragments will enable the establishment of robust protocols in our lab for the complete characterization the full-length receptor.

In this context, we have started the project presented herein with the aim to determine the structure of different fragments and the entire human neuropeptide Y4 receptor, whose peptide ligands regulate several important functions including food intake, circadian rhythms, mood, blood pressure, intestinal secretion, and gut motility. The fragment consisting of N-TM1-TM2-TM3 helices has been expressed in fusion with a soluble tag that localizes it into the *E.coli* inner membrane, enabling the purification in the natively folded form.

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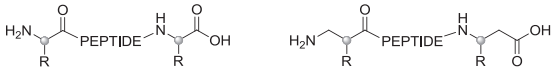
Medicinal Chemistry **142**

Terminal Homologation and Proteolytic Stability of Physiologically Active Peptides

A. Łukaszuk[1], J. Gardiner[1], K. Patora-Komisarska[1], D. Podwysocka[1], H. Demaegdt[2], P. Vanderheyden[2], G. Vauquelin[2], D. Tourwé[3], C. Rougeot[4], P. Gmeiner[5], J. C. Reubi[6], D. Seebach[1]

[1]Laboratory of Organic Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland, [2]Department of Molecular and Biochemical Pharmacology and [3]Department of Organic Chemistry, Vrije Universiteit Brussel, Brussels, Belgium, [4]Laboratory of Pharmacology of Neuro-Endocrine Regulations, Pasteur Institute, Paris, France, [5]Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Erlangen, Germany, [6] Institute of Pathology, University of Berne, Berne, Switzerland

β-Homo-amino acids differ from α-amino acids by having an extra methlene group (homo) either between the carboxyl group and the α-carbon (β^3h-amino acid) or between the amino group and the α-carbon (β^2h-amino acid). While most Fmoc-β^3hXaa(PG)-OH with proteinogenic side chains are commercially available the β^2-analogues may require appreciable synthetic efforts. Incorporation of such β-homo-amino acids into peptides has been used to create β-peptidic peptidomimetics that not only retain biological activity, but also are resistant to proteolysis [1].



In this study we have now incorporated β^2-homo-amino acids on the *N*-termini and β^3-homo-amino acids on the *C*-termini of Angiotensin IV, Neurotensin (8-13), Opiorphin, and Neuropeptide Y. Physiological activities and proteolytic stabilities will be reported [2].

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