Analytical Chemistry **73**

Indicator Displacement Assays as Molecular Timers

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Indicator displacements assays (IDAs) have emerged as powerful analytical tools. They are based on dyes which compete with analytes for binding to synthetic receptors. So far, IDAs have primarily been used to determine the identity and/or the quantity of certain analytes.
We show that a multicomponent IDA can also be employed to obtain information about the history of chemical inputs. A simple mixture of three commercially available dyes and the organometallic complex $[(Cp^*RhCl_2)_2]$ is employed to time the addition of ADP and ATP with good resolution [1].

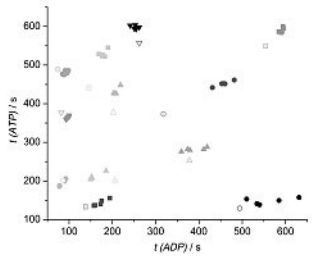

Fig.1.: The ADP and ATP addition times determined by the molecular timer in comparison with the real addition times for 12 test samples. The predictions are shown as filled symbols (5 measurements each) and the real addition timed are indicated by empty symbols.

The signal of the timer is read by UV/Vis spectroscopy and the data is analyzed via a multivariate analysis.

[1] A. Buryak, F. Zaubitzer, A. Pozdnoukhov, K. Severin, submitted.

Analytical Chemistry **74**

Real-time, on-line monitoring of organic chemical reactions using extractive electrospray ionization tandem mass spectroscopy

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Extractive electrospray ionization mass spectrometry (EESI-MS)[1] for real-time, on-line monitoring of organic chemical reactions was demonstrated for a well established pharmaceutical process reaction (one-step Michael addition reaction with phenylethylamine (PEA) and acrylonitrile in ethanol)[2] and a widely used acetylation reaction in the presence of a nucleophilic catalyst, 4-DMAP[3] (multiple-step acetylation reaction of benzyl alcohol with acetic anhydride catalyzed by 4-DMAP in dichloromethane). EESI-MS, with a commerical quadrupole-time-of-flight (Q-TOF) mass spectrometer, provides real-time information that allows determining the optimum time for terminating the reaction based on the relative intensities of the precursors and products. In addition, analysis via EESI-MS permits on-line validation of proposed reaction transients, which appears during the catalytic pathway of 4-DMAP, relying on tandem MS. The relatively simple setup allows this method to be implemented on any type of MS instrument with ESI / APCI interface. The EESI-MS features an instant response (<0.2s) and does not require sample pre-treatment, making it a powerful and convenient tool for on-line characterization and full control of chemical and pharmaceutical reactions, resulting in maximized product yield and minimized environmental costs.

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Analytical Chemistry **75**

Calcilytics – A New Treatment for Established Osteoporosis

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Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissue that leads to fragility and increased risk of fractures. Traditional therapies for osteoporosis inhibit bone resorption and prevent further bone loss. However, as many osteoporosis patients have already lost a substantial amount of bone at the time of diagnosis, there is a need for agents that stimulate new bone formation. The only anabolic treatment for osteoporosis, approved for the US and EU markets, is Forteo®/Forsteo® (Teriparatide, the 1-34 fragment of parathyroid hormone (PTH)) which causes a significant increase in bone mass and reduces vertebral fracture risk substantially. This peptide must be administered by daily subcutaneous injection and the therapy is costly. An orally active, low molecular weight compound with the same efficacy would be a highly attractive alternative for the patient.
Instead of applying exogenous PTH, mobilization of endogenous stores of the hormone can be envisaged. PTH is stored in relatively large amounts in parathyroid cells and its secretion is controlled by a calcium-sensing receptor (PCaR) located on the cell surface. Antagonists of PCaR (calcilytics) mimic a state of hypocalcemia and stimulate PTH release to the blood stream.
The starting point for the Novartis calcilytics project was a proprietary structure found in a HTS screen using a functional assay in the FLIPR format based on recombinant human PCaR. Optimization of the series resulted in an increase in *in vitro* potency by a factor of >100. First oral applications in rats with these highly potent calcilytics were rather disappointing with regard to PK/PD parameters. The presentation will focus in the second part on how these limitations were overcome. PK/PD data in rats and dogs will be shown that mark the best of our derivatives attractive for development as oral calcilytics. There is excellent correlation of drug exposure and PTH release. High levels of PTH are reached in plasma within minutes in both species after p.o.. application which revert to baseline in about 1-2 hours. This profile is a prerequisite for bone anabolic action, since it is well known that persistently elevated levels of PTH stimulate not only osteoblasts (bone forming cells) but also osteoclasts, the bone resorbing cells. The net result is increased bone turnover rather than the desired gain in bone mineral density (BMD).

Medicinal Chemistry **76**

Total Synthesis of the Resorcylic Lactone-based Kinase Inhibitor L-783277

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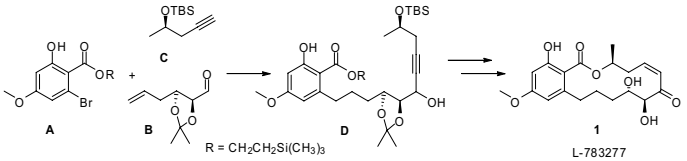
Kinases have emerged as important drug targets in cancer and inflammatory disease and several low-molecular-weight kinase inhibitors have now been introduced into clinical practice.[1] The natural product L-783277 (**1**) belongs to the family of resorcylic acid lactones (RALs), which includes compounds such as zearalenone, C292 (LL-Z1640-2), hypothemycin, or radicicol, and which exhibit a diverse range of biological activities.[2] L-783277 (**1**) is a potent inhibitor of the Ser/Thr kinase MEK.[3] We have accomplished the first total synthesis of macrolactone **1**, which is based on the consecutive assembly of the key fragments **A**, **B**, and **C**.[4] The development of an efficient enantioselective synthesis of **1** and a more detailed characterization of its biological effects are the primary goals of this research project. This presentation will discuss the details of the synthesis of **1** and the preparation of a number of analogs. Preliminary data on the in vitro biologically activity of these compounds will also be presented.



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doi:10.2533/chimia.2008.577 CHIMIA **2008**, *62*, No. 7/8

Medicinal Chemistry **77**

NMR Derived Binding Epitopes of Myelin-Associated-Glycoprotein Antagonists

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Following injury to the adult CNS, regeneration is inhibited by a signal transduction pathway initiated by three proteins, Nogo-A, Omgp and MAG, interacting with the Nogo receptor. It has been shown that blocking the binding site of MAG diminishes the inhibition of regeneration [1].

Herein, we provide an interpretation of the mode of binding of related MAG antagonists. This interpretation is achieved through a synergistic use of STD NMR [2] and antagonist affinities. The method allows the characterization of the mode of binding, i.e. the contribution of different parts of the antagonists to the overall affinity. The binding was determined through three methods, surface plasmon resonance, Hapten inhibition assays and competitive NMR binding experiments. The potential of the method to complement medicinal chemistry SAR analyses will be described. One major advantage is that the structure of the receptor is not required.

[1] A.A. Vyas, O. Blixt, J.C. Paulson, R.L. Schnaar, *J. Bio. Chem.* **2005**, *280*, 16305.
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Medicinal Chemistry **78**

The First Nonpeptidic, Small Molecule, Highly Selective Somatostatin 5 Receptor Antagonists: A Chemogenomics Approach

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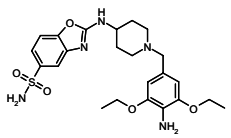
Somatostatin (SST) or somatotropin release-inhibiting factor (SRIF) is a peptide hormone distributed throughout the body exhibiting multiple biological functions, including antisecretagogue, antiproliferative and neurotransmitter activities. SST acts *via* five distinct G-protein coupled receptors (SST1-5), which all have been cloned and characterized. Particularly, SST acting via SST5 receptors has been found to activate and up-regulate NMDA receptor function and to control hormonal secretions (*e.g.*, growth hormone). So far no small-molecule, selective SST5 receptor antagonists have been described. Therefore, receptor specific antagonists are of high interest as tools to study the diverse pharmacology of SST. In order to identify chemical entry points a chemogenomics strategy based on screening known GPCR ligands related to SSTR5 (*via* sequence similarity analysis of the transmembrane consensus drug binding site) was employed. From various hits, the well-known H1 ligand Astemizole was chosen as the most promising starting point and successfully transformed into the first small molecule SST5 receptor antagonists showing nanomolar binding affinity, high selectivity and promising physicochemical properties.[1]



hSST5	Ki = 13 nM
hSST1	Ki = 1.75 μM
hSST2	Ki > 10 μM
hSST3	Ki > 10 μM
hH1	Ki = 2.64 μM

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Medicinal Chemistry **79**

Defining PET-like properties to support radiotracer discovery and their use in drug development

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The development of radiotracers for in vivo Positron Emission Tomography (PET) is quite expensive and still essentially progressing by trial and errors. The identification of a number of factors that can predict the potential of a radiotracer candidate before radiosynthesis would therefore provide substantial savings in time and efforts. We have surveyed the literature, compiled and completed a set of properties describing clinically used radiotracers as well as unsuccessful candidates using assays and methods routinely used in drug discovery programs. Among others, we will discuss the impact on PET imaging of physicochemical and permeation properties, pharmacokinetics and nonspecific binding.

Finally some of the main applications of PET imaging in drug development will be exemplified: 1) The use of a specific radiotracer to optimize the therapeutic dose and achieve efficacy, while minimizing the side effects. 2) The use of radiolabeled drug to confirm brain distribution and to identify relationship among dose, plasma concentration and brain concentration. 3) The investigation of pharmacodynamic effects using PET imaging, correlating receptor binding and regional effects on physiological parameters.

Medicinal Chemistry **80**

Efficient ligand affinity calculations using novel computational methods

Holmfridur B. Thorsteinsdottir, Michael Podvinec, Torsten Schwede, Markus Meuwly

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In recent years virtual screening tools are becoming increasingly important for drug discovery. However, the scoring functions many of these methods use often lack predicting power. Additionally the poses generated by the docking algorithms are often sub-optimal. Previously a MM-GBSA method to calculate binding free energies has been validated using the well characterized HIV-1 protease and 16 known ligands as a test system [1]. Here, we extend this method to distinguish correct from incorrect poses and secondly to improve the correct poses generated from virtual screening methods by molecular dynamics simulations. Furthermore MM-GBSA is used as a more advanced scoring technique to obtain a more reliable ranking of the ligands.

One of the main obstacles in the routine application of molecular dynamics simulations in large-scale virtual screening projects is the calculation time that is required. GRID computing is a possible solution to this problem. This work aims to develop a method for parallelization and show that parallelized molecular dynamics is able to reproduce binding free energies in a comparable way to classical molecular dynamics simulations and can therefore be applied to virtual screening projects.

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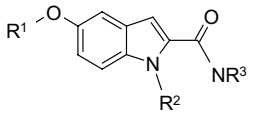
Medicinal Chemistry **81**

Histamine-3 Receptor Inverse Agonists for the Treatment of Obesity: Validation of the Target and Identification of Novel Series

Jean-Marc Plancher, Pascale David Pierson, Christian Freichel, Silvia Gatti, Cornelia Hertel, Jörg Huwyler, Peter Mohr, Toshito Nakagawa, Matthias Nettekoven, Susanne Raab, Hans Richter, Olivier Roche, Rosa María Rodríguez Sarmiento, Monique Schmitt, Franz Schuler, Tadakatsu Takahashi, Sven Taylor, Christoph Ullmer, Ruby Wiegand

F. Hoffmann-La Roche Ltd; Pharmaceuticals Division; B092/4.18C; CH-4070 Basel; Switzerland

Obesity is a major risk factor in the development of conditions such as hypertension, hyperglycemia, dyslipidemia, coronary artery disease and cancer. Several pieces of evidence, including data in primates, have highlighted the beneficial effects of H_3R inverse agonists in the regulation of food intake and body weight.
A pharmacophore model, based on selected published H_3R ligands, and validated by previous investigations[1], was used to identify several scaffolds. Among those, the indole-2-carboxamide appeared to be of great interest as a novel series of H_3R inverse agonists. Extensive structure activity relationship investigations, rewarded by the identification of several compounds reversing (*R*)-α-methyl-histamine-induced water intake increase, and reducing food intake in rats, are presented. Biochemical, pharmacokinetic and pharmacodynamic characteristics are discussed.



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Medicinal Chemistry **82**

Identification and Characterization of Highly Potent Activators of the NR4A2 Pathway - a Novel Approach to Treat Parkinson's Disease?

S. Hintermann [1], M. Chiesi [1], M. Tintelnot-Blomley [1], U. von Krosigk[1], B. Hengerer [1], W. Le [2], S. Ametamey [3], M. Honer [3]

[1] Novartis Institutes for BioMedical Research, GDC, 4002 Basel, Switzerland; [2] Baylor College of Medicine, Houston, Texas 77030, USA; [3] Center for Radiopharmaceutical Science of ETH, PSI and USZ, 8093 Zürich, Switzerland

The nuclear receptor NR4A2 (Nurr1), a member of the family of orphan nuclear hormone receptors, plays a critical role in the differentiation and survival of midbrain neurons. $Nurr1^{-/-}$ mice have no differentiated dopaminergic neurons in the substantia nigra and striatal dopamine (DA) levels are reduced by 98% leading to death shortly after birth. In heterozygous Nurr1-deficient mice, striatal DA levels and locomotor activity progressively decrease with age and the vulnerability of midbrain DA neurons to neurotoxins is increased [1,2]. Nurr1 was shown to be involved in the regulation of genes coding for e.g. aromatic amino acid decarboxylase, tyrosine hydroxylase and the DA transporter [3]. These observations triggered the hypothesis that activating the remaining Nurr1 protein in degenerating DA neurons might delay the onset of Parkinsonian symptoms.
Recently, highly potent, brain penetrable activators of the Nurr1 signaling pathway were disclosed [4]. The hit identification and SAR studies leading to these isoxazolo-pyridinones as well as their profiling *in vitro* and *in vivo* in different models will be discussed.

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Medicinal Chemistry **83**

Identification and Characterization of High-affinity Myelin-associated Glycoprotein (MAG) Antagonists

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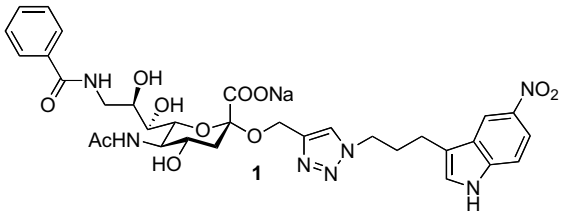
Following an injury of neurons in the central nervous system (CNS) of adult mammalians, functional repair is not possible. The two main obstacles to regeneration are inhibitor proteins located in the myelin sheaths and the formation of glial scars. MAG, a member of the Siglecs family (sialic acid-binding immunoglobulin-like lectins) is one of these inhibitor proteins [1]. It was shown that blocking the inhibitory activity of MAG leads to neuron regeneration [2]. In previous studies, by employing the Fragment-based In Situ Combinatorial Approach, we have successfully identified the high affinity antagonist **1** [3].
Herein, we report the optimization of lead **1** and the evaluation of the new antagonists by surface plasmon resonance experiments.



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Medicinal Chemistry **84**

Inspiration from nature_EMD!

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Enamel Matrix Derivative (EMD) is the active ingredient in Emdogain® (BIORA AB, Straumann, Malmö, Sweden), a commercially used product for regeneration of periodontal tissue. The main component of the derivative is amelogenin, which is secreted by ameloblasts into the enamel compartment. Extraction of EMD for the Emdogain® preparation is carried out from the molar teeth of 6-month-old pigs. At this time, amelogenin corresponds to approximately 90% of total tissue protein, and the stages of secretion and early maturation in enamel formation have been reached.

The effects of EMD in the periodontal area on cementum, periodontal ligament, bone formation, and wound healing have been studied quite extensively over the years [1-23]. Recently, researchers have started to look more at the bioactive parts of EMD (sometimes also referred to as EMP; Enamel Matrix Proteins) [20-22]. Even though EMD mainly consists of amelogenin (20 kDa) [11,16,24-26], it cannot be ruled out that other proteins besides amelogenin have biological activity. Therefore, in order to understand the overall biological activity of EMD, it is essential to investigate its components.

Medicinal Chemistry **85**

Discovery of Selective 5-HT_{5A} Antagonists

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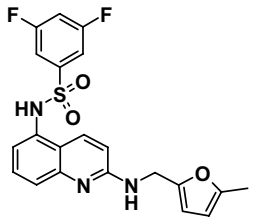
Selective quinoline 5-HT_{5A} antagonists based on 2-aminoquinoline derivatives were discovered with the help of site directed mutagenesis and receptor pharmacophore modeling of the 5-HT_{5A} binding pocket. It was predicted that the addition of groups in position 5 would potentially lead to improved potency and selectivity. This was confirmed when the non-selective 5-HT_{5A} antagonists initially interacted with only a few of the binding pocket amino acids, whereas when a new exit vector in position 5 of the quinolines was introduced, this led as predicted to the interaction with other amino acids within the binding pocket and an increase in potency and selectivity. Quinoline 1 will be presented as a selective 5-HT_{5A} antagonist with good pharmacokinetic properties.



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Medicinal Chemistry **86**

Inhibition and Dispersion of *Pseudomonas Aeurginosa* Biofilms by Glycopeptide Dendrimers

Emma Johansson[1], Shanika Crusz[2], Steve Diggle[2], Miguel Camara[2], Paul Williams[2], Tamis Darbre[1], Jean-Louis Reymond[1]*

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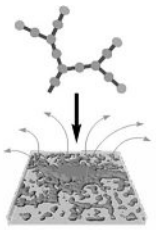

The antibiotic-resistant pathogenic *Pseudomonas aeruginosa* (PA) bacterium causes lethal respiratory tract infections in cystic fibrosis patients by means of biofilms, whose formation depends on several factors including lectins. [1] We recently discovered high-affinity ligands for one of these lectins, LecB, based on the multivalent display of C-fucosides on a peptide dendrimer framework.[2]. Here we report *in vitro* studies showing for the first time potent biofilm formation inhibition and biofilm dispersion of clinical PA strains via specific inhibition of LecB. The approach might lead to a new therapeutic strategy against PA infections.

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Medicinal Chemistry **87**

Azanorbornanes as Potent Inhibitors of Plasmepsins

Martina Zürcher[a], Luzi Barandun[a], Pascal Wyss[a], Solange Meyer[b], Daniel Bur[b], François Diederich[a]

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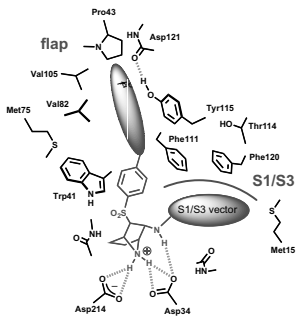
The aspartic proteases plasmepsins of *Plasmodium falciparum* are interesting antimalarial targets [1]. We found that azanorbornanes of type **1** inhibit these enzymes with IC_{50} values down to the nanomolar range [2,3]. The compounds are designed to occupy both the flap as well as the S1/S3 pocket with vectors that can be attached to the scaffold. In order to access a wide variety of molecules, a modular, facile synthesis was developed.



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Medicinal Chemistry **88**

Rational Design of T315I BCR-Abl Inhibitors for the Treatment of Drug-Resistant Chronic Myelogenous Leukemia (CML): BGG463

Paul W. Manley, Josef Brüggen, Andreas Floersheimer, Pascal Furet, Michael R. Jensen, Jürgen Mestan, Carole Pissot, Sandra Cowan-Jacob

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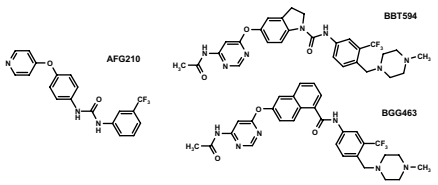
CML is caused by the dysregulated Abl tyrosine kinase activity of the BCR-Abl oncoprotein. Although well managed with 1st-line imatinib and 2nd-line nilotinib or dasatinib therapy, some patients relapse due to the emergence of clones expressing drug-resistant mutant forms of BCR-Abl. Of these, T315I BCR-Abl is the most resistant, where mutation of the gatekeeper Thr residue to Ile abrogates the activity of the inhibitors through loss of a binding interaction to the side-chain hydroxyl, coupled with unfavourable steric interactions. Based upon our understanding of the requirements for BCR-Abl inhibition and the crystal structure of sorafenib in complex with RAF, we postulated that a diarylurea might provide a good pharmacophore element to bind to BCR-Abl, without having any interaction with Thr315. This was confirmed from the structure of AFG210 in complex with the kinase domain of *wild-type* Abl. Although neither sorafenib nor AFG210 showed activity in transfected Ba/F3 cells, structural modifications led to BBT594 which inhibited T315I BCR-Abl autophosphorylation (IC_{50} 44 nM) and proliferation (GI_{50} 117 nM) in Ba/F3 cells expressing this mutant. Further optimisation gave BGG463 possessing good oral efficacy at well-tolerated doses in a murine model of CML.



Medicinal Chemistry **89**

Full Automation for Microwave Synthesis Workflows

Amira Abou-Hamdan, Ph.D.

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Since microwaves have been introduced as an alternative source of heating in organic chemistry laboratories in the late 90's, the number of publications on Microwave Assisted Organic Synthesis (MAOS) has been growing exponentially.

One of the most eye catching advantages of MAOS compared to classical methods are reaction times significantly being reduced from typically several hours to just minutes.
Although, this achievement has resolved a very important bottleneck in the discovery research, other tedious steps of the workflow now became the "rate determining step":

- dispensing of solid and liquid chemicals
- automated capping and crimping
- transfer of vials, from and to the Microwave syntheziser
- sampling and work-up of the reaction mixture

Experimental Methods
Fully unattended, automated synthesis via Wittig reaction was done on a fully automated microwave workstation, the SWAVE.

Results and Discussions
Based on selected applications examples from worldwide renowned "CombiChem" labs we will show in this presentation, how the Chemspeed SWAVE Microwave synthesis platform, not only accelerates the reaction step, but also dramatically increases the productivity for all pre- and postsynthetic steps of a typical Microwave chemistry workflow.

Conclusion
Microwave assisted syntheses were successfully automated and results were in accordance with literature and theory [1].

[1] www.biotagepathfinder.com - Wittig Olefin Synthesis

Medicinal Chemistry **90**

Glycomimetics as Potential Anti-Cancer Agents

Claudia Bello, Pierre Vogel

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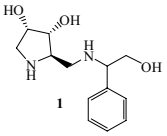
During the past decade, research into the causes of human cancer, the molecular basis of malignant transformation and gene-environment interactions that contribute to individual cancer risks, has made significant progress. Unfortunately, cancer remains a major disease burden worldwide. Further efforts have to be done in order to improve our knowledge about the mechanisms of carcinogenesis and tumour development with the aim to find new therapies and to develop new anticancer drugs.



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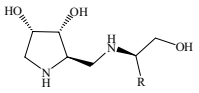
A library of sugar mimetics that are analogues of swainsonine has been developed by Vogel and co-workers. These new α-mannosidase inhibitors (e.g. **1**) have shown a high affinity for a target enzyme involved in cancer progression i.e.: Golgi α-mannosidase II. [1,2] Inhibition of this key enzyme of protein glycosilation pathway has been proposed to be a useful approach to cancer treatment



R = 4'-(trifluoromethyl)biphenyl (**2**)
biphenyl (**3**)
allyloxy (**4**)

Modification of the aromatic moiety of compound **1** has led to a series of products that show inhibitory activities toward α-mannosidase (from jack bean) similar to that of **1**. These new inhibitors[3] (e.g. **2**-**4**) were tested on different cancer cell lines and were found to inhibit cancer cell growth (e.g. **2** has IC_{50} = 48□M on SKBR3). Compound **3** and, to a lesser extent, **4** also exhibited growth inhibitory activities. Another series of analogues containing the same dihydroxypyrrolidine moiety, but with more differentiated substituents was synthesized and tested against several cancer cell lines. These show even better anti cancer activity than **1**-**4**. More extensive studies are now in progress in order to elucidate the mechanism of action of these compounds. Data suggest that the α-mannosidase inhibitory activity is not the main cause of cancer cell growth inhibitory activity.

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Targeting Glycopeptide Dendrimers-Cytotoxic Agent Conjugates Towards Tumor Cells

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Dendrimers are being investigated by various groups as selective drug delivery vehicles for cancer cells [1]. We showed recently that covalent glycopeptide dendrimer conjugates of colchicine exhibit selective cytotoxicity towards HeLa cells compared to MEF cells, presumably by means of receptor-mediated endocytosis and activation by metabolic degradation in the dividing tumor cells [2]. Here we present the improvement of the systems by modifying the glycopeptide dendrimer structures systematically using positive, neutral and negative amino acid residues.

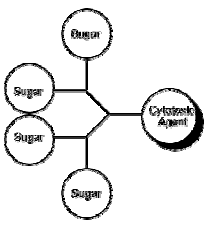

Figure 1

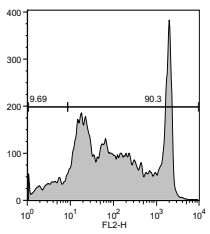

Figure 2

Figure1: Schematic structure of a conjugate. Figure 2: Representative example of a histogram from flow cytometry experiment, showing percentage of viable cell (9.69) at the left of the marker, non-viable at right (90.3).

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Medicinal Chemistry **92**

Analysis of GDB and ZINC Databases using Color-Coded Self-Organizing Maps

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The classification and content analysis of large databases of molecules is intrinsically complex. Here we used self-organizing maps (SOMs) to analyze the contents of the chemical universe database GDB[1] (26.4 M structures) and the ZINC database[2] (2 M drug-like molecules), using a 200x200 and 100x100 set of neurons, respectively. The SOM based analysis automatically organizes the databases according to structural features such as molecular weight, rotatable bond count, ring count, or polar surface area. A multidimensional color-coding scheme was used to color these SOMs according to the average descriptor values per neuron of as the main color (Hue value), the standard deviation as a grey scale (saturation value), and the neuron occupancy as the color intensity (lightness value). The color-code results in sharp and intense colors for well populated neurons with well defined values only, while scattered values or poorly occupied neurons appear grey or white respectively. This allows a straightforward and detailed insight into the databases.

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Medicinal Chemistry **93**

Which Residues Dictate Src-Specific Conformational Plasticity?

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Tyrosine kinases were among the first to be discovered as human oncoproteins. Src tyrosine kinases are key signaling protein tyrosine kinases that are of considerable interest as drug targets in cancer and many other diseases. The catalytic domains of different tyrosine kinases adopt strikingly similar structures when they are active. By contrast, crystal structures of inactive kinases have revealed a remarkable plasticity that allows the adoption of different conformations in response to interactions with specific regulatory domains or proteins. Therefore, investigating on the conformational states of Src tyrosine kinase might short cut the drug design for both effective and highly specific compounds.
A comparative structural analysis has been performed using molecular modeling. The results of this analysis depicted a pool of residues at the interface of the two lobes that appears to be important for the protein conformation and motion. With the aim of assessing the findings of the comparative analysis biochemical and biophysical studies of the conformational plasticity of the c-Src kinase domain have been performed. The results of these studies together with the comparative structural analysis will be presented.

Medicinal Chemistry **94**

Synthesis of Novel Cationic NSAID Derivatives to Facilitate Electrically-assisted Transdermal Delivery

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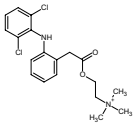
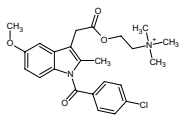
NSAIDs (Nonsteroidal Anti-Inflammatory Drugs) are commonly used for the treatment of inflammation and pain; however, their oral administration is associated with potentially fatal gastrointestinal side effects.[1] Improved transdermal delivery would increase their local bioavailability and expand the range of indications for targeted, topical therapy. The aim of this study was to synthesise novel cationic ester NSAID derivatives optimised for transdermal administration by anodal iontophoresis; the skin has an isoelectric point (pI) of ~4-4.5, and is permselective to cations.[2] Our hypothesis is that the cationic prodrugs will electromigrate into the skin under the influence of the applied iontophoretic current. When they reach the viable epidermis and dermis they will be subject to hydrolysis by endogenous esterases and hence release the active molecule.[3]

The charged esters (**1a**, **1b**) were synthesised by a carbodiimide condensation using dicyclohexylcarbodiimide (DCC).[4,5] The main challenge encountered during the synthesis was the removal of the reaction by-product dicyclohexylurea (DCU). Esterification of the carboxylic acid groups in diclofenac and indomethacin with choline, a charged aminoalcohol, introduced a pH-independent positive charge into the molecule through the quaternary ammonium. In the next part of the project we will investigate the iontophoretic delivery kinetics of the newly synthesised molecules.





1a: Diclofenac-choline ester **1b**:Indomethacin-choline ester

Medicinal Chemistry **95**

Investigation of the Ribose binding motif in Catechol-*O*-Methyltransferase (COMT)

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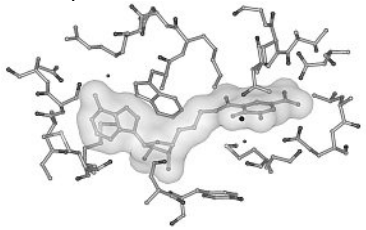
Catechol-*O*-Methyltransferase (COMT) is one of the key enzymes involved in catecholamine catabolism. Therefore it is one of the main targets for the treatment of CNS disorders such as Parkinson's disease [1]. Highly potent bisubstrate Inhibitors have been developed by *de novo* design [2], but the exact binding motif at the ribose moiety still requires further explanation [3]. In our ongoing work we investigate these interactions and compare this ubiquitous motif to other proteins.



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Medicinal Chemistry **96**

Description and Comparison of Classical Cyclic Voltammetry with HT-Cyclic Voltammetry for the determination of Redox Potentials

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Electrochemistry is comprised of several aspects of molecular sciences [1-4]. Besides the movement of charges in an electrical field, electron-transfer induced processes are an important issue in chemistry and life-sciences [1-4]. Among other electroanalytical methods such as differential pulse voltammetry or paleography, cyclic voltammetry is one of the techniques with its largest scope as it provides information of thermodynamic data, kinetic parameters or stability issues.
Standard cyclic voltammetric measurements are usually performed with macro electrodes in a single electrochemical cell.
An external collaboration with Gatlik (Gatlik Ltd., Basel/CH) gave rise to the **E**lectroactive **P**harmaceutical **S**creening **S**ystem (EPSS), a novel HT-cyclic voltammetric screening/profiling system which allows electrochemical determinations under physiological conditions in the 96-well format.
Therefore, the focus of the current study was the development of a new application based upon the EPSS system with a subsequent technical modification to measure molecules under physiological-like conditions, and a comparison of classical and HT- cyclic voltammetry.

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Medicinal Chemistry **97**

Molecular Modeling Of Glycopeptide Dendrimers Ligands Of The Fucose Binding Lectin PA-IIL From *Pseudomonas aeruginosa*

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The fucose-specific lectin PA-IIL of the opportunistic pathogen *Pseudomonas aeruginosa* is implicated in tissue binding and colony formation leading to lethal airways infections in cystic fibrosis patients. In view of developing novel anti-infective PA-IIL inhibitors, recently our laboratory developed multivalent ligands to this target [1-2]. To study the importance of multivalency for ligand binding, molecular docking and molecular dynamics at nanosecond timescale were carried out with the protein-dendrimer complex to further explore the binding mode of the dendrimer to the lectin (Figure 1).

Figure 1. Molecular model of the peptide dendrimer binding to the fucose-specific lectin PA-IIL from *Pseudomonas aeruginosa*

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Medicinal Chemistry **98**

Design and Synthesis of Strongly Binding Trna-Guanine Transglycosylase (TGT) Inhibitors Undergoing Charge-Assisted Hydrogen-Bonding

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[1] P. C. Kohler, Dr. S. R. Hörtner, Prof. Dr. F. Diederich, Laboratory of Organic Chemistry, ETH Zürich, Hönggerberg, HCI, 8093 Zürich, Switzerland.
[2] T. Ritschel, B. Stengl, Prof. Dr. G. Klebe, Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marbacher Weg 6, 35032 Marburg, Germany.
[3] B. Wagner, Dr. M. Kansy, Pharmaceuticals Division, Discovery Chemistry, F. Hoffmann-La Roche AG, 4070 Basel, Switzerland.

Bacillary dysentery, or shigellosis, is a diarrheal disease occurring worldwide, but mainly in developing countries. The emergence of multi-resistant bacterial strains makes the development of new specific antibiotics an urgent issue. The bacterial enzyme tRNA-guanine transglycosylase (TGT) has been identified as a viable target for new drugs [1].

In collaboration with the group of Prof. *G. Klebe* a class of inhibitors with K_i values down to the single-digit nanomolar range has been developed [2,3]. These compound are based on a *lin*-benzoguanine scaffold that is substituted at the 2-position. The rational drug design is based on computational modeling done with the program MOLOC (*F. Hoffmann-La Roche*) [4].

Introduction of an amino substituent at the 2-position has increased the binding affinity by a factor of 50–70 as compared to the unsubstituted scaffold [2,5]. This finding can be rationalized by the establishment of an additional hydrogen bond and, more importantly, an increase in basicity of the imidazole moiety. The corresponding pK_a value is raised from 5.2 (unsubstituted *lin*-benzoguanine) to substantially higher values of 5.9–6.7, depending on the substituent. We therefore assume protonation of the

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Synthesis and conformational analysis of phenyl-*a*-CH_2 and CF_2-galactosides. Interactions with the plant lectin viscumin.

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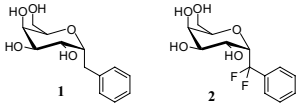
The ongoing study of protein-carbohydrate interactions has created the nee for the preparation of non-native saccharides in order to block or to inhibi certain biological events.[1] *C*-glycosides, where the anomeric oxygen ha been replaced by a methylene unit, are interesting mimetics due to their sta bility. Nevertheless, the absence of the anomeric effect results in an in creased flexibility of these pseudosaccharides, giving rise to the questio whether the entropic excess can be compensated by the binding with a re ceptor protein. We are attempting to answer this question by examining dif ferent substitution on the methylene linker. Substitution by two fluorin atoms is expected to alter significantly the conformational behaviour of th molecule relative to the hydrogen substitution, as well as to the native lin kage.[2] Therefore, two simple phenyl galactosides, **1** and **2**, have been pre pared and their conformer distribution in solution has been evaluated using combination of force field calculations and NMR studies. Interactions wit the plant lectin viscumin, which is a galactoside-specific enzyme, are als described.



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Medicinal Chemistry **100**

Interactions of Polymyxin M with Bacterial Lipopolysaccharides

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Polymyxins present a group of cyclic polycationic polypeptides isolated from various strains of Bacillus polymyxa and related species. They are capable of inhibiting the growth of a wide variety of Gram-negative bacteria and also possess anti-endotoxin activity. LPS, a structural component of the outer membrane of Gram-negative bacteria, elicit a wide range of toxic effects in a variety of organism with the human among the most susceptible species(1). Polymyxins are peptides that can effectively kill bacteria as well as neutralize free LPS (2). The precise mode of its binding to LPS and the structural features involved therein are unknown.

An understanding of the structural aspects of its binding with LPS in a membrane-mimicking environment would be very useful for the rational development of compounds with anti-endotoxin activity. Accordingly we chose to characterise interactions of Polymyxin M (Mattacin) produced by *Paenobacillus kobensis* M with Re LPS (obtained from the D31me4 strain of *E. Coli*) in the presence of phospholipid (DPC) micelles. In our study, we have purified Re LPS and Polymyxin M, and studied the interaction between them by various NMR techniques. These studies include aspects of how PMX and LPS are oriented on the phospholipid micelles.

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Medicinal Chemistry **101**

Structure Activity Studies for Hypermodified Epothilone Analogs with Potent *in vitro* Antitumor Activity

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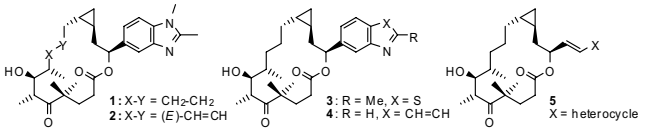
Epothilones are microtubule-stabilizing agents with potent *in vitro* and *in vivo* antitumor activity. Although the SAR of this highly promising compound class has been extensively investigated, specific aspects still remain unaddressed[1]. In a synthetic endeavor to deviate more and more strongly from the original natural product leads we discovered that the hypermodified *trans* cyclopropyl epothilone analogs **1** and **2** inhibit human cancer cell growth *in vitro* with low- to sub-nM IC_{50} values. Most remarkably their activity is significantly more pronounced for the multidrug resistant KB-8511 cell line than for the corresponding drug-sensitive KB-31 line. With this potentially highly active macrocycle in hand the aim is to further probe the effects of side chain modifications for this new chemotype. With an efficient synthesis for structures of type **1-4** available[2], a convergent new synthetic strategy for the generation of macrolide scaffold **5** was developed. The omission of the naturally occurring methyl group at C16 has been shown not to be connected with major activity losses for natural epothilones and is thus expected to produce highly active analogs in combination with the newly designed macrolide structure and various heterocycles as the aromatic side chain.



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Medicinal Chemistry **102**

The non-globular domain in *P. falciparum* enoyl-ACP reductase (PfFABI) and its role in substrate specificity and turnover

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The analysis of complete genomes has shown that Intrinsically Unstructured Proteins (IUPs) are common among organisms. Thanks to their structural flexibility, non-globular domains are involved in several cellular activities carried out by protein-protein, protein-DNA and protein-RNA interactions.
Genes of *Plasmodium falciparum* are known to be characterized by long insertions called Low Complexity Regions (LCRs), which often give rise to regions lacking a well-defined 3D structure. [1] These regions were recently shown to be important for protein functions in this parasite. [2]
This work aims at elucidating the role plaid at a molecular recognition level by the non-globular domain present in *P. falciparum* enoyl-ACP reductase (PfFabI), an enzyme involved in the type-II Fatty Acid Synthesis (FAS-II) pathway. For this purpose, a mature form deletion mutant lacking the PfFabI 43-resuidues long LCR was created, and kinetic parameters were compared to those of the mature, wild type PfFabI, using crotonoyl-CoA and enoyl-ACPs. Structural and stability studies were also performed in presence and absence of the cofactor NADH.
The results of this study show that the non-globular domain of PfFbaI is not important in maintaining the overall structure of the enzyme, but directly influences the affinity of PfFabI for its artificial and natural substrates and the catalytic efficiency of the enzyme. Moreover, the deletion mutant showed to be more susceptible to NADH stabilization.

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Medicinal Chemistry **103**

Control of abnormal metal-protein interactions in neurodegenerative disorders by metallothionein-3

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In the brain, the zinc and copper homeostasis is regulated by a small metalloprotein, metallothionein-3 (Zn_7MT-3), which is down-regulated in neurodegenerative disorders such as Alzheimer (AD), Creutzfeldt-Jacob and Parkinson. These diseases share common pathological hallmarks including misfolding of amyloid-β (Aβ), prion protein and α-synuclein, the formation of protein aggregates, abnormal metal-protein interactions and oxidative stress. In AD, Cu(II) and Zn(II) are involved in the disease progression by modulating the formation and toxicity of soluble and insoluble oligomers and aggregates of the Aβ peptide. Whereas the copper-induced Aβ aggregation is related to the ROS production and neurotoxicity, the zinc-induced Aβ aggregation is neuroprotective. The protective effect of extracellular Zn_7MT-3 from Aβ toxicity in neuronal cell cultures is not understood. We show that Zn_7MT-3 not only scavenges free Cu(II) ions [1], but also removes Cu(II) bound to Aβ [2]. A metal swap between Zn_7MT-3 and soluble and aggregated Aβ-Cu(II) is the underlying mechanism by which the ROS production and related cellular toxicity is abolished. In this process, copper is reduced by the protein thiolates forming $Cu(I)_4Zn_4$MT-3, in which an air stable $Cu(I)_4$-thiolate cluster and two disulfide bonds are present [2]. To examine whether the discovered effect represents a general protective role in other metal-linked neurodegenerative pathologies, similar studies using prion peptides in complex with Cu(II) were conducted. We show that Zn_7MT-3 by a similar metal swap reaction removes abnormally bound Cu(II) from the prion protein, impeding the ROS production. This finding signifies a so far unrecognized protective role of this protein in the brain.

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Medicinal Chemistry **104**

Thermodynamic and Kinetic Binding Properties of MAG-Antagonists

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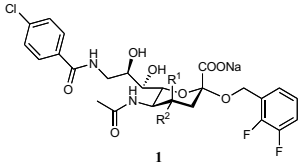
The injured adult mammalian central nervous system is an inhibitory environment for axon regeneration due to specific inhibitory proteins. One of these neurite outgrowth inhibitors [1] is the myelin-associated glycoprotein (MAG) [2]. It belongs to the siglec family (sialic-acid binding immunoglobulin-like lectin). In previous studies, we identified high affinity antagonists [3, 4]. However, they showed unsatisfactory kinetic binding properties, i.e. fast off-rates and therefore short residence times.
We therefore investigated the influence of various modifications on the insufficient kinetic properties of these MAG antagonists. For this purpose, different substitutions were introduced to the 4 position of the neuraminic acid derivative **1**.



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The thermodynamic and kinetic properties of the new antagonists were analyzed by surface plasmon resonance studies.

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Medicinal Chemistry **105**

Discovery of NMDA-Glycine Site Inhibitors from the Chemical Universe Database GDB

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One possibility to discover new small molecule drugs would be to search exhaustively through the entire chemical space using virtual screening tools and identify promising ligands for synthesis and testing. Herein we report the first example of such an approach for ligands of the NMDA-receptor glycine site, an important drug target implicated in synaptic plasticity, neuronal development, learning and memory. Starting with the Chemical Universe Database **GDB** [1], which contains all organic molecules of C, N, O, F up to 11 atoms possible under consideration of simple chemical stability and synthetic feasability rules, a Bayesian classifier trained with 61 known NMDA-receptor ligands was used to identify 15'061 virtual hits, which were expanded to 69'367 stereoisomers and docked into the glycine site of the NMDA-receptor [2] (pdb: 1PB7). From the 712 ligands docking stronger than glycine, 23 compounds were selected and either purchased or synthesized. Activity screening using NMDA receptor radioligand binding assays gave 5 hits not previously reported to interact with this receptor, two of which were expanded to 8 further active analogs, suggesting that a new lead structure has been found. Electrophysiological data show that the compounds act as glycine antagonist at the glycine site.

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Medicinal Chemistry **106**

Natural Product-Like Furo[3,4-*c*]pyranones: Lead Structures for Novel Anticancer Agents

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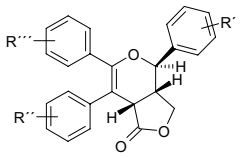
Natural products have played an eminent role in the discovery and development of new drugs.[1]
We recently reported the synthesis of different natural product-like furo[3,4-*c*]pyranones.[2] The furopyranone scaffold **1** contains the *cis*-stilbene motif, as we can find it in stilbenoids, including resveratrol and combretastatin A-4. The *cis*-stilbene motif was identified as the pharmacophore in the compounds, which exhibited interesting anticancer properties in different human cancer cell lines.[3] To conduct a more detailed structure-activity relationship (SAR) study, strategies were carried out to synthesize symmetric and asymmetric methoxy substituted α-diketones to introduce new substitution patterns in the furopyranones scaffold.
In addition, increased antiproliferative activity in HEK293 and SJSA1 cancer cells was observed with a benzyl ester substituent at the phenyl ring. The influence of new substitution patterns at the phenyl ring to refine our structure-activity relationships (SAR) studies, is also investigated.



(+/-) **1**

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Medicinal Chemistry **107**

Structure-Activity Relationships of C12-C13-Oxazoline Derivatives of Epothilone A

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The inhibition of cancer cell growth by epothilones (Epo) (**1**), natural products isolated from the myxobacterial strain *Sorangium celluosum*, is based on the stabilization of cellular microtubules, *i.e.* they exhibit a "taxol-like" mechanism of action. The widespread interest in the chemistry and biology of these macrolide-based microtubule-stabilizing agents, expedited by their potent *in vitro* and *in vivo* antiproliferative activity, has resulted in an advanced understanding of the structure-activity relationships for epothilones. The advances in the synthesis and semi-synthesis of epothilones lead to numerous active analogues of which at least seven have entered clinical evaluation as potential anticancer drugs and one of them has recently obtained FDA approval for the treatment of breast cancer [1].
One such epothilone analogue, *trans*-epothilone A (**2**), was shown to exhibits antiproliferative activity in a similar concentration-range as the natural epothilone A (**1a**) itself [2]. As a further extension of our previous work on *trans*-epoxide and *trans*-cyclopropane-based analogs of Epo A we now present the synthesis and biological evaluation of a series of epothilone derivatives that are characterized by the presence of a 2-substituted *trans*-fused C12-C13-oxazoline ring (**3**). Some of these derivatives show antiproliferative activities and tubulin-polymerizing potencies that are comparable with those of the parent compound Epo A. A clear structure-activity relationship can be delineated for these analogs with respect to the nature of the 2-substituent on the oxazoline ring. Possible interaction modes of these new epothilone derivatives with tubulin have been investigated by molecular-modeling.

Medicinal Chemistry **108**

Modulation of Antibacterial Activity by Substituted Siderophores

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Gram-negative bacteria (GNB) are becoming increasingly resistant towards β-lactam antibiotics. Reduced permeability of the outer membrane due to mutations in porins is one of the factors leading to resistance. Recently, it was demonstrated that BAL0019764 (PTX2416, fig. 1, R_1 = OH, R_2 = H, [1]) displays enhanced antibacterial activity, especially against *P. aeruginosa* species compared to aztreonam, a monobactam antibiotic commonly used for the treatment of GNB infections. The presence of siderophore moiety hydroxypyridone is considered to enhance the penetration through the membrane. The new antibiotic possibly forms a complex with iron (Fe^{3+}), which is recognized and taken up into bacteria by specific transport systems [2].

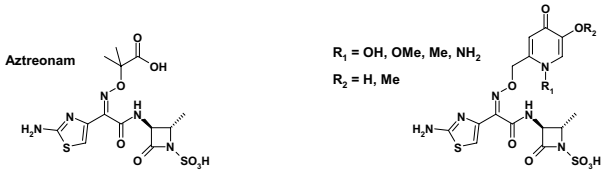

Fig. 1

It was observed that modifications of substituents R_1 and R_2 present on the siderophore moiety dramatically influenced the antibacterial activity, especially in *P. aeruginosa* strains. Preparation of those compounds as well as a more detailed table of activities will be presented.

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Medicinal Chemistry **109**

The Cation–π Interaction in the S4 Pocket of Factor Xa

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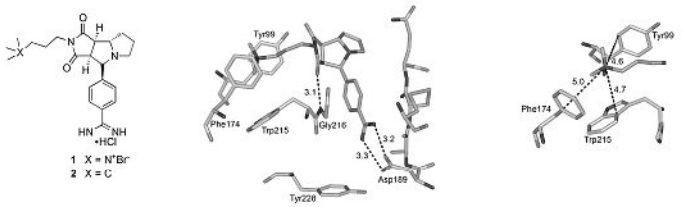
Thrombin was a target in molecular-recognition studies in the Diederich group. Inhibitors **1** and **2** were synthesized to investigate the cation–π interaction in the D pocket of thrombin. Surprisingly, the onium ion inhibitor **1** did not show much activity against thrombin but was found to be a highly active factor Xa inhibitor. The corresponding noncationic inhibitor **2**, however, showed reversed activity. Presumably, the cation–π interaction between Trp215 in the D pocket of thrombin and the onium ion is not strong enough to compensate for the desolvation energy lost during binding. In the S4 pocket of factor Xa, the aromatic box formed by the three aromatic residues Phe174, Tyr99, and Trp215 is able to compensate for the loss in desolvation energy. The cation–π interaction was quantified by comparing the binding affinities of **1** and **2**. A second, different ligand series will also be reported[1].



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Medicinal Chemistry **110**

Computer Simulation of PleD Dimerization

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The bacterial second messenger cyclic di-guanosine monophosphate (c-diGMP) is implicated in pathogenesis of many bacteria and its cellular level is tightly controlled by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). DGC activity to synthesize the symmetric signaling molecule is controlled by protein allostery and dimerization.[1,2] Bound c-diGMP in an allosteric binding site prevents product formation, presumably by altering the protein dynamics, i.e. less flexibility in inhibition- and active-site.[3] Based on microbiology experiments on PleD of {\it C. crescentus}, DGC dimerization is a further level of activity control.
PleD activation with ${\rm BeF_3 ^-}$ shows structural rearrangements in the domains D1/D2 to improve the dimerization interface and possibly facilitating dimer formation.[4]

The dimerization process is investigated for truncated PleD (D1D2 domains) in its activated and inactivated state using molecular dynamics (MD) simulations. Residual contribution to the interaction energy is studied to identify key features. In addition the role of the preserved residue Tyr26 in the dimerization process is probed.

{[1]} & B. Christen {\it et al.}, \emph{J. Biol. Chem.}, \textbf{2006}, \emph{281}, 32015. \\
{[2]} & R. Paul {\it et al.}, \emph{J. Biol. Chem.}, \textbf{2007}, \emph{282}, 29170. \\
{[3]} & F. F.-F. Schmid {\it et al.}, \emph{J. Mol. Biol.}, \textbf{2007}, \emph{374}, 1270. \\
{[4]} & P. Wassmann {\it et al.}, \emph{Structure}, \textbf{2007}, \emph{15}, 1. \\

Medicinal Chemistry **111**

Computational Study of Ligand-induced Effects in Dengue Methyltransferase

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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. No vaccinations or specific drug treatments are available.[1] The Dengue virus is an enveloped virus with a single-stranded, positive sense RNA genome, bearing a 5' cap 1 structure. The viral RNA capping machinery is essential for RNA stability and for viral replication, making it an attractive target for drug design.[2] A previous study focused on the inhibition of the activity of the methyltransferase (NS5MTase),[3] which catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the immature viral RNA cap structure. The NS5MTase enzyme posses two adjacent binding sites, one for SAM and one for the RNA cap structure.
In this work, the molecular mechanism of ligand binding to the NS5MTase is investigated at an atomistic level, using computer simulations. We focus on ligand-induced effects on the structure and flexibility of one binding site when a ligand binds to the adjacent cavity. Such information is highly valuable both for a more thorough understanding of the biological system as well as for further computer-aided drug development efforts where the incorporation of protein flexibility is crucial to improve computational predictions.[4]

[1] D.J. Gubler, *Novartis Found Symp*, **2006**, *277*, 3.
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Medicinal Chemistry **112**

HPLC Microfractionation in the Search for Antimalarial Compounds: an Application of the Haematin Polymerization Assay

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A screening assay based on the inhibition of synthetic hemozoin (beta-haematin) formation [1] to identify novel antimalarial substances was adapted to the screening of complex mixtures.
Initially, the test was applied to plant extracts and fractions. This included a specific sample preparation followed by SPE pre-purification in order to improve solubilisation of the samples and avoid interference with unwanted material such as tannins. A standard operating protocol has been developed for routine screening in both eppendorf tubes and 96-well plates.
In order to obtain fast results for a bioguided isolation, a microfractionation procedure was established for the aqueous extract of *Syzygium cumini* (L.) Skeels (Myrtaceae).
S. cumini is a tropical tree which has been extensively used in traditional medicine, mostly for antidiabetic purposes, in countries like India, the Philippines and Brazil [2]. An antiparasitic activity has already been demonstrated in the genus *Syzygium* against *Plasmodium falciparum* [3].
The preliminary screening of the aqueous extract obtained from the stem bark of *S. cumini* demonstrated a marked inhibition of beta-haematin formation.
An HPLC-UV-MS analysis of this extract was performed, followed by the evaluation of the collected fractions in the beta-haematin polymerization assay.
The work described here provides a one-step method which enables a direct correlation between activity with specific known and unknown chemical entities.

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Medicinal Chemistry **113**

Bifunctional Glycodendrimers as Ligands for Lectins of Pathogenic *Pseudomonas Aeruginosa*

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Pseudomonas aeruginosa, an important opportunistic pathogen associated with lethal infections in cystic fibrosis patients, synthesizes two lectins, PA-IL, specific for D-galactose, and PA-IIL, specific for L-fucose, which are responsible for the biofilm formation by these bacteria.[1,2] One of the latest developments in this area is the study on neoglycoconjugates bearing multivalent carbohydrates in their structure.[3] Recently we reported the discovery of tetravalent *C*-fucosyl peptide dendrimers which exhibit high affinity for the PA-IIL protein.[4] Heterofunctionality of these macromolecules should enable simultaneous inhibition of both PA-IL and PA-IIL. A combinatorial library of second-generation heterofunctional dendrimers, bearing both fucose and galactose, was constructed using split-and-mix protocol. The ligands with the highest affinity to the lectins were identified by on-bead screening of the library and resynthesized. The dendrimers' inhibition properties and their bifunctionality potential will be presented.

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Medicinal Chemistry **114**

Structural basis for silver-mediated killing of the human pathogen *Vibrio cholerae*

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Silver compounds are widely used as microcidals in the clinic and for public health hygiene. We show that Ag^+ targets a respiratory, Na^+ - translocating NADH:quinone oxidoreductase (Na^+ -NQR) of *Vibrio cholerae*, the causative agent of the diarrheal disease cholera. Both Ag^+ (K_i = 8 nM) and Hg^{2+} (K_i = 90 nM) acted as competitive inhibitors with respect to NADH oxidation by the holo-complex and its individual FAD domain. Crystals of the FAD domain diffracted to 1.8 Å, and phases were determined by MAD of a crystal soaked with $K_2Pt(NO_2)_4$. The structure of the FAD domain with Hg^{2+} revealed a heavy metal binding site which blocks access of NAD(H) to the FAD cofactor. *Pseudomonas sp.* (causing wound infections) and *Legionella sp*. (contaminants of drinking water) contain Na^+ -NQRs which are highly related to the *V. cholerae* enzyme studied here. The novel structure of the FAD domain opens new strategies to combat these human pathogens.

Medicinal Chemistry **115**

Structural Analysis of Serum Albumin using Artificial Sensor Anions

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Serum albumin is essential to the transport of metal ions, fatty acids and other small molecules or ions in the blood, comprising 60% of the plasma proteins [1]. Only human serum albumin, the crystal structure was determined and other albumin types than human was not analyzed, probably due to the imperfect crystallization. In this study, using stable metal complex anions as a sensor, local structures of some serum albumins and binding structure images of the sensor anions around a binding site were clarified. The sensor anions are assumed to bind the proteins in the area defined as Site I in subdomain IIA of serum albumin [2]. The binding mode was examined through calorimetric experiments and docking studies by computational methods.

The binding constants between serum albumins and sensor anions were determined in aqueous buffer solutions (pH=7.1, 7.4) by the calorimetric method. The binding energies were estimated by molecular mechanical calculations after docking simulations of the sensor anion with structural optimization of protein side chains (without that of backbone structures). Albumin structures of bovine, sheep and pig are obtained from human structures (PDB structures) by changes of the amino acid sequence. Then, we compared the binding free energy of the sensor anions with these types of serum albumins to the binding constants in the same system. A new type of structure-activity relationships study will be presented.

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Medicinal Chemistry **116**

Navigating in Tanimoto Space

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Virtual chemical space exploration has gained interest for drug discovery.[1] Recently we reported *Chemical Space Travel* as a tool for generating analog libraries.[2] Herein we report the analysis and docking study of such libraries spanning chemical space between AMPA and CNQX, two ligands of the AMPA-*R*, an important neurological drug target.

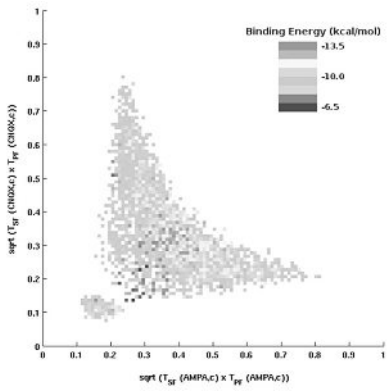

Fig. 1: Binding Energies of the 13'146 analog library compounds as function of their Tanimoto similarity coefficients to AMPA and CNQX.

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Medicinal Chemistry **117**

Aggregation of Chlorin-based Potential Photosensitizers in Aqueous Solution and their Interaction with Phospholipid Vesicles

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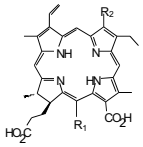
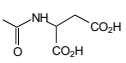
Chlorins are porphyrinic compounds with one of the pyrrole ring double bonds hydrated leading to a red-shift of the electronic absorption maxima. This makes chlorins interesting candidates as potential photosensitizers in Photodynamic therapy (PDT) [1]. However, chlorins tend to self-associate in aqueous solutions thereby reducing their water solubility and membrane incorporation efficiency, both crucial factors in PDT. In the present study, derivatives of the naturally derived compound chlorin e6 (I) bearing various substituents at the ring periphery (R_1, R_2) are characterized NMR spectroscopically and compared with respect to their aggregation behavior and membrane interaction. Aggregate formation, previously shown for (III) [2], is studied by analysis of ^{1}H NMR spectroscopic signal broadening and characteristic ring-current induced shifts of the chlorin signals. Moreover, association with dioleoyl-phosphatidyl-choline (DOPC) vesicles in solution is monitored by spectral perturbation mapping of the DOPC ^{1}H NMR signals. The data indicate that the formation of large and stable aggregates strongly reduces membrane incorporation efficiency. On the other hand, oligomer formation still enables the chlorins to efficiently distribute into the vesicular phase. pH and concentration effects are discussed in this context.



(I) $R_1 = CH_2COOH$ $R_2 = CH_3$

(II) $R_1 = CH_3$ $R_2 = CH_3$

(III) R_1 =  $R_2 = CH_3$

(IV) $R_1 = CH_2COOH$ $R_2 = CHO$

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Medicinal Chemistry **118**

Grafting of extracellular loops of Y receptors onto a β-barrel scaffold

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G-protein coupled receptors (GPCRs) belong to the class of seven-transmembrane proteins and play a pivotal role in signal transduction processes. The GPCRs share a common structural motif of an extracellular N-terminal segment, linked to a bundle of seven membrane-spanning helices that are connected through three loops on the intra- and extracellular sides, followed by an intracellular C-terminal segment[1,2].

Unfortunately, wild-type GPCRs are very difficult to produce recombinantly. In this work we aim at developing a small, preferably soluble protein, which possesses a stable core determining the global fold, and comprises surface-exposed loops that can be modified without compromising folding. In such a system the extracellular portions of a GPCR could be transferred onto that core resulting in a chimeric protein that could be considered as a "minireceptor". Such a protein contains all the parts of the receptor hypothesized to be important for ligand binding (hence the term "receptor"), but at the same time displays the favourable characteristics of small, soluble proteins (hence the term "mini").

Our efforts are concentrated on the construction and characterization of such a minireceptor for the so-called Y-receptor[3] family of GPCRs, that is targeted by peptides of the neuropeptide Y (NPY) family. The scaffold is derived from β-barrel proteins, and NMR techniques are used to assess to which extent the global fold is retained in the loop-modified mutants.

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Medicinal Chemistry **119**

Selective DNA-binding of metal complexes embedded within a protein host: a novel technology for improved anticancer therapy

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Bioinorganic drugs, such as the platinum-drug oxaliplatin, are important anticancer compounds. However, they are highly toxic and cause serious side-effects, due to lack of selectivity to their DNA target.

We hypothesized that the incorporation of a well-defined protein environment around a DNA-binding complex would increase selectivity toward a specific DNA target through second coordination interactions. Here we describe a chemogenetic screen to identify variants of metal complexes embedded within a protein scaffold exhibiting increased DNA-binding activity *in vitro*.

We used streptavidin as a 'targeting host-protein' and a biotinylated metal complex as a 'non-selective drug'. We found that the nature of the complex as well as defined single point-mutations around the 'active site' of the protein-host influenced target-recognition and binding to cancer-specific DNA-sequences.

These proof-of-principle studies represent, to our knowledge, the first example of increased selectivity of bioinorganic drugs toward cancer-specific DNA sequences using a protein carrier. We believe that this novel technology may lead to improved anticancer (bio)chemotherapy.

Medicinal Chemistry **120**

Studies of the Mechanism for Activation of Caspase-8 by NMR

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Apoptosis is a specific form of programmed cell death that plays a vital role in multicellular organisms. Two different pathways are known for triggering apoptosis: The extrinsic (extracellular) and the intrinsic (intracellular) pathway [1]. In the extrinsic pathway the Fas ligand binds to the Fas receptor resulting in receptor oligomerization followed by the recruitment of FADD. FADD can then bind to a variety of other proteins, such as the initiator caspases -8 and -10. Activated caspase-8 is released from the DISC and activates the executioner caspases-3 and -7.
Generation of an active Caspase-8 requires limited proteolysis. During this activation process Caspase-8 is autoproteolytically cleaved at two Asp sites. The active site of the enzyme contains an catalytically important Cys residue. Caspase activation is believed to be induced by dimerization (the induced proximity model) [3].
In this work we have determined the solution structure of a 266 residue mutant of Caspase-8, in which the catalytic Cys was replaced by Ala. This mutant cannot undergo autoproteolysis, and remains monomeric. In particular, the position of a long loop, which contains the cleavage sites, could be defined in the structure. Processing of Procaspase-8 was followed in the NMR tube by adding small amounts of active Caspase-8. Moreover, properties of mutants, in which the cleavage site Asp residues are replaced by Ala while preserving the active-site Cys are presented. Finally, a mutant that contains the Asp and Cys residues, but in which dimerization is impaired could shown to be similar in structure.

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