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Medicinal Chemistry Chemical Biology **MC001**

SOM230: A new therapeutic modality for Cushing's disease

Ian Lewis, Janos Pless, Rainer Kneuer, Antonio P. Silva, Daniel Hoyer, Gisbert Weckbecker, Christian Bruns and Herbert A. Schmid

Global Discovery Chemistry Department Exploratory Medicinal Chemistry Unit and Autoimmune, Oncology and Neuroscience Research Departments, Novartis Institutes of Biomedical Research, CH-4002 Basel, Switzerland

The somatostatin (SRIF, somatotropin release inhibiting factor) field has been a success story in terms of medicinal chemistry and drug discovery offering a variety of therapeutic opportunities, e.g. acromegaly, gastrointestinal neuroendocrine tumors, whole body imaging and radiotherapy. Indeed, a rational medicinal chemistry approach capitalising on structure activity relationships led to the discovery of SOM230, a stable cyclohexapeptide somatostatin mimic which exhibits unique binding to human SRIF receptors (sst1-5). This approach involved transposing functional groups, in the form of unnatural amino acids, from SRIF-14 into the stable, reduced size cyclohexapeptide template. Further, the hydroxyproline urethane extension of SOM230 has been functionalized with the chelators DTPA and DOTA, which is a necessary prerequisite for the possible development of ligands which could be used for whole body imaging. Uniquely, SOM230 exhibits binding with a 30 to 40 times higher affinity than Sandostatin® to the sst1 and sst5 receptors and exhibits higher efficacy in preclinical models in lowering Growth Hormone, Insulin-Like Growth Factor-1, ACTH and corticosterone than Sandostatin®. Recently, phase III clinical studies have established the therapeutic potential of SOM230 / Pasireotide (Signifor®), as the first pituitary directed medical therapy for Cushing's disease[1] leading to registration of SOM230 by both EMEA and FDA in 2012.

[1] Annamaria Colao, Stephan Petersenn, John Newell-Price, James W. Findling, Feng Gu, Mario Maldonado, Ulrike Schoenherr, David Mills, Luiz Roberto Salgado and Beverly M.K. Biller for the Pasireotide B2305 Study Group, A 12-Month Phase 3 Study of Pasireotide in Cushing's Disease, N Engl J Med **2012**;366:914-24.

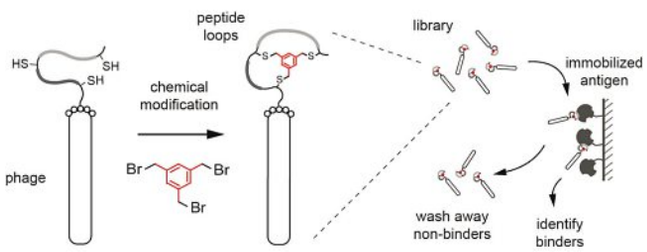
Medicinal Chemistry Chemical Bioloy **MC002**

Directed evolution of bicyclic peptides for therapeutic application

Lisa Pollaro, Alessandro Angelini, Khan Maola and Christian Heinis

EPFL, BCH 5305, 1015 Lausanne, Switzerland

Bicyclic peptides with high binding affinity and target selectivity can be isolated from large combinatorial libraries by phage display as shown in the figure below. In brief, peptide libraries displayed on phage are cyclized in a chemical reaction and subjected to affinity selections. The bicyclic peptides combine key qualities of antibody therapeutics (high affinity and specificity) and some advantages of small molecule drugs.



Potent and selective bicyclic peptide inhibitors of the cancer-associated proteases urokinase-type plasminogen activator (uPA) and matrix metalloproteinase 2 (MMP-2) were recently developed. The properties of these inhibitors and their structures will be discussed as well as first in vivo results presented.

[1] Heinis, C., *et al*., *Nat. Chem. Biol*.. **2009**, *5*, 502.
[2] Angelini, A., *et al*., *ACS Chem. Biol*. **2012**, *18*, 817
[3] Angelini, A., *et al*., *J. Med. Chem*., **2012**, *26*, 10187

Medicinal Chemistry Chemical Biology **MC003**

Non-nucleosidic COMT bisubstrate inhibitors

Christian Lerner,[a] Caterina Bissantz,[a] Bernd Büttelmann,[a] François Diederich,[b] Alain Gast,[a] Roland Jakob-Roetne,[a] Doris Roth,[a] Markus Rudolph,[a] Daniel Schlatter[a]

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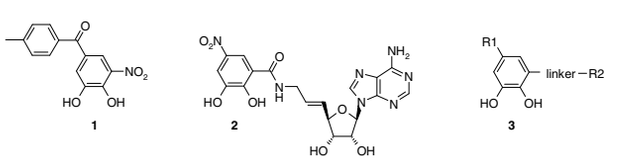
Inhibition of catechol *O*-methyltransferase (COMT) in the brain is expected to be an effective treatment of depression and in particular of disturbed cognitive performance in schizophrenia. Tolcapone (**1**) with a very low brain/plasma ratio of ~0.01 has been used as a tool compound to study the central effects of COMT inhibition in clinical trials and in animal experiments [1]. Research in collaboration with the Diederich group at the ETH Zurich led to the discovery of potent nucleosidic bisubstrate inhibitors (**2**) that bind to both, the catechol substrate pocket and *S*-adenosylmethionine (SAM) cofactor pocket of COMT [2]. In this presentation, we show non-nucleosidic bisubstrate inhibitors (**3**) that were discovered using parallel chemistry based on virtual docking and chemical intuition.



Tolcapone — nucleosidic bisubstrate inhibitor — non-nucleosidic bisubstrate inhibitors

[1] J. A. Apud, D. R. Weinberger, *CNS Drugs* **2007**, *21*, 535.
[2] C. Lerner, A. Ruf, V. Gramlich, B. Masjost, G. Zürcher, R. Jakob-Roetne, E. Borroni, F. Diederich, *Angew. Chem.* **2001**, *113*, 4164; *Angew. Chem.-Int. Edit.* **2001**, *40*, 4040.

Medicinal Chemistry Chemical Biology **MC004**

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

Thomas Jack*, Marc-David Ruepp*, Oliver Mühlemann*, Andrew J. Thomson‡, Sarah C. R. Lummis‡ and Martin Lochner*

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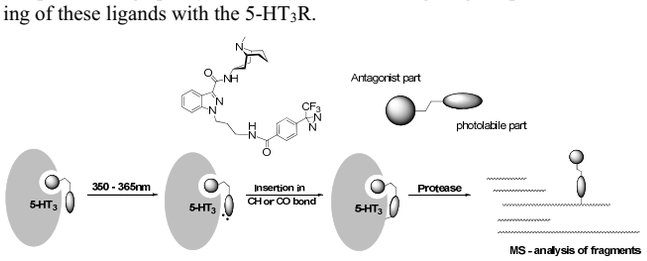
‡University of Cambridge, Tennis Court Road, CB2 1QW Cambridge, UK

The 5-HT$_3$ receptor (5-HT$_3$R) is an important ion channel responsible for the transmission of nerve impulses in the central nervous system.[1] It is difficult to characterize transmembrane dynamic receptors with classical structural biology approaches like crystallization and x-ray. The use of photoaffinity probes is an alternative approach to identify regions in the protein that are important for the binding of small molecules. Therefore we synthesized a small library of photoaffinity probes by conjugating photophores via various linkers to granisetron which is a known antagonist of the 5-HT$_3$R. We were able to obtain several compounds with diverse linker lengths and different photolabile moieties that show nanomolar binding affinities for the orthosteric binding site. Furthermore we established a stable *h*5-HT$_3$R expressing cell line and a purification protocol to yield the receptor in a high purity. Currently we are investigating the photo crosslinking of these ligands with the 5-HT$_3$R.



[1] Thompson, A. J.; Lummis, S. C., *Expert Opin. Ther. Targets* **2007**, *11*, 527.

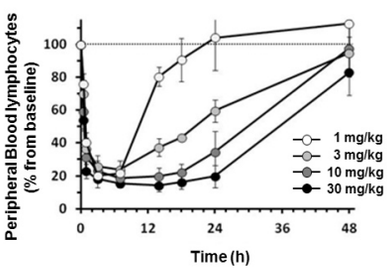
Medicinal Chemistry Chemical Biology **MC005**

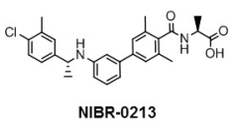
Discovery of Highly Potent $S1P_1$ Receptor Antagonists With In Vivo Efficacy

Jean Quancard, Birgit Bollbuck, Daniela Angst, Philipp Janser, Frédéric Berst, Peter Buehlmayer, Markus Streiff, Christian Beerli, Volker Brinkmann, Danilo Guerini, Paul A. Smith, Tim Seabrook, Martin Traebert, Klaus Seuwen, René Hersperger, Christian Bruns, Frédéric Bassilana, Marc Bigaud

Novartis Institutes for BioMedical Research, Basel, Switzerland

We present the identification of a novel $S1P_1$ receptor antagonist scaffold by HTS, followed by a successful optimization to afford single digit nanomolar tool compounds for subcutaneous administration. First *in vivo* studies in rats demonstrated immunomodulatory properties, kicking-off a broad strategy to address the scaffold inherent bioavailability issues. These efforts ultimately led to the discovery of NIBR-0213. Oral administration of this highly potent and selective $S1P_1$ receptor antagonist induces long lasting reduction of peripheral blood lymphocyte counts leading to efficacy in relevant animal models of autoimmune diseases.





NIBR-0213

Medicinal Chemistry Chemical Biology **MC006**

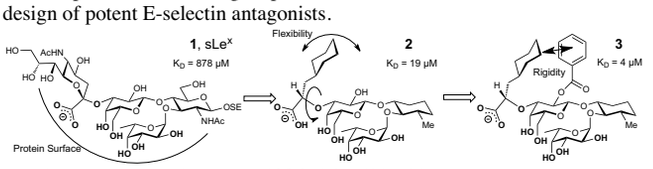
Structural Insights into E-selectin-Ligand Interactions

Roland C. Preston[1], Florian P.C. Binder[1], Mirco Zierke[1], Roman Jakob[2], Timm Maier[2], Beat Ernst[1]

[1]Institute of Molecular Pharmacy, Pharmazentrum, University of Basel, Klingelbergstr. 50/70, CH-4056 Basel
[2]Biozentrum, University of Basel, Klingelbergstr. 50/70, CH-4056 Basel

The lectin E-selectin is involved in a variety of inflammatory diseases by acting as leukocyte-adhesive protein on the cardiovascular endothelium. E-selectin has extensively been under investigation as drug target.

We investigated the binding of sialyl Lewis^x (**1**, sLe^x) and the affinity-improved glycomimetic **2** by X-ray crystallography. Co-crystallization of ligand and protein revealed significant conformational alterations of the binding site upon binding, when compared to published apo- and sLe^x (**1**) soaked structures [1, 2]. In the induced conformation, additional hydrogen bonds between the protein and the ligand are observed. We further investigated the binding of antagonist **3** with a fivefold improved affinity over **2**. The structural data reveals a sigma-pi interaction of the benzoate moiety in the 2-position of the D-Gal moiety with the (*S*)-cyclohexyl lactic acid, leading to an improved preorganization of the carboxylic acid in its bioactive conformation. In addition, a minor shift of the (*S*)-cyclohexyl lactic acid moity was observed, allowing for closer contact of this moiety in antagonist **3** to the protein. These insights provide the basis for future rational *in silico* design of potent E-selectin antagonists.



[1] Graves, B.J. *et al*. *Nature*, **1994**, *367*, 532-538.
[2] Somers, W.S. *et al*. *Cell*, **2000**, *103*, 467-479.

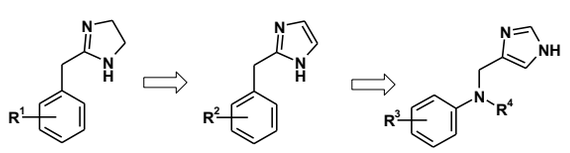
Medicinal Chemistry Chemical Biology **MC007**

Imidazolines and Imidazoles as TAAR1 Agonists

Guido Galley, Annick Goergler, Marius C. Hoener, Roger D. Norcross and Henri Stalder

F. Hoffmann-La Roche AG, pRED, CH-4070 Basel, Switzerland

2-Benzylimidazolines have been identified as submicromolar ligands for the Trace-Amine Associated Receptor TAAR1 by high-throughput screening. Further optimization led to the discovery of various classes of highly active imidazoles. An improved selectivity against the adrenergic alpha 2 receptor was observed especially for 2-aminomethyl substituted imidazoles [1].



The availability of selective ligands is regarded as an important step forward to study and further understand the role TAAR1 in biological systems [2].

[1] G. Galley, H. Stalder, A. Goergler, M. C. Hoener, R. D. Norcross, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5244.
[2] F. G. Revel, C. A. Meyer, A. Bradaia, K. Jeanneau, E. Calcagno, C. B. Andre, M. Haenggi, M. T. Miss, G. Galley, R. D. Norcross, R. W. Invernizzi, J. G. Wettstein, J. L. Moreau, M. C. Hoener, *Neuropsychopharmacology* **2012**, *37*, 2580.

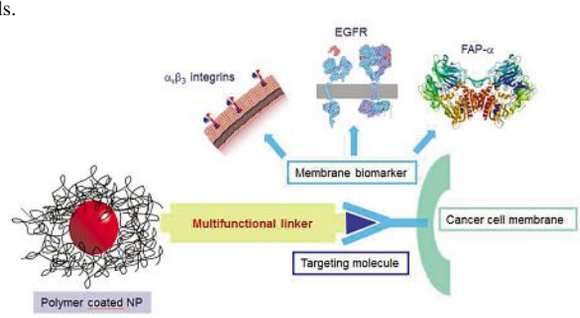
Medicinal Chemistry Chemical Biology **MC008**

Functionalized nanomaterials for cancer diagnosis

Solène Passemard[1], Davide Staedler[1], Giona Sonego[1], Joachim Loup[1], [2]Lucienne Juillerat-Jeanneret, Sandrine Gerber-Lemaire[1]

[1]EPFL, SB, ISIC, LSPN, Batochime, CH-1015 Lausanne.
[2]University Institute of Pathology, CHUV-UNIL, CH-1011 Lausanne

Medical imaging is a major tool for the prevention and detection of cancer. Conjugation of small organic ligands targeting specific cancer cells biomarkers to inorganic nanoparticles (NPs) presenting versatile optical properties could provide increased level of sensitivity and accuracy for the early diagnosis of malignant diseases. We herein present the design, synthesis and biological evaluation of several targeting molecules and their conjugation to polymer coated NPs for the specific labeling of human cancer cells. In particular, fibroblast activation protein-α [1] was selected as extracellular biomarker for the detection of human-derived breast, lung and prostate cancer cells.



[1] Lawandi, J.; Gerber-Lemaire, S.; Juillerat-Jeanneret, L.; Moitessier, N. *J. Med. Chem.* **2010,** *53*, 3423. Juillerat-Jeanneret, L.; Gerber-Lemaire, S. *Mini Rev. Med. Chem*. **2009**, *9*, 215.

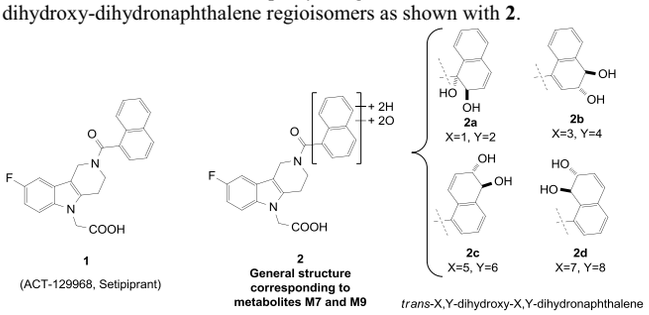
Medicinal Chemistry Chemical Biology **MC009**

Structure elucidation of two major metabolites of CRTh2 antagonist ACT-129968 (Setipiprant)

Philippe Risch, Heinz Fretz, Thomas Pfeifer, Julien Pothier

Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, 4123 Allschwil, Switzerland

Two major metabolites **M7** and **M9** were detected after incubating ^{14}C labeled CRTh2 antagonist **1** with human hepatocytes. Analysis of the mass spectra (LC-MS/MS) indicated that the tetrahydropyridoindole core of **1** remained intact whereas its naphthyl ring seemed to be converted to two dihydroxy-dihydronaphthalene regioisomers as shown with **2**.



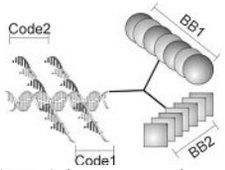
Based on literature precedence, **1** was assumed to be transformed in two consecutive enzymatic steps to **M7** and **M9**, two distinct vicinal *trans*-X,Y-dihydroxy-X,Y-dihydronaphthalene regioisomers. Consequently, the four most plausible regioisomers **2a-d** were synthesized in racemic and enantio-enriched form. Structure and absolute stereochemistry of the two metabolites **M7** and **M9** could unequivocally be assigned by comparing analytical and spectral data of the synthetic samples with the biological extracts.

Medicinal Chemistry Chemical Biology **MC010**

Development and Evaluation of DNA-Encoded Compound Libraries as an Effective Drug-Discovery Method

Raphael M. Franzini[1], Willy Decurtins[1], Moreno Wichert[1], Florent Samain[2], Jörg Scheuermann[1], and Dario Neri[1]

[1]Institute of Pharmaceutical Sciences, ETH Zürich, Wolfgang-Pauli-Str. 10, 8093 Zürich; [2]Philochem AG, Libernstr. 3, 8112 Otelfingen



DNA-encoded libraries (DELs) have emerged as a promising approach for drug development because of a unique profile of speed and cost-efficiency.[1] DELs are compound collections in which each structure is unambiguously defined by a unique nucleic acid barcode. Similar to phage-display technology, panning these libraries against immobilized target proteins provides enriched binders; high-throughput sequencing of the corresponding DNA-barcodes enables the identification of these structures.
In this presentation, we report on our ongoing efforts in the development of DEL technology. Our design philosophy is to generate structurally compact DELs of high purity and chemical diversity. In particular, we describe a library comprising over 100'000 encoded molecules, based on pairs of 650 structural fragments, which was assembled by a novel solid/liquid-phase synthesis strategy. Preliminary screening experiments against varied protein targets have established the effectiveness of this library for rapid and cost-effective identification of hit compounds. Selected examples of identified protein binders illustrate the utility of this method as a drug discovery tool.

[1] J. Scheuermann, and D. Neri *ChemBioChem*, **2010**, *11*, 931.

Medicinal Chemistry Chemical Biology **MC011**

Identification of novel P3-P1' macrocyclic inhibitors of HCV NS3/4A protease

Oliver Simić, Trixi Brandl, Pascal Rigollier, Ursula Bodendorf, Nikolaus Schiering, Claus Ehrhardt, Ulrich Hassiepen, Kai Lin, Julie Flynn

Expertise Platform Proteases/Novartis Institutes for Biomedical Research, 4002-Basel, Switzerland

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that subsequently can lead to cirrhosis, carcinoma and liver failure. Around 170 million people worldwide are chronically infected with HCV which is the leading cause of liver transplants. Approval of telaprevir and boceprevir in 2011 as direct acting HCV NS3/4A protease inhibitors complements the previous standard combination therapy of injectable pegylated interferon-a (PEG IFN-a) and the antiviral drug ribavirin.
While both, telaprevir and boceprevir, act as covalent reversible inhibitors bearing a ketoamide warhead, our efforts were focused on the identification of non-covalent inhibitors.
Based on x-ray crystallographic data analysis of acyclic ligands in complex with HCV NS3/4A the discovery of novel macrocyclic acyl-sulfonamide based inhibitors will be highlighted and a concise structure activity relationship along with optmization efforts to improve ADME properties will be described.

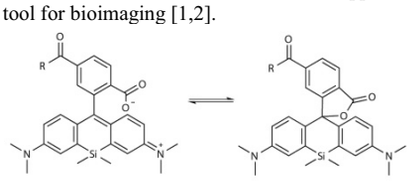
Medicinal Chemistry Chemical Biology **MC012**

Applications of silicon-rhodamine for the imaging of living cells

Gražvydas Lukinavičius, Keitaro Umezawa, Luc Reymond and Kai Johnsson

Ecole Polytechnique Fédérale de Lausanne (EPFL), Institute of Chemical Sciences and Engineering (ISIC), 1015 Lausanne, Switzerland

The ideal fluorescent probe for bioimaging is bright, absorbs at long wavelengths and can be flexibly implemented in living cells and in vivo. However, the design of synthetic fluorophores that combine all of these properties has proven to be extremely difficult. Here, we introduce a biocompatible near-infrared silicon-rhodamine probe that can be specifically coupled to proteins using different labeling techniques. Importantly, its high permeability and fluorogenic character permit imaging of proteins in living cells and tissues, while its brightness and photostability make it ideally suited for live-cell superresolution microscopy. The excellent spectroscopic properties of the probe combined with its ease of use in live-cell applications make it a powerful new tool for bioimaging [1,2].



[1] G. Lukinavicius, K. Umezawa, N. Olivier, A. Honigmann, G. Yang, T. Plass, V. Mueller, L. Reymond, I.R. Correa, Jr., Z.G. Luo, C. Schultz, E.A. Lemke, P. Heppenstall, C. Eggeling, S. Manley, and K. Johnsson, *Nat Chem.* **2013**, 5, 132-9.
[2] K. Umezawa, G. Lukinavičius and K. Johnsson, *International patent application.* **2013,** WO2011EP64750

Medicinal Chemistry Chemical Biology **MC013**

Discovery of highly potent and selective covalent reversible cathepsin S inhibitors

W. Haap, G. Hartmann, R. Alvarez Sanchez, L. Anselm, D. W. Banner, R. Ecabert, U. Grether, H. Kuehne, B. Kuhn, T. Luebbers, J. U. Peters, J. M. Plancher, A. Rufer, B. Spinnler

Pharma Research and Early Development,
F. Hoffmann-La Roche AG, Grenzacher Str. 124,
4070 Basel, Switzerland

Cathepsin S (CatS) is a cysteine protease involved in the antigen presentation process and in extra cellular matrix degradation. Dysregulated CatS activity is a driver for chronic inflammation, destructive proteolysis, arterial calcification and increased neoangiogenesis. Therefore CatS is an interesting target for several indications such as autoimmune diseases, cancer or cardio vascular diseases. One approach to inhibit the proteolytic activity of CatS is to block its active site with covalent reversible inhibitors which react with the cysteine present in the catalytic triad. This presentation will focus on the identification and optimization of covalent reversible CatS inhibitors via X-ray structure elucidation and molecular modeling to improve potency, selectivity, reversibility, and ADME properties.

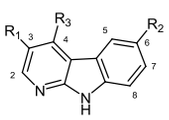
Medicinal Chemistry Chemical Biology **MC014**

Synthesis of inhibitors of oncogenic fusion protein NPM-ALK for *in vitro* and *in vivo* assays.

Sébastien Tardy[a], William H Bisson[a], Luca Mologni[b], Peter Goekjian[c], Carlo Gambacorti-Passerini[b], Leonardo Scapozza[a]

[a]Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, Quai E. Ansermet 30, 1211 Geneva, Switzerland
[b]Department of Clinical Medicine, University of Milan-Bicocca, Italy
[c]Laboratory of Organic Chemistry, ICBMS, University of Lyon 1, France

Anaplastic large cell Lymphomas (ALCL) is a cancer affecting mostly children and young adults. It is driven by the kinase activity of the oncogenic fusion protein NPM-ALK resulting from t(2;5) (p23;q35) chromosomal translocation. Using structure-based design we have shown that the skeleton pyrido [2,3-b] indole could be a lead scaffold for the preparation of potential tyrosine kinase inhibitors of NPM-ALK. This core, commonly called α-carboline, appears in a number of natural products and molecules of pharmacological interest [1].



The objectives of this work were to synthesize a library of tyrosine kinase inhibitors starting from the azacarbazole scaffold by modifying it with many different substitutions at position *C*-3 and *C*-6 or *C*-4 and *C*-6. In order to functionalize the aromatic ring, the methodology consists in sequentially use chemo- and regioselective Suzuki-Miyaura, Sonogashira, and Buchwald palladium-catalyzed cross-coupling reactions [2]. The medicinal chemistry effort resulted in NPM-ALK inhibitors active *in vitro* and *in vivo*.

[1] Scapozza, L. *et. al*, *Appl. Int. WO2010025872A2*, **2010**.
[2] Goekjian, P., G. *et. al*, *Eur. J. Org. Chem.*, **2010**, 6665-6677.

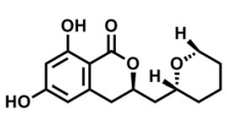
Medicinal Chemistry Chemical Biology **MC015**

Total Synthesis of the Antimalarial Agent Cladosporin.

Laure C. Bouchez*, Marion Rusch, Maude Patoor, Madeleine Livendahl, Christophe Bodenreider, Dominic Hoepfner.

Novartis Institute for Biomedical Research, Fabrikstrasse 22,
CH-4056, Basel, Switzerland

Cell-based screening has recently been shown to be an attractive way to find new leads for malaria drug development. Next generation antimalarials needs to be active against drug-resistant parasites and efficacious against both liver- and blood-stage infections. We screened a natural product library to identify inhibitors of *Plasmodium falciparum* blood- and liver-stage proliferation. [1]
Cladosporin, a fungal secondary metabolite was identified as a potent antiparasitic agent with a striking activity against both blood and liver stages (IC_{50} in the nanomolar range).
Thus, we designed a route for de novo synthesis of Cladosporin, with the flexibility to potentially generate a (wide-ranging/diverse) collection of analogues. [2] and [3]



Cladosporin

[1] Dominic Hoepfner *et al*., *Cell Host & Microbe* **2012**, *11*, 654-663.
[2] *Reported synthesis*- Huaji Zheng *et al., J. Org. Chem.* **2012**, 5656-5663.
[3] Laure Bouchez *et al. Article in preparation.*

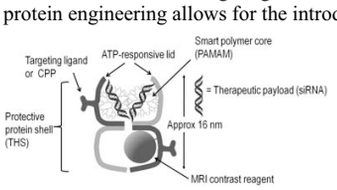
Medicinal Chemistry Chemical Biology **MC016**

Thermosome – a cage protein acting as a versatile delivery platform

Martin G. Nussbaumer, Alba Mascarin, Thomas Mindt & Nico Bruns

University of Basel, Klingelbergstr. 80, 4056 Basel, Switzerland

Protein cages represent a versatile platform for a variety of functions such as targeted delivery of drugs or contrast agents. These proteins protect the drug from premature degradation and enhance uptake in desired tissue [1]. The versatility stems from the different modification sites of protein cages. Various cargoes can be packed in the interior of such protein cages. The exterior can be modified with cell-targeting and/or –penetrating moieties. Furthermore, protein engineering allows for the introduction of additional linking sites.



To this end, we use the thermosome (THS), a chaperonin from *T. acidophilum* [2]. THS features two cavities that can accommodate guest molecules. Polyamidoamine (PAMAM) was covalently bound to the genetically introduced cystein residues in the interior. This cationic polymer is able to bind and stabilise small interfering RNA (siRNA) by electrostatic interactions [3]. Oligonucleotides, such as siRNA, are normally rapidly degraded in serum. Our experiments showed the uptake of siRNA into our hybrid as well as the enhanced stability of siRNA to RNase. To achieve cell-targeting, THS was modified on its outer surface with the cell penetrating peptide TAT. With TAT a 75-fold increase of cell uptake was observed.
Experiments are underway to elucidate the delivery of siRNA to cells. Additionally, we can show uptake of metal nanoparticle into THS, which can be used for MRI contrast purposes.

[1] Y. Ma et al., *Adv. Drug Deliver. Rev.* **2012,** *64(9),* 811-825.
[2] N. Bruns et al., *Angew. Chem.* **2009**, *121*, 5776-5779.
[3] J.H. Zhou et al., *Chem. Comm.,* **2006**, 2362.

Medicinal Chemistry Chemical Biology **MC017**

Inert Ruthenium Complexes as Anticancer Drug Candidates

Vanessa Pierroz,[1,2] Tanmaya Joshi,[2,3] Anna Leonidova,[2] Cristina Mari,[2] Leone Spiccia,[3] Stefano Ferrari[1] and Gilles Gasser[2]

[1]Institute of Molecular Cancer Research, University of Zurich, Winterthurerstrasse 190, CH-8057, Switzerland
[2]Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057, Switzerland
[3]School of Chemistry, Monash University, Victoria 3800, Melbourne, Australia

Since its FDA approval in 1978, cisplatin has been one the most used anticancer drugs in the world. However, this particular platinum based complex as well as its other derivatives display severe side-effects including nephrotoxicity.[1] To overcome these drawbacks, several metal-based complexes as drug candidates are being investigated; Ruthenium complexes emerging as promising alternatives.[2,3] Recently, the *in vitro* behavior of two substitutionally inert Ru(II) complexes bearing a derivative of the ligand 2-(2'-pyridyl)pyrimidine (CppH) have been investigated in our groups. Complex **1**, in particular, shows cytotoxicity comparable to that of cisplatin on different cancer cell lines and, importantly, lower toxicity than cisplatin on healthy cells. Using several biochemical assays, we could demonstrate that this Ru(II) complex exerts its cytotoxicity by triggering a mitochondria-mediated apoptosis.[4]

[1] Dhar, S.; Lippard, S. J. In *Bioinorganic Medicinal Chemistry*; Alessio, E., Ed.; Wiley-VCH: Weinheim, **2011**, 351.
[2] Keppler, B. K., Hartinger, C. *et al., Bioinorganic Medicinal Chemistry,* Alessio, E., Ed.; Wiley-VCH: Weinheim, **2011,** 151.
[3] Dyson, P. J. *et al. Int. J. of Oncology* **2006,** *29,* 261.
[4] Pierroz, V.; Joshi, T.; Leonidova, A.; Mari, C.; Schur, J.; Ott, I.; Spiccia, L.; Ferrari, S.; Gasser, G. **2012**, *J. Am. Chem. Soc*.**134**, 20376.

Medicinal Chemistry Chemical Biology **MC018**

Antiprotozoal Isoflavan Quinones from *Abrus precatorius*

Yoshie Hata[1], Maria De Mieri[1], Melanie Raith[1], Samad Ebrahimi[1], Stefanie Zimmermann[1,2], Tsholofelo Mokoka[3], Dashnie Naidoo[3], Gerda Fouche[3], Vinesh Maharaj[3], Reto Brun[2], Marcel Kaiser[2], Matthias Hamburger[1]

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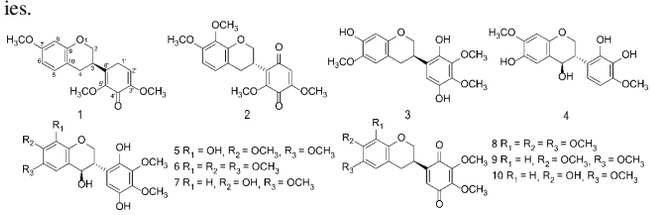
A CH_2Cl_2/MeOH (1:1) extract of the whole plant of *Abrus precatorius* (Fabaceae) showed strong *in vitro* activity against *Trypanosoma brucei rhodesiense.* The active compounds were tracked by HPLC-based activity profiling. Ten compounds were isolated, including five new natural products [1]. Their structures and relative configuration were established by NMR (^{1}H, ^{13}C, COSY, HMBC, HSQC, NOESY). The absolute configuration was determined by comparison of electronic circular dichroism (ECD) spectra with calculated ECD data. Abruquinones I (**1**) and B (**8**) showed strong *in vitro* activity (IC_{50}s of 0.30 ± 0.1 µM and 0.16 ± 0.1 µM, respectively). Selectivity indices (SI) as calculated from cytotoxicity data in L-6 cells were 78.3 and 61.3. These compounds are promising candidates for *in vivo* studies.



[1] Y. Hata, M. Raith, S. Ebrahimi, S. Zimmermann, T. Mokoka, D. Naidoo, G. Fouche, V. Maharaj, M. Kaiser, R. Brun, M. Hamburger, *Planta Med.* **2013**, DOI: 10.1055/s-0032-1328298.

Medicinal Chemistry Chemical Biology **MC019**

Combinatorial chromophore assembly inside a DNA four-way junction

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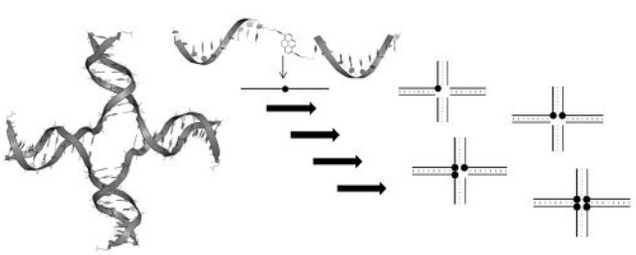
In 1964 first proposed by Robin Holliday as a mechanistic model to solve the mystery of how genetic information is exchanged in yeast, the DNA four-way junction or Holliday junction (HJ) was proofed to be the key intermediate in homologous recombination and became an important tool in the field of DNA origami, computation and nanomachines. Herein we use the assembly of four modified nucleic acid strands into the planar square conformation of this higher order DNA structure to demonstrate in a proof of principle manner the cumulative effect of pyrene moieties interacting inside the junction.[1][2]



[1] R. Holliday, *Genetical Research*, **1964**, 5, 282 - 304.
[2] Deshmukh N. Gopaul et al., *The EMBO Journal*, **1998**, 17, 4175 - 4187

Medicinal Chemistry Chemical Biology **MC020**

Specific Targeting of Cancer Cells using Cytotoxic Re(I) Complexes

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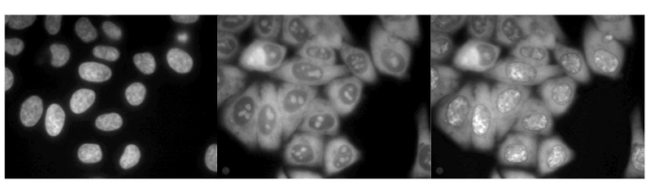
Minimizing the effect of anti-cancer drugs on healthy cells has become, nowadays, one of the major concerns in medicinal chemistry. Some strategies, such as in photodynamic therapy, involve the use of external triggers to select the area of drug activation, while other approaches rely on conjugating the active compound to a moiety specifically targeting cancer cells. In recent years, several Re compounds have been shown to be cytotoxic on various cancer cell lines.[1] All of them, however, indiscriminately affect both cancer and healthy cells. In this study, we screened a series of *N, N*-bis(quinolinoyl) Re(I) tricarbonyl complexes for antiproliferative activity. The cytotoxicity of some of these compounds increases significantly upon light irradiation.



Due to the fluorescent properties of the Re(I) complexes employed, their localization in cells could be studied in cervical cancer (HeLa) cells. Importantly, the metal complexes were attached to specific-targeting biomolecules to further enhance the selectivity of bioconjugate towards cancer cells. [2]

[1] G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.,* **2010**, *54*, 3 and references therein.
[2] A.Leonidova et al., **2013**, *submitted*.

Medicinal Chemistry Chemical Biology **MC021**

Phototoxic Evaluation of Novel DNA Intercalating Ru(II) Complexes

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Photodynamic Therapy (PDT) is a medical technique relying on the synergistic action of light and a non-toxic photosensitizer to induce cell death through the formation of reactive oxygen species.[1] PDT offers the opportunity to kill tumor cells with a spatial and temporal control, without causing severe side-effects, as is observed with other chemotherapeutic agents such as cisplatin. The current synthetic photosensitizers in the market, though, are porphyrin based and suffer from several drawbacks including tedious synthesis and purification as well as prolonged light sensitivity. Here, we report the phototoxic behavior of six easy-to-synthesize Ru(II) complexes (Fig. 1), all of them showing strong DNA binding affinity and a good nuclear uptake. Moreover, the complexes exhibit luminescence enhancement with DNA intercalation and a high singlet oxygen production in hydrophobic environments. The biological behavior of the complexes, evaluated in the dark and upon irradiation with light of different wavelengths (essential to map their photoactivity), shows one of the compounds to rapidly induce tumor cell death upon light activation.[2]

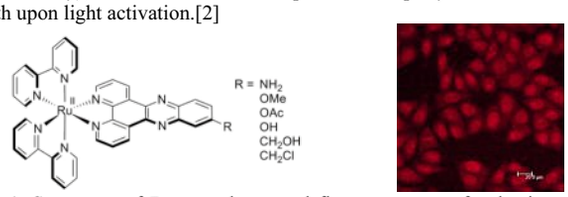

Fig. 1. Structures of Ru complexes and fluorescence confocal microscopy image of HeLa cells incubated with one of the complexes.

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[2] Mari, C. *et al.*, **2013**, *submitted.*

Medicinal Chemistry Chemical Biology **MC022**

Synthesis and Incorporation of Diazirine-Modified Uridine Triphosphate

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A diazirine-modified uridine triphosphate analogue designed to detect RNA –RNA binding protein (RBP) interactions was synthesised and its use in T7 RNA transcription assays is described.

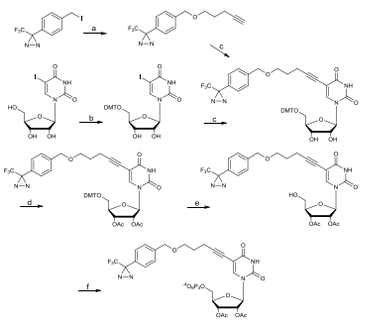

Figure 1. Synthesis of triphosphate **Y**.

3-(*α*-iodo-*p*-tolyl)-3-(trifluoromethyl)-3H-diazirine was synthesized according to Bayley *et al*[1]. *a)* 4-pentyn-1-ol, NaH, THF, 65% ; *b)* DMTCl, Py, 77%; *c)* CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 61%; *d)* Ac_2O, DMAP, Py, 71%; *e)*dichloroacetic acid, CH_2Cl_2, 48%; *f)* Triphosphorylation[2].

[1] Shih, L. B.; Bayley, H. *Anal. Biochem.* **1985**, *144*, 132-141.
[2] Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631-635

Medicinal Chemistry Chemical Biology **MC023**

Towards Novel Organometallic Antischistosomal Drug Candidates

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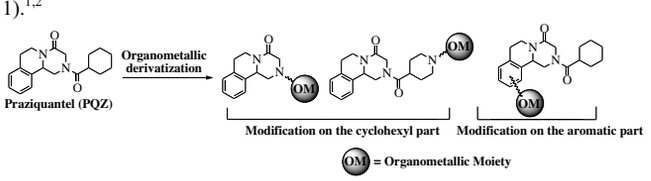
Schistosomiasis is a major human health problem caused by parasitic worms of the genus *Schistosoma*. Annually, more than 280 000 deaths are reported, mostly in tropical regions of developing countries. In addition, more than 207 million people are infected and nearly 800 millions are at risk of being infected. The infective disease is currently controlled by an organic drug, Praziquantel (PZQ), which, despite its success, suffers from several drawbacks (e.g. metabolic stability, inactivity against the juvenile stage of *Schistosoma*). With this in mind, our group has initiated a program to derivatize PZQ with organometallic moieties using three different strategies (Scheme 1).[1,2]



Scheme 1. Strategies for the design of organometallic derivatives of PZQ.

Impressive *in vitro* activity, in the nanomolar range, against *Schistosoma mansoni* was reported for two of our organometallic derivatives of PZQ. Our recent *in vivo* results will be also discussed.[1,2]

(1) Patra, M. et al., *Chem. Eur. J.* **2013**, *19*, 2232 and references therein.
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Medicinal Chemistry and Chemical Biology **MC024**

Synthesis of fluorescent probes for the study of the 5-HT_3A receptor : applications in imaging and fluorescence polarization

Jonathan Simonin[1], Thomas Jack[1], Marc-David Ruepp[1], Daniel Hothersall[2], Christopher Connolly[2], Andrea Chicca[3], Jürg Gertsch[3], Andrew Thompson[4], Sarah Lummis[4] and Martin Lochner[1]

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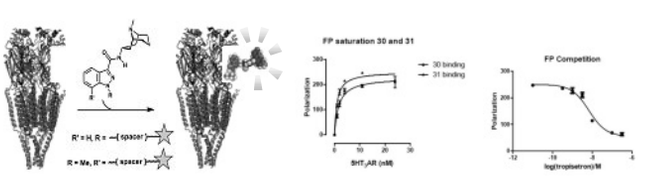
University of Dundee, [2]Ninewells Hospital Dundee DD1 9SY, UK.
University of Cambridge, [4]Department of Biochemistry, Cambridge CB2 1QW, UK.

The 5-HT_3 receptor is a ligand-gated ion channel (LGIC) and a member of the Cys-loop family of receptors. High affinity fluorescent probes can be used to study such a membrane receptor which structure is difficult to solve by X-ray diffraction method. In this work, a small library of fluorescent probes was synthesized using granisetron[1] as a core to attach diverse fluorophores. The fluorescent probes have been characterised by radioligand binding (K_i) and fluorescence spectroscopy (λ_{max} Abs, λ_{max} Em, ε and Φ_f). They were tested in live cell imaging, resulting in two high affinity probes containing fluorescein, being specific for the 5-HT_3A homomeric receptor[2] and useful for fluorescent polarization. Research for more stable fluorophores led to suitable probes for imaging studies. Our efforts to produce and isolate the 5-HT_3A receptor in micelles and in good purity were rewarded by successful fluorescence polarization experiments. Based on these results, a robust binding assay is currently being developed that could be used to determine binding affinities for compounds targeting the 5-HT_3R.



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[2] J.Simonin; S.K.V.Vernekar; A.J.Thompson; J.D. Hothersall; C.N. Connolly; S.C.R.Lummis; M.Lochner, *Bioorg. Med. Chem. Lett*., **2012**, 22, 1151.

Medicinal Chemistry Chemical Biology **MC025**

Reactions of C-centered Amino Acid Radicals with Ascorbate

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[1]Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland
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Aerobes are under constant exposure to partially reduced oxygen species (PROS). Proteins are major potential biological targets for PROS, because of their high reactivity. This causes formation of C- and O-centered radicals located on the amino acid residues of the proteins. Both radical types can propagate biological damage. Ascorbate, the principal endogenous low-molecular antioxidant, can repair tyrosine and tryptophan radicals in model compounds and in various proteins *in vitro* [1-3] and may thereby prevent reaction with O_2. It also reduces the peroxyl radicals of glycine, alanine and proline derivatives [4].

We studied the reactions of ascorbate with C-centered amino acid radicals in the model compounds α-methylalanine (α-metAla) and *N*-acetylamide derivatives of glycine (*N*-ac-Gly-amide), alanine (*N*-ac-Ala-amide) and proline (*N*-ac-Pro-amide).

The radicals of α-metAla and *N*-ac-Pro-amide were repaired by oxidizing ascorbate to the ascorbyl radical with rate constants close to 10^6 M^{-1} s^{-1}; the repair of the *N*-ac-Ala-amide radical was slower. The reaction was followed mainly at 360 nm, where ascorbyl radical has an absorption coefficient of 3300 M^{-1} cm^{-1}. In contrast, no repair of the radicals of *N*-ac-Gly-amide was observed, for which we estimated an upper limit of 10^4 M^{-1} s^{-1} for the corresponding rate constant. Our results suggest that in living tissues, ascorbate cannot inhibit the reaction of protein C-centered radicals with O_2.

[1] B.M. Hoey and J. Butler *Biochim. Biophys. Acta* **1984**, *791*, 212-218.
[2] R. Santus et al. *Free Radic. Res.* **2000**, *33*, 383-391.
[3] A.S. Domazou et al *Free Radic. Biol. Med.* **2009**, 46, 1049-1057.
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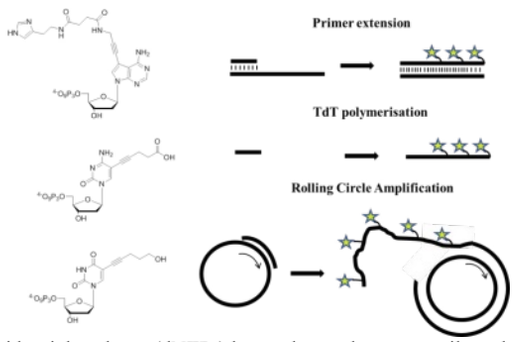
Medicinal Chemistry Chemical Biology **MC026**

Modified nucleoside triphosphates – a gateway for the generation of long chemically modified oligonucleotides

Marcel Hollenstein

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



Nucleoside triphosphates (dNTPs) have advanced as a versatile and convenient vector for supplying nucleic acids with additional chemical functionalities.[1,2] Modified dNTPs bearing side-chains that can mediate organocatalysis[3] or mimic the residues of the active site of proteases[4] are shown to be excellent substrates in rolling circle amplification and TdT-mediated polymerization, for the generation of long and heavily modified single-stranded oligonucleotides.

[1] Hocek, M., Fojta, M., *Org. Biomol. Chem*. **2008**, *6*, 2233-2241.
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[3] Hollenstein, M., *Chem. Eur. J*. **2012**, *18*, 13320-13330.
[4] Hollenstein, M., *Org. Biomol. Chem*. **2013**, submitted.

Medicinal Chemistry Chemical Biology **MC027**

Bloch Surface Wave Based Platform for Real-time Amyloid Aggregation Kinetics Investigation

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The misfolding and aggregation of specific proteins has been associated with incurable diseases such as Alzheimer's or Parkinson's disease [1]. In the specific case of Alzheimer's disease, recent studies have shown that it is the soluble oligomeric forms of aggregates appearing in the early stages of aggregation that cause cell toxicity, rather than insoluble fibrils. Research on new strategies of diagnosis is imperative to detect the disease prior to the onset of clinical symptoms [2].
Here, we propose the use of an optical method for protein aggregation kinetic studies using a Bloch surface wave (BSW) sensor made of a periodic stack of silicon oxide and silicon nitride layers [3]. The aim is to detect the early dynamic events of protein aggregation and fibrillogenesis of the amyloid-beta peptide Aβ42, which plays a central role in the onset of the Alzheimer's disease. The detection principle relies on the refractive index changes caused by the interaction of soluble oligomeric aggregates with the sensor silicon nitride surface.
We demonstrate the efficacy of the BSW sensor by monitoring in real-time the formation of the first toxic oligomeric forms of Aβ42. Furthermore, we provide new insights into the complex mechanism of aggregation of this protein system in the presence of small molecular probes able to interfere with the kinetics of amyloid formation.
As a control method, Transmission Electron Microscopy (TEM) has been used to morphologically characterize the sample during aggregation.

[1] P.T. Lansbury and H.A. Lashuel, *Nature* **2006**, *443*, 774-779.
[2] H. Hampel et al., *Nat Rev Drug Discov* **2010**, *9*, 560–574.
[3] V. Paeder et al., *Sens Act B* **2011**, *157*, 260-264.

Medicinal Chemistry Chemical Biology **MC028**

Effects of A-minor interactions in the folding and catalytic activity of DNAenzymes

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University of Berne, Department of Chemisty and Biochemistry, Freiestrasse 3, 3012 Berne, Switzerland

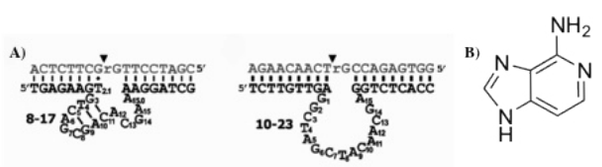

Fig. 1 A) DNAzymes 8-17 and 10-23 [3]. B) 3-deazaadenine

DNAzymes are single stranded DNA molecules, which display catalytic functions similar to that of protein enzymes[1]. The DNAzyme 8-17 and 10-23 (Fig.1 A) are two important members of artificially selected DNAzymes. To get a better insight into the mechanism and their particular folding, the adenine analogue shown in Fig. 1 B was used and incorporated in the catalytic core of both DNAzymes. All adenine bases were exchanged as single modifications with N-3-deazaadenine and the obtained modified DNAzymes were used to assess the effect of A-minor interactions on the catalytic efficiency.[2, 3]

[1] Breaker, R. R.; Joyce, G. G., A DNA enzyme that cleaves RNA. *Chem. Biol*. **1994**, 1, 223-229.
[2] Salandrian, K.; et al., Stability of DNA Containing a Structural Water Mimic in an A-T Rich Sequence. *J. Am. Chem. Soc*. **2011**, 133, (6), 1766-1768.
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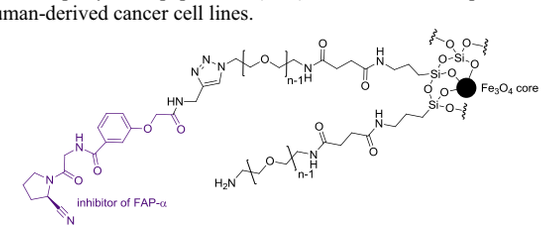
Medicinal Chemistry Chemical Biology **MC029**

A new inhibitor of extracellular prolyl-endopeptidases for nano-theranostic applications

[a]Davide Staedler, [a]Solène Passemard, [a]Guillaume Stéphane Schneiter, [a]Giona Sonego, [a]Joachim Loup, [b]Lucienne Juillerat-Jeanneret and [a]Sandrine Gerber-Lemaire

[a]EPFL, SB, ISIC, LSPN, Batochime, CH-1015 Lausanne.
[b]University Institute of Pathology, CHUV-UNIL, CH-1011 Lausanne

In the past decade, various nanotechnologies and their applications in biology have gained prominence as new options in cancer diagnosis and therapy (so called theranostic) [1]. Particularly, inorganic nanoparticles (NPs) composed by optically versatile materials, showed interesting characteristics for cancer theranostic as probes for non-invasive imaging [2]. We present herein the functionalization of iron oxide (Fe_3O_4) NPs with a competitive and irreversible prolyl-endopeptidases (PEP) inhibitor for the specific labeling of human-derived cancer cell lines.



The presented approach can be applied to others metal-based NPs, such as Bismuth ferrite ($BiFeO_3$, BFO) SHG NPs, which are optically versatile.

[1] Zuo, L.; Wei, W.; Morris, M.; Wei, J.; Gorbounov, M.; Wei, C. *Med Clin North Am* **2007,** 91, 845.
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Medicinal Chemistry Chemical Biology **MC030**

Toward New Inhibitors of *E. coli* PaPG-II adhesins: Crystallography, ITC and Modeling - Aided Drug Design

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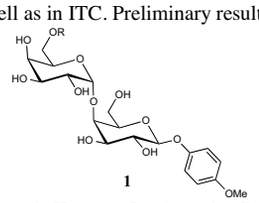
The PapG-II adhesins of *E. coli* are surface proteins involved in adhesion to host cells in the urinary tract. Their expression is highly correlated with the risk of development of pyelonephritis in humans[1], a potentially life-threatening disease. Since bacterial resistance to conventional antibiotics is rapidly increasing, there is a urgent need of new therapeutic options. In an effort to find new antagonists as antiadhesive lead structures, we critically examined all previously published structures with reported affinity for *E. coli* PapG-II adhesins[2]. However, the best antagonist, 4–methoxyphenyl α–D–Gal(1-4)β–D–Gal (**1**), exhibits only micromolar affinity[1]. We co-crystallized **1** with purified PapG-II and solved the structure of the protein-ligand complex by X-ray. Based on this information, new antagonists were rationally designed, synthesized and biologically evaluated in a polymer-based affinity assay as well as in ITC. Preliminary results are presented.



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Medicinal Chemistry Chemical Biology **MC031**

Stilbenoids from *Pholidota chinensis* as new scaffold for $GABA_A$ receptor modulators

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In a search for new natural product-derived $GABA_A$ receptor modulators, we screened a plant extract library on *Xenopus laevis* oocytes expressing recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors, by means of a two-microelectrode voltage clamp assay [1]. A dichloromethane extract of stems and roots of *Pholidota chinensis* (Orchidaceae) enhanced the GABA-induced chloride current (I_{GABA}) by 132.75% ± 36.69% at 100 µg/mL.

By means of an HPLC–based activity profiling approach [2], the three structurally related stilbenoids coelonin (**1**), batatasin III (**2**), and pholidotol D (**3**) were identified. Dihydrostilbenoid **2** enhanced I_{GABA} by 1512.19% ± 176.47% at 300 µM, with an EC_{50} of 52.51 ± 16.96 µM, while compounds **1** and **3** showed much lower activity, suggesting conformational flexibility as in **2** to be crucial for receptor modulation. This was confirmed by a study on a series of 11 commercially available stilbenoids and their corresponding semisynthetic dihydro derivatives. Dihydrostilbenoids showed higher activity in the oocyte assay than their corresponding stilbenes. The dihydro derivatives of tetramethoxy piceatannol and pterostilbene were the most active, with modulations comparable to that of compound **2**. Dihydrostilbenoids represent a new scaffold for $GABA_A$ receptor modulators.

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Medicinal Chemistry Chemical Biology **MC032**

Design and Synthesis of novel Matrixmetalloproteinase-Inhibitors[$]

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[$]2013 Dr. Max Lüthi Award of the Swiss Chemical Society

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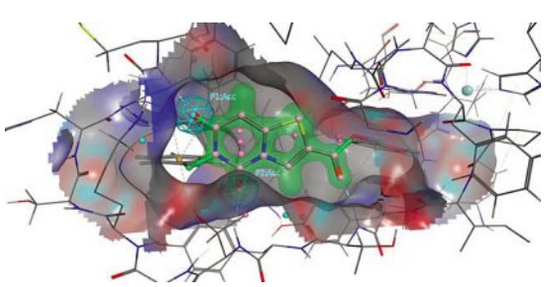
Matrixmetalloproteinase-13 (MMP-13) is a Zn^{2+}-dependent protease that catalyzes the cleavage of type II collagen, the main structural protein in articular cartilage. Excess MMP-13 activity causes cartilage degradation in osteoarthritis; hence, MMP-13 is a promising therapeutic target protein. However, due to their lack of selectivity clinical MMP inhibitors have been associated with serious side effects like the musculoskeletal syndrome.[1]



The aim of the presented study was the development of selective MMP-13 inhibitors. The potential inhibitors were designed with cheminformatics methods, subsequently synthesized and their biological activity determined.

[1] J. A. Sparano et al. *J. Clin. Oncol.* **2004**, *22*, 4683-4690.
[2] A. R. Johnson et al. *J. Biol. Chem.* **2007**, *282*, 27781-27791.

Medicinal Chemistry Chemical Biology **MC033**

Synthesis of labelled peptides to study the mechanism of a bacterial oligosaccharyltransferase

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N-linked protein glycosylation is an essential post translational protein modification in eukaryotes that is involved in many cellular processes. The enzyme oligosaccharyltransferase (OST) embedded in the ER membrane, catalyses the *en-bloc* transfer of a lipid-linked oligosaccharide (LLO) to an Asn side-chain located in the recognition sequon Asn-X-Thr/Ser (X ≠ Pro). With the first X-ray structure of a full-length bacterial OST enzyme, the PglB protein from *Campylobacter lari*, essential insights into the principles of glycosylation sequon recognition and the catalytic mechanism of OST were obtained. To study the mechanism of PglB, linear peptides labelled with 5-carboxyfluorescein were synthesized by Fmoc solid-phase peptide synthesis (SPPS). Based on the acceptor substrate sequence, linear peptides with systematic modification of the -2 (Asp), the 0 (Asn) and the +2 (Thr) position were designed. For further insight of the enzymatic mechanism, modified amino acids were included as well. Most of them were obtained by the direct modification of the amino acid side chains on solid support. In the other cases, pre-modified amino acid building blocks were introduced during the SPPS.
Using fluorescence anisotropy and gel fluorescence, these peptides allowed to quantify acceptor substrate binding affinities and glycosylation turnover rates by PglB.

[1] a) Lizak C., Gerber S., Numao S., Aebi M., Locher K.P., *Nature* **2011**, *474*, 350. b) Gerber S., Lizak C., Michaud G., Bucher M., Darbre T., Aebi M., Reymond J.L., Locher K.P., *JBC* **2013**, *288*, 8849.

Analytical Chemistry **MC034**

Monitoring intra- and inter-day human breath metabolic signatures: evidence for the existence of individual phenotypes

Pablo M-L Sinues[1], Lukas Meier[1], Christian Berchtold[1], Noriane Sievi[2], Giovanni Camen[2], Malcolm Kohler[2], Renato Zenobi[1]

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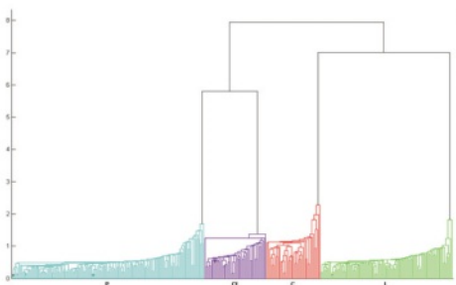
[2]University Hospital Zurich, Switzerland

A groundbreaking NMR study has suggested the existence of stable individual metabolic phenotypes in urine [1]. The analysis of breath is an attractive alternative approach to confirm the existence of such individual metabolic signatures because breath analysis is completely non-invasive.

Four subjects breathed through the counterflow inlet of a commercial mass spectrometer. The breath samples encountered an electrospray of water, whereby some exhaled compounds were ionized and readily detected. A total of 341 mass spectra collected during four days were analyzed statistically (see figure).



Dendrogram resulting from hierarchical cluster analysis of 341 breath samples donated by 4 subjects. In full agreement with [1], clustering according to donor is readily observable.

Our results support the existence of individual breath metabolic signatures.

[1] M. Assfalg *et al*, *PNAS* **2008**, *105(5)*, 1420.

Medicinal Chemistry Chemical Biology **MC035**

Protein-Ligand Affinity using Long-Lived States and Long-Lived Coherences

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During the last decades, powerful NMR techniques have emerged that can identify lead compounds for drug discovery and investigate the strength of protein-ligand interactions.
In this context, we are exploiting the peculiar properties of the so-called Long-Lived States (LLS) [1] and Long-Lived Coherences (LLC) [2] in order to increase the sensitivity of NMR for drug screening [3].
The only requirement is to have a weak ligand with an isolated AX spin system that can carry an LLS or an LLC. We have covalently attached 'spy groups' like bromothiophene (thanks to S. Passemard, A. Laguerre and S. Gerber, LSPN, EPFL) to a weak peptide ligand, in order to render LLS and LLC screening procedures applicable to arbitrary ligands. We have demostrated the possibility to perform drug screening by determining the best inhibitors for trypsin among a small library of candidates.
Currently we are exploring the possibility to use these techniques in combination with dynamic nuclear polarization, in order to enhance the signals and decrease the amounts of protein and ligands used.

[1] Carravetta M., Levitt M.H. *J Am Chem Soc* **(2004)** 126(20):6228-6229.
[2] Sarkar R., Ahuja P., Vasos P.R., Bodenhausen G. *Phys Rev Lett.* **(2010)** 104(5):053001.
[3] Salvi N., Buratto R., Bornet A., Ulzega S., Rentero Rebollo I., Angelini A., Heinis C., Bodenhausen G. *J Am Chem Soc* **(2012)** 134(27):11076-11079.

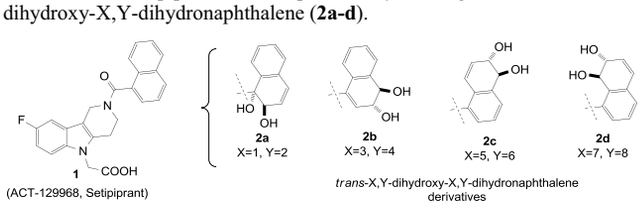
Medicinal Chemistry Chemical Biology **MC036**

Syntheses of *trans*-X,Y-dihydroxy-X,Y-dihydronaphthalene derivatives for the identification of two major metabolites of CRTh2 antagonist ACT-129968 (Setipiprant)

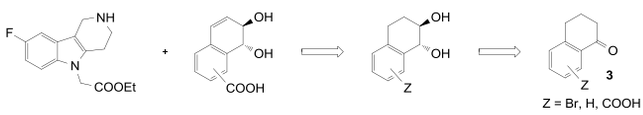
Philippe Risch, Heinz Fretz, Thomas Pfeifer, Julien Pothier

Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, 4123 Allschwil, Switzerland

According to spectral results and mechanistical hypotheses, metabolites **M7** and **M9** of Setipiprant **1** are presumably among vicinal *trans*-X,Y-dihydroxy-X,Y-dihydronaphthalene (**2a-d**).



Their syntheses start from tetralones **3** and involve an epoxydation/hydratation sequence to build the *trans*-diol moiety, a benzylic oxydation/Bamford-Stevens elimination sequence to build the olefin moiety and a lithium mediated carboxylation. The use of Jacobsen's chemistry for the intermediate epoxydation delivers enantio-enriched compounds.



Finally, the confrontation between the synthesized candidates and the biological extracts allowed for the identification of metabolites **M7** and **M9** and the assignment of their absolute configurations.

Medicinal Chemistry Chemical Biology **MC037**

Specific RNA Labeling Strategies for Fluorescence Applications

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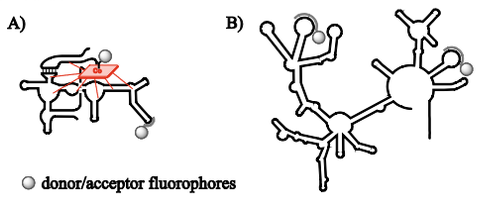
The role of RNA in living organism is manifold. For example, riboswitches and ribozymes can act as regulatory and catalytic elements. They specifically bind to metabolites and thereby undergo structural changes. In order to study the mechanism of functional RNAs, we use fluorescence techniques such as FRET (Förster Resonance Energy Transfer). We investigate the structural rearrangements of the *btuB* riboswitch of *E. coli* upon B_{12} binding (Figure A) and of the group II intron ai5γ of *S. cerevisiae* upon Mg^{2+} binding (Figure B).[1,2] As the labeling of such large RNAs is challenging, we developed novel strategies. We are currently using non-natural nucleic acids that have an extremely strong and specific binding affinity to RNA/DNA sequences. To study the process of RNA folding upon binding of the metabolite, we have also labeled the B_{12} itself with various fluorophores suitable for FRET. These strategies present the opportunity to investigate the folding mechanism, and associated ligand binding properties of functional RNAs in unprecedented detail.



[1] M. Steiner, K. S. Karunatilaka, R. K. O. Sigel, D. Rueda, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13853-13858.
[2] S. Gallo, M. Oberhuber, R. K. O. Sigel, B. Kräutler, *ChemBioChem* **2008**, *9*, 1408-1414.

Medicinal Chemistry Chemical Biology **MC038**

Enhancement of oral bioavailability of FimH antagonist via a prodrug approach

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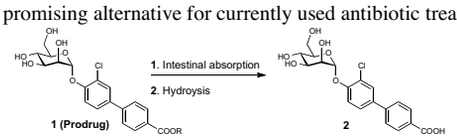
Urinary tract infection (UTI) is the most common bacterial infection worldwide. This, together with high risk of reoccurrence after initial acute infection, makes UTIs significantly problematic in both medical and economical terms. UTI is predominantly induced by uropathogenic *Escherichia coli* (UPEC). FimH is a mannose-specific adhesin located on the tip of type 1 pili, an organelle expressed by the bacteria. It mediates binding to the bladder surface and enables colonization of the host's urinary tract. FimH antagonists are promising alternative for currently used antibiotic treatments.



Our group has published one of the most potent FimH antagonist reported up-to-date (→ **2**) [1]. However, **2** suffers from poor pharmacokinetic properties. For an improvement of the PK properties a prodrug approach was envisaged [2]. Here, we present synthesis and impact of these modifications on solubility, permeability and lipohilicity.

[1] Klein, T.; Abgottspon, D.; Wittwer, M.; Rabbani, S.; Herold, J.; Jiang, X.; Kleeb, S.; Lüthi, C.; Scharenberg, M.; Bezençon, M.; Gubler, E.; Pang, L.; Smiesko, M.; Cutting, B.; Schwardt, O.; Ernst, B. *J. Med. Chem.* **2010**, 53, 8627–8641.
[2] Beaumont, K.; Webster, R.; Gardner, I.; Dack, K.;, *Curr drug Metab.* **2003**, 4, 461-485.

Medicinal Chemistry Chemical Biology **MC039**

Dendritic Antimicrobial Peptide against Pathogenic Bacteria

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University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

Many antimicrobial peptides (AMPs) act by disrupting microbial membranes.[1] The discovery of antimicrobial peptide dendrimers in which positive charges are provided by the multiple amino termini at the dendrimer periphery was carried out with a diverse 6750-membered combinatorial library[2] of 3rd generation peptide dendrimers. These sequence types are particularly resistant to proteolysis[3], and were prepared by split-and-mix solid phase peptide synthesis (SPPS) on a photolabile resin suitable for off-bead assays.[4]
Third generation peptide dendrimers with hydrophobic amino acids in their branches and multiple free amino termini as the source of positive charges possess significant antimicrobial activities against Gram positive and Gram negative bacteria. They show remarkably low haemolytic activity and act as membrane targeting agents on synthetic unilamellar vesicles assembled from *E. coli*, phosphatidylglycerol and phosphatidylcholine lipids.[5]
New approaches to develop even more potent antimicrobial peptides dendrimers against the opportunistic pathogen *P. aeruginosa* are under progress.

[1] T. Darbre, J.-L. Reymond *Org. Biomol. Chem.* **2012**, *10*, 1483-1492.
[2] N. Maillard, A. Clouet, T. Darbre, J.-L. Reymond *Nature Protoc.* **2009**, *4*, 132-142.
[3] P. Sommer, V.S. Fluxa, T. Darbre, J.-L. Reymond, J.-L. *ChemBioChem* **2009**, *10*, 1527.
[4] N. Maillard, T. Darbre, J.-L. Reymond *J. Comb. Chem.* **2009**, *11*, 667.
[5] M. Stach, N. Maillard, R. U. Kadam, D. Kalbermatter, M. Meury, M. G. P. Page, D. Fotiadis, T. Darbre, J.-L. Reymond *Med. Chem. Commun.* **2012**, *3*, 86.

Medicinal Chemistry Chemical Biology **MC040**

Bioisosteric Modifications to Extend the Antiadhesive Effect of FimH Antagonists

Lijuan Pang, Simon Kleeb, Daniela Abgottspon, Katrin Lemme, Beat Ernst*

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Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Urinary tract infections (UTIs), primarily caused by uropathogenic *E. coli* (UPEC), affect millions of people and account for significant morbidity and high medical costs. The key step in the pathogenesis of UTIs is the bacterial adhesion to urothelial cells, which is mediated by the virulence factor FimH located on type 1 pili. Blocking FimH and therefore the adhesion with FimH antagonists offers a new therapeutic approach for the prevention and treatment of UTIs. However, the antagonists developed so far have hardly met the requirements for clinical applications due to poor pharmacokinetic (PK) properties. *In vivo* studies indicated that with biphenyl α-D-mannosides as FimH antagonists, high doses were necessary to achieve the minimal concentrations required for antiadhesive effects in the bladder [1, 2]. To improve oral bioavailability and renal excretion, we chemically modified the aglycone by replacing the carboxylate with various bioisosteres. The affinity and the thermodynamic profile of these FimH antagonists were evaluated by a cell-free competitive binding assay as well as isothermal titration calorimetry (ITC). Furthermore, the PK properties were determined by different *in vitro* and *in vivo* assays. As a result, this strategy led to FimH antagonists with an excellent pharmacological profile regarding both duration and effectiveness of the treatment.

[1] Klein, T. *et al. J. Med. Chem.* **2010**, *53*, 8627.
[2] Cusumano, C. K. *et al. Sci. Transl. Med.* **2011**, *3*, 109ra115.

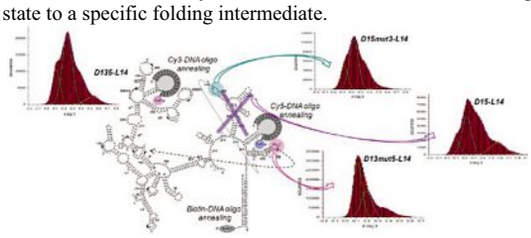
Medicinal Chemistry Chemical Biology **MC041**

A revised folding pathway of group II introns: Assigning specific structures to the individual FRET states

Erica Fiorini[1], Lucia Cardo[1,2], Danny Kowerko[1] and Roland K. O. Sigel[1]

[1]University of Zurich, Institute of Inorganic Chemistry, Winterthurerstrasse 190, Zurich, Switzerland; [2]University of Birmingham, Institute of Biomedical Research, Birmingham, B15 2TT, UK

Group II introns belong to the class of self-splicing ribozymes, are mobile genetic elements, and are found within the genes of bacteria and lower eukaryotes.[1] We designed a derivative of *S. Cerevisiae* ai5γ group II intron (~900 nts), labeled with the Cy3-Cy5 fluorophore pair and biotin for surface immobilization. This construct enables a detailed characterisation of the folding pathway by smFRET using TIRF microscopy, since it preserves the dynamics and the catalytic activity of the parent ribozyme.[2] Previous studies revealed a linear three-state folding pathway from the unfolded to the native state devoid of kinetic traps.[2] Our studies revealed an additional folding step. Furthermore we made four mutants each carrying a distinct mutation that interrupts a specific tertiary interaction, known to be involved in crucial inter-domain tertiary interactions. This enabled us to assign each FRET state to a specific folding intermediate.



[1] A.M. Pyle, *Crit. Rev. Biochem. Mol. Biol.*, **2010**, *45*, 215.
[2] M. Steiner, K.S. Karunatilaka, R.K.O. Sigel, D. Rueda, *Proc.Nat. Acad. Sci.*, **2008**, *105*, 13853.

Medicinal Chemistry Chemical Biology **MC042**

First *Cis*-Dichloro Dithiolato-Bridged Rh(III) Half-Sandwich complexes

Gajendra Gupta,[a] Benjamin S. Murray[b], Paul. J. Dyson,[b] Bruno Therrien*[,a]

[a]Institute of Chemistry, University of Neuchatel, Ave de Bellevaus 51, CH-2000 Neuchatel, Switzerland; [b]Institut des Sciences et Ingénierie Chimiques, EPFL, CH-1015 Lausanne, Switzerland

Treatment of arene Rh(III) and Ir(III) half-sandwich dichloride dimers with different thiols (HSR, R = CH_2Ph, CH_2CH_2Ph, $CH_2C_6H_4$-*p*-^{t}Bu) leads to the formation of a series of neutral and cationic thiolato bridged complexes. The molecular structures of the neutral complexes reveal that the two rhodium atoms are bridged by two thiolato ligands without any metal-metal bond and the pentamethylcyclopentadienyl and chloride ligands are *cis* to each other, an unexpected conformation for such dinuclear complexes and seems to be the first example of a *cis*-dichloro dithiolato Rh complex. The cytotoxicity of the complexes were determined in the A2780 and A2780cisR human ovarian cancer cell lines and are found to be highly cytotoxic. In fact, the Ir complexes are found to be the most active (IC_{50} < 0.025 μM), even more than the ruthenium and rhodium analogues. Interestingly, they appear to be the most active pentamethylcyclopentadienyl iridium complexes evaluated to date. Furthermore, computational studies to rationalize the formation of the *cis*-isomer as the most stable configuration are under way.

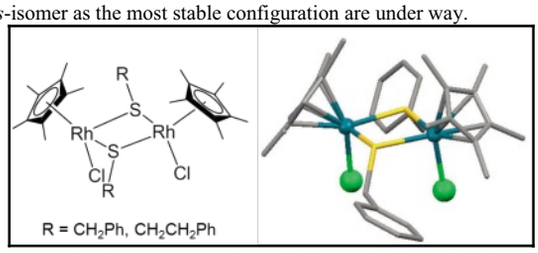


Figure 1. Molecular structure of *cis*-dichloro rhodium dithiolato-bridged complexes

Medicinal Chemistry **MC043**

Phosphorylation-dependent Molecular Switches, the PleD Protein Case

Juvenal Yosa[1], Markus Meuwly[1]

[1]University of Basel, Klingelbergstrasse 80, CH-4056 Basel

PleD protein contains an intrinsic nucleotide cyclase activity, [1] which converts two molecules of GTP into bis-(3'→5')-cyclic diGMP (c-di-GMP). c-di-GMP is a molecule of great interest which regulates surface-adhesion properties and motility in bacteria. In addition, c-di-GMP is involved in bacterial biofilm formation and persistence. Because c-di-GMP is found exclusively in bacteria makes it a potential target for medicinal applications. PleD is a homodimer protein, where each monomer contains two receivers domains; Rec1 and Rec2, Rec1 serves as a phosphoryl acceptor, whereas, Rec2 was proposed to act as an adaptor for dimerization. Also PleD contain GGDEF domain or catalytic domain which is a conserved amino acid sequence motif with dyguanilate cyclase activity (DGC). [2] The signaling c-di-GMP is synthesized from two GTP molecules in a symmetric condensation reaction. Each monomer accommodate one GTP in the catalytic domain. It has been demonstrated by cross-linking experiments that exclusively dimeric PleD is catalytically active.[3]

Because dimerization is fundamental for the enzymatic activity, and this process is coupled with the phosphoryl transfer to REC-1, here we investigate, using molecular dynamics simulations MD and bias molecular dynamics simulation BMD, the pathway whereby the two monomers are repacking, and specifically the role of the phosphorylation to form a functional protein. Molecular modeling on PleD protein can serve as a model to understand the dynamical process and the molecular mechanism in bacterial cell cycle.

[1] Jenal, U. and Malone, J., *Annu. Rev. Genet.* **2006**, *40*, 385.
[2] Wassmann W., et al. *Structure.* **2007**, *15*, 915.
[3] Paul, R., et al. *J. Biol. Chem.* **2007**, *282*, 29170.

Medicinal Chemistry Chemical Biology **MC044**

Bioactive compounds from the *Spondias tuberosa* fruits

M. L. Zeraik[1], E. F. Queiroz[2], I. Castro-Gamboa[1], M. Cuendet[2], J. -L. Wolfender[2], M. Q. Paulo[3], V. S. Bolzani[1]

[1]NuBBE, UNESP, 14800-900, Araraquara, São Paulo, Brazil. [2]School of Pharmaceutical Sciences, UNIGE, 1211, Geneva 4, Switzerland. [3]Laboratory of Natural Products, Dept. of Chemistry, UFPB, Paraiba, Brazil.

The Northeast of Brazil is a source of great variety of edible fruits used as food by the local population. In most cases, these fruits have only been poorly studied for their chemical constituents and their biological activities [1]. The purpose of this study was to evaluate the chemical composition of the methanolic pulp extract of Umbú (*Spondias tuberosa* Arr. Camara, Anacardiaceae), as well as measure its anti-aging activities. At 40 μg mL^{-1}, the extract presented high antioxidant activity in the DPPH (89%), ABTS (97%) and ORAC (64%) assays, as well as 61% inhibition of acetylcholinesterase activity. The analytical HPLC-UV condition was geometrically transferred to the preparative medium pressure chromatography (MPLC-UV) by chromatographic calculations. In a second step, MPLC-UV was used to isolate the active compounds. All of the MPLC fractions were monitored by UHPLC/TOF/MS given a 2D LC/LC plot of the MPLC separation. In a single step six compounds were isolated for the first time in this plant and a new compound derived from gallic acid was found. The structures of the isolated compounds were elucidated by classical spectroscopic methods including 2D NMR and HR-MS.

Acknowledgement: BSJRP BRAZILIAN/SWISS Joint Research Programme grant to J.L.W., E.F.Q, I.C.G., M.C. and V.S.B.

[1] da Silva AR, de Morais SM, Marques MM, de Oliveira DF, Barros CC, de Almeida RR, Vieira ÍG, Guedes MI. *Pharm. Biol.* **2012**, *50*, 740.

Medicinal Chemistry Chemical Biology **MC045**

Phytochemical investigation of the bark of *Tetrapteris mucronata* a plant use in the ayahuasca preparation

M. M. F. Queiroz[1], E. F. Queiroz[2], G. Marti[2], L. Marcourt[2], I. Castro-Ganboa[1], V. Bolzani[1], J. L. Wolfender[2]

[1]NuBBE, Department of Organic Chemistry, Institute of Chemistry, São Paulo State University (UNESP), P. O. Box 355, 14800-900, Araraquara, São Paulo, Brazil. [2]School of Pharmaceutical Sciences, University of Geneva, 1211, Geneva 4, Switzerland.

Tetrapteris mucronata Cav. (Malpighiaceae) is a plant used in some regions of Brazil in ayahuasca preparation a psychotropic plant decoction [1]. To assess the phytochemical composition of *T. mucronata* and understand its traditional use, the aqueous and methanolic extracts were investigated. Their HPLC-PDA-ESI-MS profiles have been determined. The isolation of the main compounds of the methanolic extract was performed by a direct transfer of analytical HPLC conditions to medium pressure liquid chromatography (MPLC). This resulted in the isolation of six alkaloids and one new phenanthrene derivative. Since tryptamine and β-carboline alcaloids are known to have powerful hallucinogenic activities, an HPLC-ESI-MS/MS method has been developed for their quantification in *T. mucronata* water and methanolic extracts. These results provide a rational support for the traditional use of *T. mucronata* stem bark in ayahuasca preparations, since β-carboline alkaloids are present in a relevant amount.

[1] Ott, J., *Ayahuasca analogues : Pangœan entheogens*. 1st ed.; Natural Products Co.: Kennewick, WA, **1994**; p 127.

Medicinal Chemistry Chemical Biology **MC046**

Isolation of new antifungal compounds from *Swartzia simplex*

Q. Favre-Godal[1], E.F. Queiroz[1], L. Marcourt[1], M.P. Gupta[2], J.-L.Wolfender[1]

[1]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland. [2]Center for Pharmacognostic Research on Panamanian Flora - CIFLORPAN, University of Panama, Apartado 10767, Estafeta Universitaria, Panama city, Panama.

The dichloromethane extract of the root bark of *Swartzia simplex* (Sw.) Spreng (Fabaceae) presented an interesting antifungal activity against *Candida albicans* in a bioautography assay [1,2]. In order to isolate the active compounds, bioguided isolation was undertaken using HPLC-microfractionation in 96 well plates and bioautography to localize the active compounds in the HPLC-PAD metabolite profiling of the crude extract. The analytical HPLC-PAD conditions were geometrically transferred to a preparative medium pressure chromatography (MPLC-UV) by chromatographic calculations for the efficient isolation of the active compounds at the milligram scale in one step. Using this approach ten compounds were isolated, six of them are new natural products. The structures of the isolated compounds were elucidated by classical spectroscopic methods including UV, 2D NMR and HR-MS.

Acknowledgements: SNF grant CR2313-143733 to J.L.W. and E.F.Q.

[1] Q. Favre-Godal, E.F. Queiroz, D. Sanglard, J.-L.Wolfender. Planta Med 2012; 78-PD159.
[2] Q. Favre-Godal, E.F. Queiroz, J.-L.Wolfender. JOAC 2013; in press.

Medicinal Chemistry Chemical Biology **MC047**

Vitis vinifera* canes a new source of antifungal compounds against *Plasmopara viticola, Erysiphe necator* and *Botrytis cinerea

S. Schnee[1], E.F. Queiroz[1], L. Marcourt[1], F. Voinesco[1], L. Marcourt[2], P.-H. Dubuis[1], J.-L. Wolfender[2], K. Gindro[1]

[1]Swiss Federal Research Station Agroscope Changins Wädenswil ACW, Route de Duiller 50, P.O. Box 1012, 1260 Nyon, Switzerland.[2]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland.

Worldwide the vast majority of grapevine areas are planted with *Vitis vinifera* cultivars that are all susceptible to various fungal diseases such as downy (*Plasmopara viticola*) and powdery (*Erysiphe necator*) mildews and grey mould (*Botrytis cierea*). Since *V. vinifera* canes represent an unexploited agronomical waste material, their biological activity against the major grapevine pathogens and their chemical content were investigated in order to determine if extracts or compounds could be potentially used in a sustainable manner to protect grapevines. Methanolic and ethanolic crude extracts of *Vitis vinifera* canes exhibited significant antifungal activity against the three major fungal pathogens of grapevine: *P. viticola, E. necator* and *B. cinerea*. The active extract was analysed by LC-PDA-ESI-MS and some compounds were dereplicated. Efficient targeted isolation using medium pressure chromatography (MPLC-UV) afforded in one step six pure constituents. The structures of the isolated compounds were elucidated by 2D NMR and HR-MS. Six identified compounds presented interesting antifungal activities against *P. viticola*

Medicinal Chemistry Chemical Biology **MC048**

Synthesis, structure determination and biological applications of bicyclic bridged peptides

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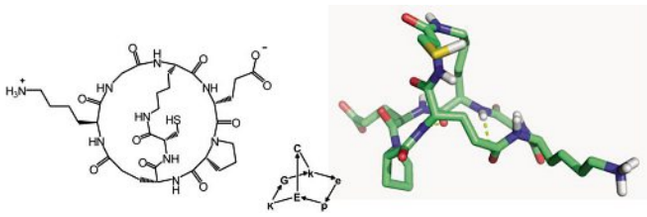
Linear peptides are poor drug candidates due to their low bioavailability and rapid proteolysis. These limitations can be overcome by rigidifying their structure through head-to-tail or side chain-involving cyclizations. The use of multiple branching amino acids in a peptide sequence, like diamino acids (as used in peptide dendrimers[1]) or amino diacids, allows to synthesize peptides resembling polycyclic alkanes using an orthogonal protection scheme.



These peptides are structurally well-defined and cover an almost pristine area of peptide topological space[2]. Their conformational rigidity was investigated by means of 2D-NMR and may offer a platform to design drugs tackling protein-protein interactions. Moreover, by a combination of appropriate amino acids, we develop cell penetrating bicyclic peptides for drug carrier design and cellular protein targeting.

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Medicinal Chemistry Chemical Biology **MC049**

"Bridging the Gap" - Adjusting the Acid Pharmacophore in sialyl Lewis^x Mimics by Ring Closing Metathesis

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The selectins play a key role in the body's defense mechanism against inflammation.[1] They form a class of three cell adhesion molecules (E-, P-, and L-selectin), which, in case of an inflammatory stimulus, are responsible for the initial steps of the inflammatory response, that is, the tethering and rolling of leukocytes on the endothelial surface of blood vessels. These steps are a prerequisite for the subsequent firm adhesion and the final extravasation of leukocytes to the site of the inflammatory stimulus. However, excessive infiltration of leukocytes into the adjacent tissue can lead to acute and chronic reactions, as observed in reperfusion injuries, stroke or rheumatoid arthritis.[2] Therefore, the antagonism of selectins is regarded as a valuable pharmaceutical option.

For the development of efficient antagonists, the low binding affinity, high polarity and structural complexity of the physiological selectin ligand sialyl Lewis^x (sLe^x) are challenges to be accomplished. In the past, we identified high-affinity sLe^x mimetics by stabilizing the Le^x-core structure.[3] Based on the structure of E-selectin co-crystallized with E-selectin antagonists,[4] different approaches with the aim to stabilize the orientation of the carboxylate of the Neu5Ac moiety are explored.

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[2] B. Ernst, J.L. Magnani, *Nat. Rev. Drug Discov.* **2009**, *8*, 661-677.
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[4] Preston Roland, *Abstract-SCS Fall Meeting Lausanne*. **2013**.

Medicinal Chemistry Chemical Biology **MC050**

Research on antagonists of Siglec-8

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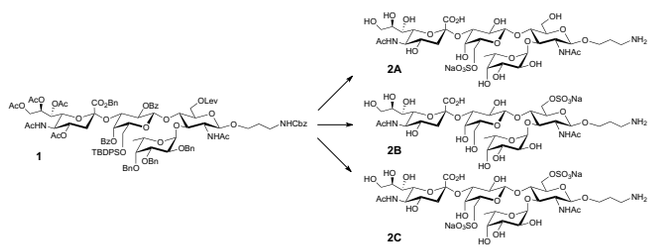
Siglecs (sialic acid immunoglobulin-like lectins) are members of the immunoglobulin gene family that contain sialoside binding N-terminal domains.[1] They are cell surface proteins found predominantly on cells of the immune system. Among them, Siglec-8 is uniquely expressed by human eosinophils and mast cells, as well as basophils. Targeting Siglec-8 with glcomimetics may thus provide a means to specifically inhibit or deplete these cell types and be ideally suited for treatment of eosinophil and mast cell-related diseases, such as asthma or chronic rhinosinusitis.[2]
To study the interactions between Siglec-8 and its ligands, three sulfated sLe^x, **2A**, **2B** and **2C** are synthesized starting from the common intermediate **1**.



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Medicinal Chemistry Chemical Biology **MC051**

Synthesis and Evaluation of Novel Cannabinoid Type 2 Receptor Tracers for PET Imaging

Roger Slavik[1], Daniel Bieri[1], Uwe Grether[2], Stjepko Čermak[1], Stefanie D. Krämer[1], Adrienne Müller[1], Luca Gobbi[2], Markus Weber[1], Roger Schibli[1], Simon M. Ametamey[1], Linjing Mu[1].

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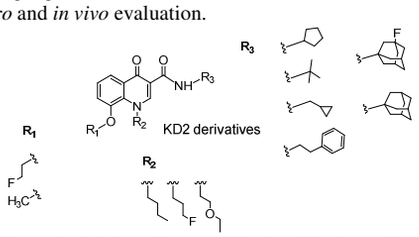
The cannabinoid receptor type 2 (CB2) has very low expression level in brain tissue under basal conditions, but it is up regulated in diverse pathological conditions. To develop a brain PET tracer towards CB2, we evaluated the potential of a promising 4-oxo-quinoline lead structure from literature [1] - designated KD2 - as an imaging agent for CB2 sites. KD2 has been synthesized and labeled with an ^{11}C-isotope. Preliminary *in vitro* / *in vivo* studies showed that [^{11}C]KD2 is a promising PET tracer for CB2. Within an optimization program with focus on lowering lipophilicity and plasma protein binding, several new KD2 derivatives were synthesized and their binding affinities towards hCB1/2 were determined. *K*i values for the CB2 receptor ranged from 0.7 – 1220 nM, with a selectivity hCB2 over hCB1 from 10 to >10'000. Furthermore, various novel CB2 ligands from a novel structural class were synthesized and tested in functional and binding assays. The most promising ligands will be radiolabeled with ^{11}C or ^{18}F isotopes for further *in vitro* and *in vivo* evaluation.



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Medicinal Chemistry Chemical Biology **MC052**

Identification and analysis of the putative AMP-activated protein kinase (AMPK) in blood stream form of *Trypanosoma brucei*

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AMP-activated protein kinase (AMPK) is a heterotrimeric complex well conserved in all eukaryotic which acts as regulatory sensor of the energy status of the cells. Its role is based mostly on sensing the AMP/ATP ratio of the cells and to restore the adenine nucleotide homeostasis [1]. In the blood stream form of *Trypanosoma brucei*, the parasite responsible for the Human African Trypanosomiasis or sleeping sickness, the AMPK complex has neither been completely identified nor characterized to date.

Previous investigations on the procyclic form of *T. brucei* allowed the identification of the β and γ subunits of TbAMPK [2]. It is the aim of this project to identify the unknown α subunit in the blood stream form of *T. brucei* via genetic modification by homologous recombination to replace the β or γ subunit by a tagged variant, followed by subsequent isolation of the complex by affinity purification. After the identification of the α subunit the role of the TbAMPK complex, but also of the identified subunits alone, will be investigated with respect to cell viability and cell metabolism using genetic an biochemical tools.

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Medicinal Chemistry Chemical Biology **MC053**

Highly potent and selective MMP-13 inhibitors: A straight forward design approach

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Matrix Metalloprotease-13 (MMP-13) is one of the enzymes belonging to the zinc-depending endopeptidase family and is involved in angiogenesis as well as in tissue remodeling. An over activity of MMPs can lead to various pathological processes such as rheumatoid arthritis or tumor growth and metastasis [1].
In our present study, we have discovered novel MMP-13 Inhibitors with nanomolar potency, as well as an attractive selectivity profile against a set of antitargets within the same enzyme family.
Starting from the co-crystal structure PDB 2OW9 [2], we designed a novel scaffold by maintaining the key attractive interactions to the protein backbone. Further, we targeted the loop which is buried deep in the S1' pocket and is referred to as the selectivity loop which furnished potent and selective MMP-13 inhibitors.

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Medicinal Chemistry Chemical Biology **MC054**

Synthesis of Cell Penetrating Peptide-Peptide Nucleic Acid (CPP-PNA) Conjugates and Evaluation of their Biological Performance

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PNAs are oligonucleotide analogues in which the sugar-phosphate backbone has been replaced by peptide backbone formed from *N*-(2-aminoethyl)-glycine units linked by amide bonds (Fig. 1A). PNA can bind to a complementary mRNA and disturb protein expression via steric hindrance. We conjugated a PNA to 15 different CPPs to the increase cellular uptake and antisense activity (Fig. 1B) [1, 2]. Their transfection efficiencies were evaluated on colon cancer cells (HT-29) stably expressing luciferase by measuring the expression of the latter. We identified 3 CPP conjugates that displayed a dose-dependent luciferase inhibition at concentrations that were not cytotoxic. These bio-reactive CPP-PNA conjugates with well-defined structure will be further modified for colon-specific delivery. This work was financially supported by Gebert Rüf Stiftung (GRS-041/11).

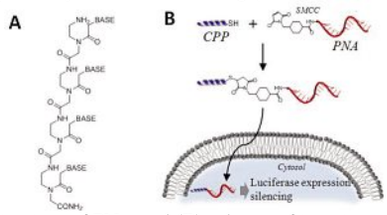

Figure 1. (A) Structure of PNA and (B) scheme of target gene silencing with PNA conjugated to CPPs.

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[2] S.H. Lee, B. Castagner, J.C. Leroux, *Eur. J. Pharm. Biopharm.* **2013**, DOI: 10.1016/j.ejpb.2013.03.021.

Medicinal Chemistry Chemical Biology **MC055**

Synthesis of novel fluorescent agonists, with selectivity for the A_1 adenosine receptor

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[2]School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK.

The adenosine receptors are members of the GPCR family and there are four sub-types: A_1, A_{2A}, A_{2B} and A_3. The A_1 adenosine receptor is involved in a range of processes in the CNS, including epilepsy and ischaemia. Despite its important role, relatively little is known about the trafficking of this receptor in neurons.[1] An agonist conjugated to a fluorophore may provide insight.

In collaboration with Professor Bruno Frenguelli at the University of Warwick we have started to design and synthesise selective fluorescent compounds. These are derived from known A_1 adenosine receptor agonists and the strategy is to attach a linker and fluorophore *via* a cyclic component at the 6 position of the adenine. In the initial stages of this project we have prepared a number of novel intermediates, with interesting activity at the A_1 adenosine receptor (Figure 1). The initial biological results and preparation of fluorescent targets will be presented.

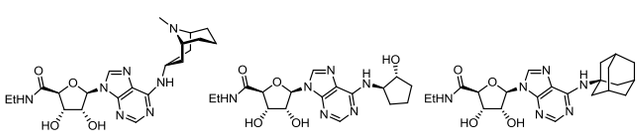

Figure 1 Novel agonists for the A_1 adenosine receptor.

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Medicinal Chemistry Chemical Biology **MC056**

Towards the site-specific modification of the hERG K^+ channel

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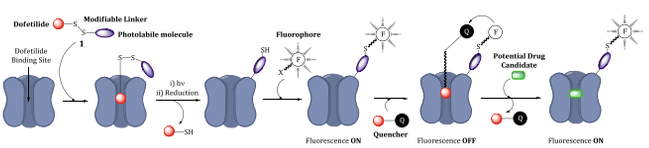
[2]Department of Clinical Research
University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

The ***h***uman ***E***ther-a-go-go ***R***elated ***G***ene (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. In consequence, every drug candidate has to be tested in order to avoid LQT side effects. The existing assays, including electrophysical patch clamp assay and radioligand-based competition binding assays, have some disadvantages and are not suitable for high-throughput screening[1]. The ultimate goal of this project is to covalently attach a small fluorophore to the hERG channel using photoaffinity probes **1** based on dofetilide, the ligand which is known to bind to the channel with high affinity[2]. Modifiable linkers will allow further post-photoaffinity labeling modification of the hERG channel in order to introduce a small fluorophore near the channel which would provide a means for sensitive and rapid detection of potential channel blockers.



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[2] G.J. Diaz et al., *J. Pharmacol. Toxicol. Methods.*, **2004**, *50*, 187.

Medicinal Chemistry Chemical Biology **MC057**

Functionalized Oligoprolines As Multivalent Scaffolds in Tumor Targeting

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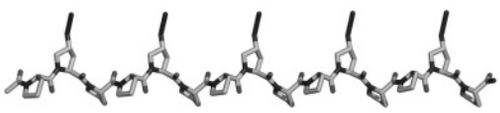
Azidoproline containing oligoprolines are conformationally well-defined, helical molecular scaffolds that can be functionalized for example by copper-catalyzed azide-alkyne cycloaddition (CuAAC) or Staudinger reduction and subsequent acylation.[1] The structural integrity of this scaffold allows for conjugating targeting vectors in defined distances towards each other.



Scheme 1 Azidoproline functionalized Oligoproline

Recent studies on radiolabeled oligoproline-bombesin conjugates that target the gastrin-releasing peptide receptor (GRP-R) showed *in vitro* and *in vivo* superior internalization compared to established monovalent ligands.[2] We are currently expanding this concept to the integrin-ligand c(RGDyK) as well as to [Tyr^3]Octreotide derived ligands.[3]

[1] (a) Y.A. Nagel, M. Kuemin, H. Wennemers, *CHIMIA*. **2011,** *65,* 264. (b) M. Kuemin, L.-S. Sonntag, H. Wennemers *J. Am. Chem. Soc*. **2007,** *129,* 466.
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[3] J.C. Reubi, *Endocrine Rev*. **2003,** *24,* 389.

Medicinal Chemistry Chemical Biology **MC058**

Expression and purification of calcium-dependent protein kinase 2, a potential target of triclosan in *Plasmodium falciparum*

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[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211, Geneva

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Triclosan (TCL) is a biocide able to kill *in vitro* cultures of *Plasmodium falciparum* with an EC_{50} of less than 1 μM [1]. Despite enoyl-ACP reductase (FabI) has long been thought to be its primary target, there is strong evidence for TCL to act through a multi-target mechanism [2, 3]. By means of computational methods, we recently identified calcium-dependent protein kinase 2 (CDPK2) as a target of TCL in *P. falciparum*.
CDPKs are a group of kinases involved in many cellular processes of the parasite. PfCDPK2 is considered to be essential, but its function is not yet known. We thus expressed and purified to homogeneity this enzyme in its non-phosphorylated form suitable for further biochemical characterization. TCL is a non-competitive inhibitor of PfCDPK2 with an IC_{50} of 48 μM. A small library of TCL derivatives was subsequently used to perform a structure-activity relationship (SAR) study, showing that the B-ring could be modified in order to improve activity.
This study represents a first step towards the understanding of the antiplasmodial effect of TCL in *P. falciparum* and a starting point for the development of new and potent CDPK2 inhibitors.

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Medicinal Chemistry Chemical Biology **MC059**

First insight into the structure and metal-ion binding sites of the human CPEB3 ribozyme

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The CPEB3 ribozyme is a highly conserved, self cleaving RNA located in the second intron of the *cpeb3* gene [1] in mammals. Most of the available knowledge about this ribozyme is based on comparative studies with the structurally and biochemically similar HDV (Hepatitis Delta Virus) ribozyme; both can be folded in the same overall pseudoknot structure [1,2] and show strong parallels in their catalysis and metal-ion requirements [1,3].
In this work, the solution structure of a well-conserved motif of the CPEB3 ribozyme, the P4 region, is determined by NMR spectroscopy, and the binding sites and structural impact of Mg^{2+} and $[Co(NH_3)_6]^{3+}$ ions are discussed. The detailed knowledge about the P4 structure and metal ion-binding preferences thus brings us closer to understanding the CPEB3 ribozyme's function in the cell. First information on metal-ion binding sites of the full-length ribozyme is provided by the results of terbium cleavage experiments of the native CPEB3. NMR studies of a segmented, partially labeled construct are also discussed.

Financial support by the Swiss National Science Foundation and from a Sciex post doctoral grant (Grant No. 11.156) to MRZ is gratefully acknowledged.

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Medicinal Chemistry Chemical Biology **MC060**

Synthesis and Biological Activity of Analogs of the Microtubule-Stabilizing Natural Product Zampanolide

M. Jordi, D. Zurwerra, F. Glaus, L. Betschart, J. Schuster, J. Gertsch, W. Ganci, K.-H. Altmann

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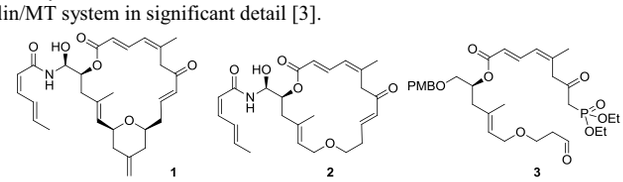
Zampanolide (**1**) is a polyketide-based macrolide that was first isolated in 1996 by Tanaka and co-workers from the marine sponge *Fasciospongia rimosa* [1]. The compound exhibits potent *in vitro* antiproliferative activity against different human cancer cell lines and was discovered recently to be a new microtubule (MT)-stabilizing agent (MSA) [2]. We have completed a total synthesis of **1** and we have characterized its interactions with the tubulin/MT system in significant detail [3].



1 **2** **3**

This presentation will discuss the synthesis of a series of zampanolide analogs and their activity against a panel of human cancer cell lines. This includes desTHP-zampanolide (**2**), which was obtained *via* a selective intramolecular HWE reaction with phosphonate/aldehyde **3** as the ring-closing step. Intriguingly, **2** retains profound antiproliferative activity. Building on the chemistry developed for the synthesis of **2** we have prepared a series of side chain-modified variants of **2** which will also be discussed here.

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Medicinal Chemistry Chemical Biology **MC061**

Stereochemical control of phosphorothioate linkages in RNA synthesis controlled by the activator

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[2] Westfälische Wilhelms-Universität, Corrensstraße 40, 48149 Münster, Germany

The routine solid-phase synthesis of oligoribonucleotides (ORN), having a fully phosphorothioated (PS) backbone, yields 2^{n-1} different diastereoisomers (ds), where n = number of nucleotides. We have performed a detailed study of the coupling mechanism and showed the influence of different activators on the ds-ratio. Specifically, we analyzed the ds-ratios of all possible 16 PS-dinucleotide motifs of RNA to give a picture of Rp- to Sp- distribution. This dinucleotide model was extended to the prediction of Rp-/Sp-ratios of an octanucleotide containing a single PS-linkage. Since Rp-/Sp-diastereoisomers have different chemical and biological properties e. g. affinity, activity and stability [1][2], this dinucleotide model represents a means to predictably modulate the ratios of Rp- and Sp-linkages and therefore their biological properties. Several commonly-used activators were tested in the dinucleotide model. Thermal denaturation experiments confirmed the results of switching the Rp-/Sp-ratios upon the destabilizing effects of sulfur introduction into PS-ORN/ORN duplexes.

[1] Maria Koziolkiewicz et al., *Antisense & Nucleic Acid Drug Development.* **1997**, 7, 43-48
[2] Agnieszka Krakowiak et al., *Nucleosides & Nucleotides*, **1998**, 17, 1823-1834

Medicinal Chemistry Chemical Biology **MC062**

Influence of metal ions on RNA structure as revealed by X-ray crystallography

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Due to the polyanionic nature of RNA, the principles of charge neutralization and electrostatic condensation require that cations help to overwhelm the repulsive forces, in order for RNA to adopt a three-dimensional structure [1, 2]. A detailed structural knowledge of RNA-metal ion interaction is thus crucial to understand the underlying mechanism of metal ions in the catalytic activity of ribozymes or regulatory functions in riboswitches [1, 3].
In our study we use an octameric RNA as model system to see a pattern in coordination of various metal ions to specific binding sites and to understand the interactions between metal ions and RNA.
New technical approaches of the synchrotron radiation facility at the Swiss Light Source (SLS) helps to obtain high-resolution data that is required for a detailed structural analysis.

Financial support by the ERC, the Swiss National Science Foundation and the University of Zurich is gratefully acknowledged.

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Medicinal Chemistry Chemical Biology **MC063**

Human RNA G-quadruplex Structures as Potential Anticancer Targets

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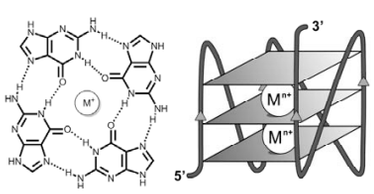
Guanine-rich nucleic acids fold into non-canonical helical structures known as G-quadruplexes (GQ), stabilized by central metal ions [1]. Human RNA GQs are relevant as potential antitumor targets. Therefore, we study the GQ in the mRNA of the NRAS gene (neuroblastoma RAS viral oncogene homolog), shown to inhibit protein synthesis *in vitro* [2], and the TERRA GQ (telomeric repeat-containing RNA), involved in telomere maintenance [3].



Currently, we are systematically assessing the stabilization of RNA GQs in the presence of monovalent and divalent metal cations by means of circular dichroism, UV melting and NMR. NRAS GQ formation and dynamics are being studied by single molecule FRET (Förster Resonance Energy Transfer) spectroscopy.

Financial support from the SBFI (COST Action CM1105), ERC (R.K.O.S.), SNF (D.D. and R.K.O.S.) and University of Zurich is gratefully acknowledged.

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MC064

Medicinal Chemistry Chemical Biology

Measurement of the tissue oxygenation *in vivo* by time-resolved luminescence spectroscopy of Ru(Phen), a poorly photosensitizing probe

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Measuring the tissue oxygen concentration (pO_2) *in vivo* is of interest for numerous fundamental and applied studies. One minimally-invasive approach to assess pO_2 is based on the measurement of the oxygen-dependent luminescence lifetime of molecular probes. The relation between the partial pressure of oxygen and this lifetime is governed by the Stern-Volmer equation. Unfortunately, virtually all oxygen-sensitive probes based on this principle induce some degree of phototoxicity [1]. Thus, we assessed the phototoxicity (wavelength: 470±20 nm; light dose: 2.5-20 J/cm^2; drug dose: 1-20 mg/kg; drug-light interval: 1min) and oxygen sensitivity of dichlorotris (1,10-phenantroline) Ruthenium (II) hydrate (Ru(Phen)) in the chicken embryo chorioallantoic membrane model (CAM). Luminescence lifetimes have been measured with a dedicated time-resolved spectrometer [2]. We demonstrated that, after intravenous injection, Ru(Phen) presents an easily detectable, pO_2-dependent, luminescence lifetime with "small" drug and light doses (1 mg/kg and 120 mJ/cm^2 @ 470 nm). With a fluence rate of 65 mW/cm^2, the phototoxic threshold was found to be at 10 J/cm^2 with the same wavelength and drug dose.

[1] Stepinac TK, Chamot SR, Rungger-Brandle E et al., *Invest Ophthalmol Vis Sci*. **2005**, *46*, 956-966.
[2] Piffaretti F, Santhakumar K, Forte E et al., *J Biomed Opt*. **2011**, *16*, 037005.

Medicinal Chemistry Chemical Biology **MC065**

Sequential combination of axitinib with photodynamic therapy delays tumor growth in preclinical model of ovarian carcinoma

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Vaso-occlusive photodynamic therapy (PDT) is considered as a promising treatment for certain types of skin and lung cancer. However, the PDT-induced microvascular damages result in tissue hypoxia, activating the hypoxia-inducible factor-1 (HIF-1) that promotes VEGF-mediated angiogenic responses and attenuates the overall PDT efficacy. As recent strategies have clearly demonstrated increased therapeutic effectiveness of combining anti-VEGF drugs with PDT, we combined Visudyne®-PDT with axitinib, a potent small tyrosine kinase inhibitor (TKI) that specifically targets the VEGF receptors 1, 2 and 3, in order to improve the clinical outcomes of the photodynamic treatment in cancer patients.
In this preclinical study in the chorioallantoic membrane (CAM) of the chicken embryo, we optimized the drug and light parameters, as well as the relative schedules of the therapies. On the physiological CAM vasculature, injecting low doses of axitinib 4 hours before PDT prolonged the vaso-occlusive effect of the treatment in a statistically significant manner. In addition, treatment of A2780 ovarian tumors grafted on the CAM with axitinib caused increased intra-tumoral oxygenation, as measured between 24 and 54 hours after drug injection. This so called “normalization window” was used to improve the vaso-occlusive PDT outcome. Combination of low dose PDT (2.5 J/cm^2 at 420 nm) with axitinib applied in various schedules resulted in synergistic tumor growth suppression.

Medicinal Chemistry Chemical Biology **MC066**

Arene-linked dinuclear RAPTA analogues

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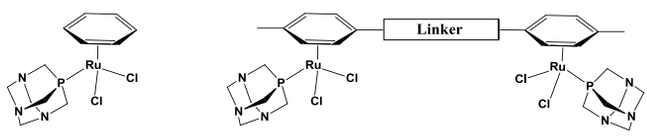
Organometallic "piano-stool" Ruthenium arene complexes are a family of promising drug compounds which exhibit antitumoural and antimetastatic properties [1, 2]. One series of these, developed in the Dyson laboratory, are the RAPTA compounds [Ru(η^6-arene)Cl$_2$(pta)] which exhibit high anti-metastatic activity combined with excellent clearance rates and low general toxicity.



One attractive feature of the RAPTA structure is the manner in which each ligand (arene, PTA, chlorides) may be modulated and elaborated upon in order to confer greater biological activity on the resultant complex [1].

In this work we present the first examples of RAPTA compounds linked via organic tethers, through the arene ligand, to yield complexes of significantly enhanced activity relative to mononuclear analogues. A series of stereoisomeric dinuclear RAPTA compounds will be introduced and the impact of the stereochemistry of the linker on the biological activity of the dinuclear RAPTA complexes will be discussed.

[1] Ang, W. H., Casini, A., Sava, G., Dyson, P. J., *J. Organomet. Chem.*, 2011, 696(5), 989-998.
[2] Bruijnincx, P. C. A., Sadler. P. J., *Adv. Inorg. Chem.*, 2009, 61, 1-62.

Medicinal Chemistry Chemical Biology **MC067**

Amino Acid Sequence Variation in a Multivalent Glycopeptide Dendrimer targeting LecA inhibiting *Pseudomonas aeruginosa* Biofilms

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The spread of antibiotic resistant bacteria is one of the most pressing problems in human health today. The gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic human pathogen causing lethal airways infections. It can form surface-attached biofilms protecting itself from classical antibiotic treatment. Biofilm formation is mediated in part by the galactose-specific lectin LecA (PA-IL) and the fucose-specific lectin LecB (PA-IIL).[1,2] Capitalizing on the well-known cluster effect observed on binding of multivalent carbohydrates to lectins, we have reported the first cases of *P. aeruginosa* biofilm inhibition with multivalent lectin inhibitors, including the galactosylated glycopeptide dendrimer **GalAG2** (βGal-KPL)$_4$(*K*FKI)$_2$ *K*HI-NH$_2$ targeting LecA.[3] Herein we report the lectin binding, biofilm inhibition and dispersal by an Ala scan series of **GalAG2** in order to determine the influence of the peptidic sequence. Some galactosyl peptide dendrimers with variations in the terminal tripeptidic sequence were also evaluated.

[1] V. E. Wagner and B. H. Iglewski, *Clinic Allerg Immunol* **2008**, *35*, 124
[2] Tielker *et. al.*, *Microbiology* **2005**, *151*, 1313
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Medicinal Chemistry Chemical Biology **MC068**

Synthesis, biochemical and structural characterisation of novel thymine derivatives as reporter probes for HSV1-tk gene expression imaging with positron emission tomography

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HSV1-TK as a reporter probe is the most studied system for the visualization of gene expression *in vivo*. C-6-alkylated pyrimidine derivatives are good substrates of HSV1-TK and exhibit no cytotoxic effects [1], thus several C-6-substituted analogs were synthesized and biochemically characterized. Their phosphorylation pattern was monitored in presence of HSV1-TK or hTK using a protocol based on HPLC-UV/DAD [2]. Synthesized compounds were phosphorylated in presence of HSV1-TK but not in presence of hTK. For the first time diphosphorylation of one non-natural substrate by HSV1-TK was observed. The crystal structures of HSV1-TK in complex with the compounds were solved by molecular replacement using the data collected at the SLS synchrotron. The refined structures unequivocally show the detailed binding mode of the compounds inside HSV1-TK. The proliferation of HSV1-TK transduced HEK293-cells was selectively inhibited by one of the compounds with an IC$_{50}$ value comparable to the one of ganciclovir. We have been using PK15 NTD cell lines expressing human equilibrative and concentrative nucleoside transporters according to the published protocol [3] to assess compounds transportability. The results of these experiments that are in progress will be shown. Concluding the presented results indicate that one of the synthesized compounds has a potential to be a new PET reporter probe and future studies will be focused on it.

[1] U.Müller, et al., *J. Nucl. Med. Bio*. **2011**, *39*, 256.
[2] P. Schelling et al., *J. Bio. Chem*. **2004**, *279*, 32832.
[3] J.L. Ward et al., *J. Bio. Chem*. **2000**, *275*, 8380.

Medicinal Chemistry Chemical Biology **MC069**

Interaction of platinum anticancer drugs and RNA

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The interaction of platinum drugs with targets other than DNA could be responsible for the toxic side effects or could play some still unrevealed role in their anticancer activity. It has already been reported that some RNA dependent activities are inhibited upon administration of platinum drugs, but little is known on the effects of platinum on RNA biology [1]. Moreover, information on RNA structural changes upon platinum binding is still very scarce. RNA structure is much more complicated than DNA and interaction with platinum drugs may lead to a different scenario than the one observed with DNA-platinum interaction.
In order to understand the effect of platinum drugs on RNA structure and activity we use as a model RNA a 27 nucleotide long hairpin derived from the mitochondrial group II intron ribozyme *Sc*.ai5γ [2]. The chosen RNA contains structural features widespread in RNAs and its NMR structure in solution is known [3], making easier a detailed investigation of the structural changes upon platinum drug interaction. We are now optimizing the reaction conditions with both cisplatin and oxaliplatin. The platinated adducts will be characterized by different techniques like mass spectrometry, circular dichroism and UV thermal melting studies as well as NMR spectroscopy, in order to evaluate the structural preferences of the binding.

Financial support by the Swiss National Science Foundation (Ambizione fellowship PZ00P2_136726 to DD), by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged.

[1] E.G. Chapman, V. De Rose, *J. Am. Chem. Soc.*, **2010**, *132*, 1946
[2] R.K.O Sigel, *Eur. J. Inorg. Chem*., **2005**, *12*, 2281
[3] D. Donghi, M. Pechlaner, R.K.O Sigel, unpublished

Medicinal Chemistry Chemical Biology **MC070**

Anti-Cancer Effect of Photodynamic Therapy Enhanced through the Combination with Low-Dose, Angiostatic Kinase Inhibitors

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Photodynamic therapy (PDT) is used as an effective clinical treatment for a number of different cancers; however, secondary tissue reactions to PDT such as hypoxia and inflammation have pro-angiogenic side effects which may counteract the desired angio-occlusive effects of PDT. The combination of PDT with clinically used anti-angiogenic drugs may provide improved anti-tumor outcome. We tested the effect of three clinically approved angiostatic tyrosine kinase inhibitors (TKIs) (sunitinib, sorafenib and axitinib) and the VEGF neutralizing anti-body based compound bevacizumab in two tumor models for the improvement of PDT outcome. The strongest anti-cancer effects were seen for the combination of PDT and low dose axitinib or sorafenib which indicated synergistic potential. Real-time quantitative PCR indicated the suppression of VEGFR-2 expression in the vasculature of tumors treated with the combination therapy of PDT and axitinib. Pre-administration of angiostatic compounds did not improve PDT outcome and increased oxygenation in axitinib treated tumors with was not identified. These result indicate that improved therapeutic outcome in combination treated tumors was not due to a vascular normalization effect. The current data imply that there is future for certain anti-angiogenic agents to further improve the efficacy of photodynamic anti-cancer therapy.

Medicinal Chemistry Chemical Biology **MC071**

Compounds with a N^+-C-C-O Backbone Catalyze the Disproportionation of Peroxynitrite to Nitrite and Peroxynitrate

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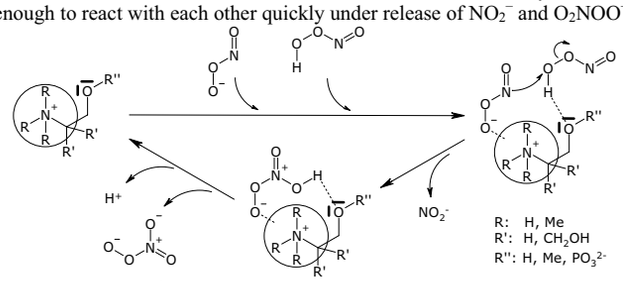
The oxidizing and nitrating agent peroxynitrite, $ONOO^-$ and ONOOH is formed *in vivo*, which has stimulated studies of its reactions with biomolecules. Peroxynitrite is unstable and decays by isomerization to NO_3^- and by disproportionation to NO_2^- and to the artificial oxidant O_2NOO^- [1]. The buffer tris(hydroxymethyl)aminomethane (Tris) catalyzes the disproportionation. 2-Aminoethanol, choline, serine and 2-aminoethylphosphate accelerate the disproportionation too while the amine analogue of Tris, the alcohol analogue of Tris and the mixture of both do not. The effect of choline indicates that the positively charged nitrogen of the N^+-C-C-O substructure – e.g. the protonated amine of a 1,2-aminol – may attract $ONOO^-$ while the vicinal oxygen forms a hydrogen bond with ONOOH. When $ONOO^-$ and ONOOH both are coordinated to the same molecule, they are close enough to react with each other quickly under release of NO_2^- and O_2NOO^-:



The substructure N^+-C-C-O is widespread among biomolecules, e.g. amino acids, phospholipids (cephalin, lecithin, phosphatidylserine), glucosamine.

[1] D. Gupta et al, *Dalton Trans.* **2009**, *29*, 5730

Medicinal Chemistry Chemical Biology **MC072**

Bis(benzoxaborole): organoboron antibacterial and antifungal agent

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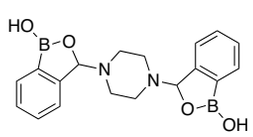
Benzoxaboroles - internal, cyclic hemiesters of phenylboronic acids - have been recently earning growing research interest mainly in terms of their bioactvity.[1] Along with their antifungal and antibacterial properties,[2] they present exceptional sugar receptor activity,[3] constituting an interesting class of organoboron compounds to be investigated.



Preparation and biological evaluation of piperazine-derived bis(benzoxaborole) is reported. The bis(benzoxaborole) system can be straightforwardly assembled starting from 2-formylphenylboronic acid. Synthesis of the bis(*para*-fluoro) derivative, as well as methods for efficient preparation of bis(phenylboronic acid) analogues are presented.

[1] S. J. Baker, J. W. Tomsho, S. J. Benkovic, *Chem. Soc. Rev*. **2011**, *40*, 4279-4285.
[2] A. Adamczyk-Woźniak, O. Komarovska-Porokhnyavets, B. Misterkiewicz, V. P. Novikov, A. Sporzyński, *Appl. Organometal. Chem*. **2012**, *26*, 390-393.
[3] M. Bérubé, M. Dowlut, D. G. Hall, *J. Org. Chem*. **2008**, *73*, 6471-6479.

Medicinal Chemistry Chemical Biology **MC073**

A modular system for point-specific labeling of native oligonucleotides

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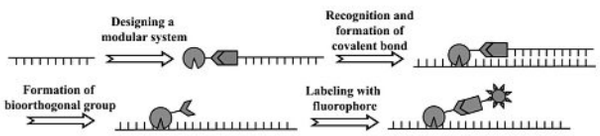
Functionalization of native ribonucleic and deoxyribonucleic acids has paramount importance in all research fields related to life sciences. A variety of bioorthogonal groups and modifications in biomolecules are in use, e.g. for targeted drug delivery or imaging of DNA and RNA [1,2]. Restriction of the lengths of such modified oligonucleotides accessible by automated phosphoramidite-based synthesis prompted us to devise an alternative method.



The basic principle relies on the annealing of a donor DNA strand that carries a reactive group to a complementary template strand [3]. This positions the reactive group close in space to the target nucleotide in the template strand allowing its site-specific modification [3]. The intermediately formed covalent cross-link between donor and template strand can be cleaved due to a specifically designed linker between the reactive group and the donor DNA. This approach will be generally applicable for different kind of modifications due to the manifold combination possibilities of reactive groups and the linkers.

Financial support by the Swiss National Science Foundation (SNSF-Professorship PP002-119106/1 to EF) is gratefully acknowledged.

[1] J. Brunner, R. Kraemer, *J. Am. Chem. Soc.* **2003**, *125*, 12410-12411.
[2] D. M. Kolpashchikov, *Chem. Rev.* **2010**, 110, 4709-4723.
[3] I. A. Oleinich, E. Freisinger, to be submitted**.**

Medicinal Chemistry Chemical Biology **MC074**

Ligand development for the vesicular monoamine transporter VMAT2: Synthesis of Analogs of Tetrabenazine

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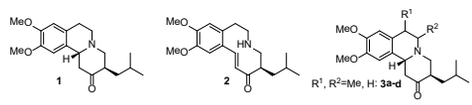
Tetrabenazine (TBZ (**1**), Fig. 1) is a tetrahydroisoquinoline derivative which is an approved drug against dyskinesia and hyperkinetic movement disorders (chorea Huntington).[1] TBZ reversibly binds to and blocks the vesicular monoamine transporter 2 (VMAT2), which is responsible for the transport of monoamines from the cytoplasm into granular vesicles of presynaptic neurons.[2] *In vivo* TBZ is converted into its pharmacologically active metabolite α-dihydrotetrabenazine (α-DHTBZ); only the (+)-enantiomer of α-DHTBZ binds to VMAT2 with high affinity.[3]



This presentation will discuss a new stereoselective synthesis of **1** that entails formation of the macrocyclic key intermediate **2** by RCM.[4] Following the same overall strategy we have also prepared monomethylated tetrabenazine derivatives **3a-d** for biological testing. The ultimate goal of this research is the discovery of more selective VMAT2 blockers than TBZ and to assess whether such compounds may exhibit an improved side effect profile *in vivo*.

[1] P. Diana, *Neuropsychiatric Disease and Treatment* **2007**, *5*, 545-551.
[2] D. R. P. Guay, *Am. J. Geriatr. Pharmacother.* **2010**, *8*, 331-373.
[3] M. R. Kilbourn, L. C. Lee, T. Vander Borght, D. Jewett, K. Frey, *Eur. J. Pharmacol*. **1995**, *278*, 249-252.
[4] M. Johannes, K.-H. Altmann, *Org. Lett*. **2012**, *14*, 3752-3755.

Medicinal Chemistry **MC075**

Pyridomycin As Lead For New Anti-Tuberculosis Agents

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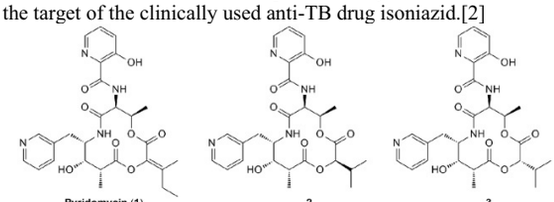
Pyridomycin (**1**) is a bacterial natural product that was first isolated from the *Streptomyces* strain no. 6706 in 1953 and was shown to exhibit significant *in vitro* anti-tubercular activity.[1] Only recently, the molecular target of **1** has been identified as the NADH-dependent enoyl-reductase InhA which is also the target of the clinically used anti-TB drug isoniazid.[2]



The objective of this work is to explore the biology and medicinal chemistry of pyridomycin with a focus on the *de novo* synthesis of analogs of the natural product. In this context we have achieved the stereoselective synthesis of two target structures **2** and **3** which are structurally conserved analogs of **1**. [3] Despite the lack of the enol ester functionality, (*R*)-analog **2** retained most of the anti-tubercular activity of **1**, while (*S*)-analog **3** showed a significantly reduced activity.

In order to initiate a structure activity relationship study around the pyridomycin scaffold, more analogs have been prepared, relying on saturation of the enol ester and on variation of the hydroxypicolinic acid moiety. The syntheses of these compounds, their biological activities and a crystal structure of an analog bound to InhA will be presented at the meeting.

[1] Maeda K. *et al*., *J. Antibiot. 6A* **1953**, 140.
[2] Hartkoorn R. C. *et al*., *EMBO Mol. Med.* **2012**, *4*, 1032.
[3] Horlacher O. P. *et al.*, *ACS Med. Chem. Lett*. **2013**, *4*, 264.

Medicinal Chemistry Chemical Biology **MC076**

Developing Protein Containers with Tailored Recognition Properties

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[2]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA.

Macromolecule encapsulation based on self-assembly of host proteins and guest molecules plays important roles in Nature. These include virus capsids, ferritin proteins, and bacterial microcompartments such as the carboxysome. We have previously developed a novel protein container, AaLS-13, derived from lumazine synthase, which was engineered to encapsulate positively charged protein cargo.[1] Creation of such unnatural protein containers is not only important for gaining a deeper understanding of natural encapsulation systems but also for providing new tools for various applications such as drug delivery, bioimaging, and nanoreactors. Here, we applied the same strategy to a computationally designed protein cage, O3-33.[2] O3-33 forms a 24-mer cage structure with octahedral symmetry, and its size (13 nm in diameter) is considerably smaller than that of AaLS-13 (35 nm in diameter). First, we introduced six glutamic acid mutations on the internal surface of each O3-33 subunit. Despite the smaller luminal volume, and thus, higher negative charge density, O3-33-neg6 assembled into a similar cage structure as O3-33. Next, we incubated O3-33-neg6 with a positively supercharged GFP variant, GFP(+36). As expected, up to 4 molecules of GFP(+36) associated with the O3-33-neg6 cage. The crystal structure of the complex revealed that GFP(+36) molecules were likely located on the internal side of the O3-33-neg6 pore. The results presented here reveal that design strategies relying on host-guest electrostatic interactions constitute a simple and versatile method to create protein containers capable of encapsulating charged macromolecules.

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[2] King, N. P. *et al. Science,* **2012,** *336,* 1171–1174.

Medicinal Chemistry Chemical Biology **MC077**

Polymer nanoreactors as an efficient source of radicals "on demand" for photodynamic therapy

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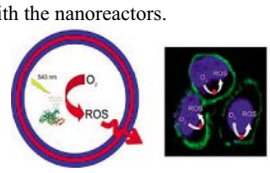
[2]Institute of Physics of Complex Matter, Faculty of Basic Sciences, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne

Photodynamic Therapy (PDT) is gaining an increasing attention for treatment of various pathologic situations, such as cancer, age related macular degeneration, burns and ulcer. However, there are still open points that are necessary to be solved, such as an increase of the efficiency of photosensitizers, and a decrease their toxicity in other bio compartments than the desired ones. To solve these drawbacks, we introduced the concept of polymer nanoreactor as a source of radicals "on demand" [1]. Nanoreactors based on the encapsulation of a highly efficient PDT photosensitizer (rose bengal-BSA) in polymer vesicles serve for local generation of radicals upon irradiation with a specific electromagnetic radiation. Polymer nanoreactors play a dual role: they protect the rose bengal-BSA conjugate, and allow it to act *in situ,* inside the cavity of vesicles. Nanoreactors produce under irradiation with appropriate wavelength reactive oxygen species inside cells and therefore induce cell death, whereas non-irradiated cells remain healthy, even if they are in contact with the nanoreactors.



[1] Patric Baumann, Vimalkumar Balasubramanian, Ozana Onaca-Fischer, Andrzej Sienkiewicz, Cornelia G. Palivan, *Nanoscale* **2013**, *5*, 247.

Medicinal Chemistry Chemical Biology **MC078**

Design and re-iterative data mining of a highly diverse chemical library used for antibacterial activity screenings

Gianpaolo Chiriano,[*a] Liudas Slepikas,[a] Agata Kranjc Pietrucci,[a] Ophelie Pattey,[a] Pierre Cosson,[a] Hajer Ouertatani-Sakouhi,[a] Thierry Soldati,[a] Sébastien Kicka,[a] Hubert Hilbi,[b] Christopher Harrison,[b] John McKinney,[c] and Leonardo Scapozza[a]

[a]University of Geneva, Geneva-Switzerland; [b]Ludwig-Maximilians University, Munich-Germany; [c]EPFL, Lausanne-Switzerland.

In this work, we aimed at the discovery of novel compounds towards the prevention or treatment of bacterial infections caused by *Klebsiella pneumoniae*, *Legionella pneumophila* or *Mycobacterium marinum* using simple but experimentally highly tractable hosts, amoebae, among which *Dictyostelium discoideum.*[1] To fulfill this goal, we designed a pathway-based highly diverse compounds library based on 18 host and/or bacteria pathways crucial for the host-pathogen interaction process. Known ligands/metabolites linked to these pathways have been used to launch a campaign of ligand-based virtual screening from ZINC lead-like database by the means of ROCS.[2] The resulting library includes 1711 selected compounds (~100 per each pathway) and 2000 compounds from the Prokinase library containing ligands binding to kinases involved in several signaling pathways and the NINDS. Ongoing screening designed to identify compounds interfering with host-pathogen interactions already led to the discovery of several *hit* candidates with significant activity at 30 μM. As result, we designed new *hits*-based libraries for further testing and establishing primary SARs. To virtually manage these libraries and perform the related data-mining, the Shared Results Database (SRD) was created by using InstantJChem[3] and will be shared via internet among the scientific community. Libraries design, SRD structure and SAR studies for the most interesting *hits* will be presented.

1. Froquet et al., *Nat Protoc* **2009**; 4 (1): 25-30
2. http://www.eyesopen.com/rocs
3. http://www.chemaxon.com/products/instant-jchem/

Medicinal Chemistry Chemical Biology **MC079**

The ionotropic serotonin receptor as scaffold for functional and structural studies

Ruud Hovius, Joachim Piguet, Horst Vogel

Laboratory of Physical Chemistry of Ploymers and Membranes
EPFL

Transmembrane proteins offer next to their own functionality, like transporter or signal transducer, the possibility to be tailored for other purposes, like scaffolds for the membrane-proximal attachment of proteins. Here, the serotonin-gated ion channel 5HT3R is used as central molecule for structural and functional studies. This receptor is homo- or heteropentameric assembly of subunits (~450 aa) that have each a highly structured N-terminal extracellular ligand-binding domain (~230 aa), four transmembrane helices and a rather un-structured large intracellular loop (~125 aa) between the 3rd and 4th TM.
Here, proteins were appended to the N-terminus of or inserted in the large intracellular loop of the receptor for imaging, e.g. fluorescent proteins (~250 aa) or SNAP-tag (~180 aa), or to engineer a membrane-bound calcium sensor (~470/ 650 aa). Despite the large relative size of additions, the fusion proteins featured, in general, unperturbed ligand-binding and channel properties, showing that 5HT3R very tolerant to major modifications.
The subunit of heteropentameric 5HT3_A+B receptor was investigated using A subunits with appropriate fluorescent proteins in the large intracellular loop enabling fluorescence resonance energy transfer (FRET).
Ligand binding to 5HT3R was quantified using a FRET assay employing a fluorescent labelled ligand and a 5HT3R carrying a fluorescent protein on its N-terminus.

Medicinal Chemistry Chemical Biology **MC080**

Pathogen inactivation lesions to platelets: comparison at the peptide level of the two main techniques available

Giona Sonego, Michel Prudent, Mélanie Abonnenc, Jean-Daniel Tissot, Niels Lion

Service Régional Vaudois de Transfusion Sanguine, Unité de recherche et Développement, Lausanne, Switzerland

Pathogen reduction technologies (PRT) for platelets concentrates have a central role in preventing transfusion-transmitted infections. The principle of PRT is based on the photochemical modification of DNA/RNA strands, inactivating pathogens. Intercept process (adopted in Switzerland) uses amotosalen as a photoactive compound whereas Mirasol uses riboflavin. In addition of modifying nucleic acids, they also produce reactive oxygen species (ROS). Although PRT are in routine use in blood centers, they are suspected of reducing the haemostatic activity of platelets[1-3]. The mechanism giving rise to this phenomenon is not clear. At the proteome level, we have shown that a few proteins are affected by the Intercept treatment[4]. Here, the impact of PRT is investigated at single unit (amino acid) level. Different sequences of amino acids were treated with the photochemically active techniques Intercept or Mirasol, which allowed to compare the impact of these two PRT. Under these oxidative conditions it was possible to observe and quantify, by liquid chromatography coupled to mass spectrometry, different kinds of oxidations, mainly on Thr, Tyr, Cys and His moieties. Moreover, differences between these two PRT were illustrated by the conversion rates of oxidized peptides after the treatment, where Mirasol exhibits the greatest impact. Investigations on proteins participating to platelet activation/aggregation and clot formation will follow.

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[3] Kerkhoffs J-LH, *Br J Haematol* **2011**, *153*, 531.
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Medicinal Chemistry Chemical Biology **MC081**

Highly Chemoselective and Practical Thio-Alkynylation

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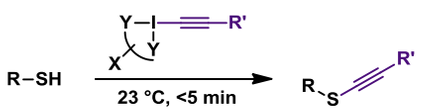
Due to the tremendous capacity of acetylene chemistry,[1] methods that selectively transfer alkyne moieties onto vastly functionalized intermediates are highly sought after. Current thio-alkynylation processes are rare in number and generally utilize nucleophilic acetylides under strongly basic conditions.[2] Consequently, these harsh conditions have resulted in a moderate scope and functional group tolerance. In this context, we have developed a highly chemoselective and practical thio-alkynylation reaction by utilizing electrophilic, hypervalent iodine-based, alkyne-transfer reagents.[3] The developed thio-alkynylation reaction readily proceeds at room temperature (*i.e.* <5 min) in an open flask, using commercially available reagents. The scope of the reaction is broad, with a variety of highly functionalized biologically and medicinally relevant phenolic-, benzylic-, heterocyclic-, aliphatic and peptidic thiols undergoing alkynylation in excellent yield. Furthermore, the utility of the thiol-alkynylation in post-synthetic elaboration has been demonstrated, for instance, *via* installment of fluorophore tags.



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Medicinal Chemistry Chemical Biology **MC082**

Development and engineering of probes for bivalent epigenetic marks

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Nucleosomes of key developmental genes in stem and progenitor cells are thought to be marked with repressive (H3K27me3) as well as activating (H3K4me3) post-translational modifications (PTMs). They hence form bivalent chromatin domains, where genes are repressed but believed to be poised for induction. This property could play a major role in developmental and differentiation processes, and could also be implicated in some cancers.
The presence of bivalent nucleosomes has been inferred from methods such as genome wide chromatin immunoprecipitation (ChIP), ChIP re-ChIP or immunopurification followed by LC MS/MS experiments. The common limitation of these approaches is their requirement for cell lysis and their reliance on antibodies. Further, no tools are available to directly show co-existence of H3K4me3 and H3K27me3 on the same nucleosome.
In order to directly visualize the colocalization of H3K4me3 and H3K27me3 in cells and thereby to detect and follow bivalent domains, we are engineering genetically encoded protein probes. These probes are based on protein domains that specifically bind to H3K4me3 and H3K27me3 and bimolecular fluorescence complementation (BiFC) of a split fluorescent protein for detection. In order to validate and optimize our probes, we are synthesizing chemically modified nucleosomes carrying the modifications as in vitro validation model. This will allow us to optimize probes sensing properties through engineering of efficient marks binding domains.
Finally, optimized probes will allow direct visualization of bivalent chromatin within the nuclei of living cells. Subsequently, this approach could be applied to develop a range of relevant multivalent probes to be used as a general tool to study post translational modifications.

Medicinal Chemistry Chemical Biology **MC083**

Chromatin conformational dynamics and heterochromatin protein 1

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Chromatin, the complex of DNA and histones in eukaryotic organisms, is involved in the regulation of cell specific retrieval and duplication of genetic information. Post-translational modifications of histones serve as recruitment platforms for chromatin effector proteins and thereby exhibit a regulatory role in the orchestration of cellular processes like transcription, replication and DNA-damage repair. An important effector is heterochromatin protein 1 (HP1), which is associated with genetically silenced chromatin in part through the recognition of histone H3 trimethylated at lysine 9 (H3K9me3).

Current knowledge about the higher-order organization of nucleosomes into fibers is limited, especially in complex with effectors, and little information is available on the dynamics of the system. Our aim is to quantify the dynamics occurring within reconstituted chromatin fibers to understand the time-scales and the distances associated with motions within chromatin. These investigations are extended to study the interplay between the chromatin fiber and HP1.

We design chromatin fibers with functionalities required for biophysical characterization by employing a combination of synthetic chemistry, enzyme-based and recombinant methodologies. Histone H3 bearing the K9me3 mark is produced by expressed protein ligation. Modified nucleosomes are incorporated into chromatin fibers containing PCR-incorporated fluorophores at defined positions. This paves the way for detailed investigations of local spatial and temporal fluctuations within chromatin fibers as a function of the modification state and HP1 binding, using fluorescence-based approaches involving FRET and FCS.

Medicinal Chemistry Chemical Biology **MC084**

Visualization and Characterization of the Splicing/Reverse-Splicing Equilibrium of single pre-mRNAs with 3-colour sm-FRET spectroscopy

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Group II introns are large self-splicing ribozymes that can be found in bacteria and organellar genes of plants, fungi and yeast. The spliced intron is able to specifically reinsert into its original RNA substrate by a so-called retrohoming process, resulting in a splicing/reverse-splicing equilibrium. To catalyse both reactions, the ribozyme folds into several stable intermediates, minimizing the activation energy of the processes. The kinetics of the conformational transitions as well as the intron/exon binding affinity play an essential role in the catalytic activity as they govern the ability to reach the native structure. To characterise structurally and kinetically the splicing/reverse-splicing equilibrium of the very large *Sc*.ai5γ group II intron by single molecule FRET spectroscopy (smFRET), we design here a system labelled with three different fluorophores. Such a labelling scheme is essential to differentiate the different products and identify the intron-exon binding events. Furthermore, we encapsulate our single reactive systems in phospholipid vesicles in order to allow the products and reactants to freely diffuse inside the small reactors. By combining the free motion of the molecules to the presence of crowding agents and co-factors, we intend to observe an *in vivo*-near behaviour, which remains unprecedented.

Financial support by the ERC, the SNF and the University of Zürich are gratefully acknowledged

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Medicinal Chemistry Chemical Biology **MC085**

RNA folding and splicing under physiological conditions

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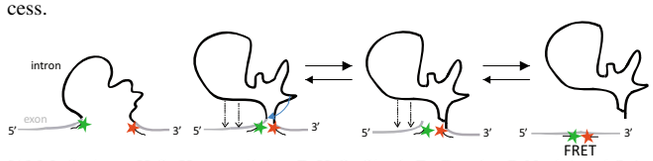
One of the biggest challenges of structural biology nowadays is to apply the current understanding about biological frameworks and interactions gained from *in vitro* studies to the complex cellular environment. This is particularly difficult for RNA, because of its functional diversity and its characteristic architecture. Our research focuses on a particular process during RNA processing called splicing and place emphasis on the complex folding pathway of the involved RNA under native conditions. The splicing mechanism is a crucial step during RNA maturation and so far investigated only by *in vitro* studies. [1] We established a series of techniques to fluorescently label the RNA of a group II intron from *S. cerevisiae* on specific regions which will be used for further *in vivo* studies (Figure). The folding pathway is investigated by single-molecule Förster Resonance Energy Transfer (smFRET) under physiological conditions and the obtained results are compared with *in vitro* data. [1, 2] Furthermore, bulk FRET experiments *in vitro* are carried out as a first step towards *in vivo* studies of the group II intron splicing process.



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Medicinal Chemistry Chemical Biology **MC086**

Evaluation of a new derivative of 5-aminolevulinic acid for vascular photodynamic therapy : *in vitro* stability study, *in vivo* fluorescence spectroscopy and *in vivo* phototoxicity

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Photodynamic therapy (PDT) based on photoactivable porphyrins (PAPs) is used to treat various dermatological conditions, including diseases associated with vascular abnormalities. The production of PAPs can be induced in the skin by topical application of their precursor 5-aminolevulinic acid (ALA). The penetration of ALA in the skin after topical application is suboptimal due to ALA's hydrophilic nature. Moreover ALA is not selectively internalized by endothelial cells of the skin blood vessels. New ALA-derivatives are developed by our group to optimize this selectivity. This approach is beneficial for treatment of conditions such as vascular malformations or modifications (telangiectasia, port-wine stain, rosacea, etc).

We studied the stability of ALA-tyrosine ester in aqueous solution at different storage conditions (pH and temperature) and at different times after solubilisation. We concluded that fresh solutions must used within 24h. In addition they must be stored at low (4 °C) temperature to prevent hydrolysis of the ester bond, especially at physiological pH. We also studied *in vivo* the fluorescence emission spectroscopy, the photostability, the photoproducts formation and the vascular phototoxicity of PAPs produced after a topical administration of ALA-tyrosine ester on the chick's chorioallantoic membrane model, and compared them with corresponding measurements obtained with ALA. Our resuts suggest that the photobleaching constant of PAPs is different with these two precursors ($p < 0.1$). If confirmed, this small difference could be explained by PAPs localization in different cells and/ or cellular compartments, or by different relative proportions of the various fluorophores produced in the heme cycle.

Medicinal Chemistry Chemical Biology **MC087**

Heterochromatin Protein 1 association dynamics studied using total internal reflection microscopy

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Post-translational modifications of histone tails play an important role in recruiting different proteins to chromatin, including the chromatin architectural protein HP1 (Heterochromatin Protein 1). This protein is considered one of the main actors in heterochromatin formation and in the consequent transcriptional silencing. However the dynamic of its interaction with chromatin has not been yet studied deeply. HP1 contains a chromodomain responsible for recognition of H3 methylation at lysine 9 (H3K9me3) and a chromoshadow domain that plays a role in the protein oligomerization. Using synthetic modified chromatin we aim at study the dynamics of HP1 association with chromatin, considering in particular how the abundance of H3K9me3 mark on histone tails can affect the accumulation of this protein, its affinity and its residence time on the chromatin. In our project we reconstitute and immobilize chromatin fibers containing multiple modified nucleosomes. Using Total Internal Reflection Fluorescence Microscopy, a powerful method to investigate single molecules dynamic, we want to determine the variation in the association and dissociation rates of HP1 and its residence time on chromatin arrays as a function of chromatin modification. In conclusion, our final goal is to set up a biophysical platform to measure the dynamic interaction of different chromatin effectors with modified chromatin arrays.

Medicinal Chemistry Chemical Biology **MC088**

Catalytic Strategies for Nucleic Acid Tagging

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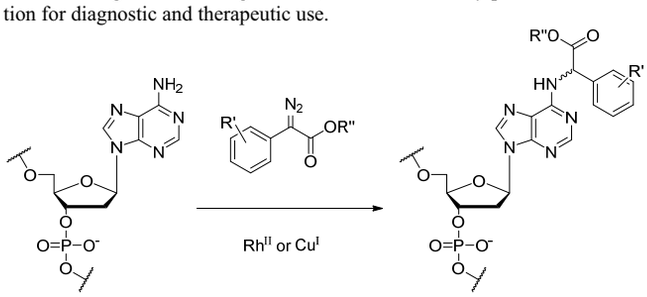
The field of nucleic acid research heavily relies on strategies for DNA and RNA tailoring in order to label and expand their functional potential. The development of such methodologies is a key step not only to the complete understanding of their biological role, but also to directly perturb their function for diagnostic and therapeutic use.



We have shown that a variety of nucleic acids can be catalytically alkylated with rhodium(II) and copper(I)-carbenoids generated from α-diazo carbonyl compounds. The alkylation takes place via an N-H insertion reaction selectively targeting the non-paired nucleobases in single-stranded DNA and RNA motifs [1]. The use of alkyne-functionalized α-diazo carbonyl compounds allows tagging of the nucleic acid substrate with a wide range of reporter groups such as fluorophores, and affinity tags via 'click' chemistry.

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Medicinal Chemistry Chemical Biology **MC089**

Does alteration of adenine purine levels lead to a loss-of-fitness phenotype in *Trypanosoma brucei* ?

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Human African Trypanosomiasis (HAT), also known as sleeping sickness, is caused by the parasites *T. brucei rhodesiense* and *T. brucei gambiense*. Available treatments for this disease still remain unsatisfactory, needing parental administration, are toxic, or parasites have acquired resistance. Thus safe and potent new drugs are needed to treat sleeping sickness.
Trypanosoma brucei adenosine kinase (*Tb*AK), an enzyme involved in the purine salvage pathway, was identified by chemical proteomics to be the intracellular target of 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine (compound 1). This molecule exhibits very good antitrypanosomal activity with an IC_{50} of 1 μM, and biochemical analysis revealed that compound 1 is a strong activator of *Tb*AK[1,2].
The aim of the current project is to understand the mechanism of action of compound 1. To this end changes in adenine purine levels of trypanosomes induced by compound 1 were measured by means of an ion-pair HPLC/UV method. Several strains of *Trypanosoma brucei* were tested, e.g. 1) wild type strain, 2) a tetracycline inducible *ak* overexpression strain W2, hypothesizing that the presence of compound 1 is similar to overexpression of *Tb*AK), and 3) a tetracycline inducible null mutant *ak* overexpression strain D299V to provide insight into the effect of non-physiological high levels of *Tb*AK.

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Medicinal Chemistry Chemical Biology **MC090**

A FRET Sensor for GABA and Synthetic $GABA_B$ Receptor Ligands

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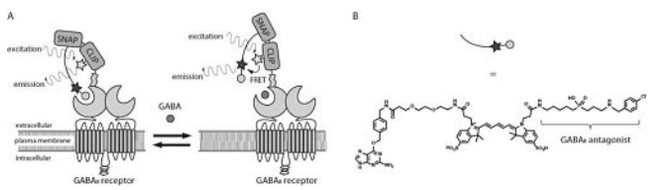
γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian nervous system. Despite its importance there is no system available that is able to measure GABA concentrations at the single cell level with good spatio-temporal resolution. The presented work describes the development of a FRET sensor for GABA based on the Snifit sensor design [1]. The GABA-Snifit displays a GABA-dependent fluorescence emission spectrum in the range of 500−700 nm that permits sensing micromolar to millimolar GABA concentrations on the surface of living mammalian cells [2]. Additionally, GABA-Snifit can be used to characterize synthetic $GABA_B$ receptor ligands such as agonists, antagonists and allosteric modulators that can be used as therapeutics.



GABA-Snifit. (A) Design principle of the GABA-Snifit based on the $GABA_B$ receptor and the labeling tags SNAP- and CLIP-tag. (B) Structure of the GABA-Snifit substrate containing a $GABA_B$ receptor antagonist.

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Medicinal Chemistry Chemical Biology **MC091**

On Homotropic Cooperativity in CYP3A4

Christian S. Müller[1,2], Tim Knehans[3], Patricia L. Bounds[1], Ursula von Mandach[2], Dmitri R. Davydov[4], James R. Halpert[4], Amedeo Caflisch[3], and Willem H. Koppenol[1]

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Cytochromes P450 (CYP) are a superfamily that can catalyse a variety of reactions, e.g. the oxidation of non-activated hydrocarbons. We investigated the mechanism of interactions of cytochrome P450 3A4 (CYP3A4), the principle human drug-metabolizing enzyme, with carbamazepine (CBZ).

A model of a CYP3A4 complex with CBZ was subjected to molecular dynamics simulations in GROMACS [1] to find preferred binding locations and orientations of the substrate, from which we chose residues S119, A370, and I369 as appropriate targets for site-directed mutagenesis. Mutants were constructed and screened for CBZ epoxide formation. Wild-type CYP3A4 and I369 mutants exhibited Michaelis-Menten dependence of the reaction rate on CBZ concentration. S119 and A370 mutants induced a change in the kinetics profiles to sigmoidal behavior that suggests homotropic cooperativity in the enzyme-substrate interaction.

Cooperativity in substrate interactions with CYP3A4 has been reported [2,3]. Our findings demonstrate that the CYP3A4-CBZ interaction is exquisitely sensitive to even modest structural changes in the substrate binding pocket and may provide useful insights regarding mechanisms of cooperative substrate interactions with CYP3A4.

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Medicinal Chemistry Chemical Biology **MC092**

Methionine promotes tyrosine oxidation

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α-Synuclein, the normal function of which is not yet well understood, may aggregate in response to cellular oxidative stress into Lewy bodies inside dopaminergetic neurons, which is a hallmark of Parkinson's disease[1].
We aim to study the oxidation chemistry of α-synuclein in real time using the pulse radiolysis technique, which allows the generation of oxidative species, e.g. $HO^{\cdot}$, in solution within 1 μs. We chose to investigate the reaction of $HO^{\cdot}$ with a synthetic tyrosine- and methionine-containing peptide corresponding to residues 124 – 130 near the C-terminus. We observed unexpectedly rapid formation of tyrosyl radicals ($Y[O^{\cdot}]$):

$$AYEMPSE + HO^{\cdot} \rightarrow AY[O^{\cdot}]EMPSE + H_2O \qquad k = 2 \times 10^6\ M^{-1}s^{-1}$$

We also investigated the reaction of $HO^{\cdot}$ with synthetic peptide from β-synuclein, corresponding to residues 118 – 131, which contains 3 tyrosine and no methionine, where normal rates of tyrosine oxidation were found:

$$SYEDPPQEEYQEYE + HO^{\cdot} \rightarrow \text{products} \qquad k = 5 \times 10^3\ M^{-1}s^{-1}$$

We believe that the presence of a nearby methionine residue promotes oxidation of tyrosine, perhaps catalytically. Further experiments are being performed to elucidate the reaction mechanism.

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Medicinal Chemistry Chemical Biology **MC093**

NMR spectroscopic investigations of porphyrinic photosensitizers with nanoparticles as carrier systems

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Photodynamic therapy (PDT) is a cancer treatment modality, which is based on the selective uptake of a photosensitizer (PS) by cancer tissue. Upon excitation with red light the photosensitizer may react with molecular oxygen ($^3O_2$) via energy transfer to generate singlet oxygen ($^1O_2$), which is known to give rise to oxidative reactions triggering cell death. Many PS molecules, however, are hydrophobic and have a strong tendency to aggregate in aqueous solution decreasing their photosensitizing and biological effectiveness. The use of nanoparticles as PS carriers presents a promising approach for enhancing PDT efficacy [1].
A previous study was aimed at investigating the aggregation behavior of several porphyrinic compounds, which are promising PS in PDT, by NMR spectroscopic methods. Through observation of NMR resonance broadening and porphyrin ring current induced shifts, the extent of aggregation in phosphate buffered saline could successfully be assessed [2]. Our present work is concentrated on the search for an appropriate nanocarrier system for selected porphyrins. Carrier systems such as poly(DL-lactide-co-glycolide) (PLGA), polyvinylpyrrolidone (PVP) or β-cyclodextrin are currently probed by NMR spectroscopic methods for their ability to form inclusion complexes with porphyrinic compounds. Cell uptake is probed using cultured HeLa cells and fluorescence based methods. Since amino acid residues have been found to enhance the biological effects of porphyrinic compounds [3], our focus is on amino acid conjugates of the dihydroporphyrin chlorin e6.

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Medicinal Chemistry Chemical Biology **MC094**

Neuritogenic Surfaces: Towards Solid Support Mediated Neuronal Organisation

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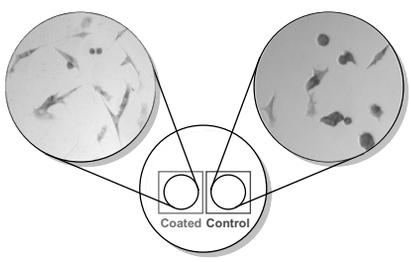
This work aims to provide new solutions for two different challenges: 1) implant based neuronal network reconstruction for spinal cord injuries and 2) improvement of connections between neurons and electronic sensors.[1,2] With our modular and straightforward method, we were able to coat glass slides with structurally diverse neuritogenic natural product analogues (NNPA) to induce neuronal differentiation in *rat pheochromocytoma* cells.[3] The robustness of this approach is underlined by the fact that reuse of already *in vitro* applied slides is possible with maintained activity. If a non-coated slide is placed in close proximity to a slide coated with NNPA within the same cell media, no enhanced cell differentiation activity could be detected on its surface. This opens the possibility of neuronal organization on solid support.



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Medicinal Chemistry Chemical Biology **MC095**

A Bioorthogonal In Vivo Ligation Reaction and its Application for Non-Invasive Bioluminescent Imaging of Protease Activity in Living Mice

Aurélien Godinat, Hyo Min Park, Stephen C. Miller, Ke Chen, Douglas Hanahan, Laura E. Sanman, Matthew Bgyo, Allen Yu, Gennady F. Nikitin, Andreas Stahl, Elena A. Dubikovskaya*

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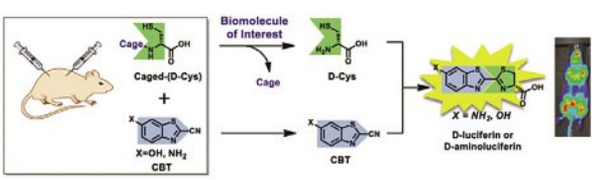
The discovery of biocompatible reactions had a tremendous impact on chemical biology, allowing the study of numerous biological processes directly in complex systems. Here we report that D-cysteine and 2-cyanobenzothiazoles can selectively react with each other in vivo to generate a luciferin substrate for firefly luciferase. First, the production of a luciferin substrate can be visualized in a live mouse by bioluminescence imaging and furthermore allows interrogation of targeted tissues using a ″caged″ luciferin approach. We therefore applied this reaction to the real-time non-invasive imaging of apoptosis associated with caspase 3/7. Caspase-dependent release of free D-cysteine from the caspase 3/7 peptide substrate Asp-Glu-Val-Asp-d-Cys allowed selective reaction with 6-amino-2-cyanobenzothiazole in vivo to form 6-amino-D-luciferin with subsequent light emission from luciferase. Moreover, the split luciferin approach enables the modular construction of bioluminogenic sensors, where either or both reaction partners could be caged to report on multiple biological events, suggesting further applications for both bioluminescence and specific molecular targeting in vivo.



Medicinal Chemistry Chemical Biology **MC096**

Convergent preparation of unsaturated acyclic nucleoside phosphonate prodrugs

Ugo Pradère, Vincent Roy, Aurélien Montagu, Jan Balzarini, Robert Snoeck, Graciela Andrei, Luigi Agrofoglio

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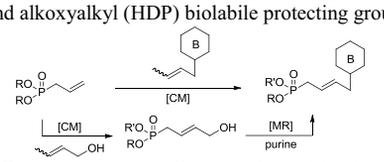
Acyclic nucleoside phosphonates (ANPs) is a key class of antiviral agent with compounds of primary importance against both DNA and retrovirus infections. Because of the ionic character of their phosphonate moiety, the ANPs have to be functionalized as prodrugs by insertion of biolabile protecting groups. Our group recently reported a new type of unsaturated ANPs bearing a (*E*)-4-phosphono-but-2'-en-1'-yl sugar like backbone. Such modified moiety proved to be well mimicking the conformation of the C1-O4-C4-C5 atoms from natural substrate by X-ray studies with thymidylate kinase. Direct conversion of nucleoside phosphonates into their prodrug form is particularly cumbersome and often low-yielding. In order to open an easy access to prodrugs and assess the antiviral potency of our ANPs, we developed convergent synthetic strategies to allow simultaneous insertion of the phosphonate moiety and the biolabile protecting groups. Cross metathesis (CM) and Mitsunobu reaction (MR) were employed for the preparation of a small library of unsaturated ANP prodrugs bearing carbonyloxymethyl (POM, POC) and alkoxyalkyl (HDP) biolabile protecting groups [1,2].



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Medicinal Chemistry Chemical Biology **MC097**

Efficient conversion of nucleoside analogs to 5'-methylene-phosphonate prodrug derivatives

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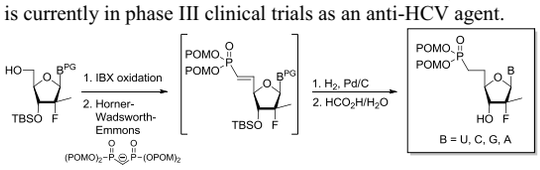
Nucleoside phosphonates that contain a phosphonate bioisostere of a natural 5'-phosphate group have received considerable synthetic and medicinal chemistry attention over the years. However, the ionic character of these phosphonic acid derivatives is generally an obstacle for cellular penetration, which often translates to a lack of biological activity for these nucleoside analogs. In order to mask the negative charges, phosphonate prodrugs bearing biolabile protecting group such as pivaloyloxymethyl groups (POM) have been developed. Because the preparation of such phosphonate prodrugs is generally tedious and low yielding, we developed a general and efficient method to prepare nucleoside 5'-methylene bis(POM)-phosphonate prodrugs through the condensation of tetra(POM)-bisphosphonate with protected 5'-aldehyde nucleosides *via* a modified Horner-Wadsworth-Emmons reaction. Our strategy was applied to the 2'-deoxy-2'-α-fluoro-2'-β-*C*-methyl nucleoside series whose most advanced member, PSI-7977 (GS-7977) is currently in phase III clinical trials as an anti-HCV agent.



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New therapeutic options for the treatment of life-threatening parasitic diseases

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Echinococcus multilocularis (fox tapeworm) is considered to be the most dangerous human pathogenic parasite occurring in Central Europe. Infections with the parasite are quite rare (0.5 – 2.5 cases / 100.000 inhabitants); therapy of choice is surgical removal of infected tissues. However, alternative treatments for inoperable patients are urgently needed, because there are only very few parasitostatic and no parasitocidal chemotherapeutic options available for infected persons. Progression of the untreated disease leads to almost certain death within approx. 15 – 20 years after infection.
During the last approx. 25 years, ruthenium complexes have attracted increasing interest as potential therapeutics for the treatment of cancer.[1,2] We will present our recent promising data,[3] demonstrating hydrolytically stable ruthenium complexes to be a possible therapeutic option also for the treatment of *alveolar echinococcosis*, which is the name of the disease originating from fox tapeworm infections. In addition, some new results from our research on antiparasitic agents will be shown.

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Medicinal Chemistry Chemical Biology **MC099**

RNA-Metal ions interactions by smFRET

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RNA folding and activity depends vastly on the nature of metal ions present in solution. The potential of RNA for medical and biotechnological applications grows daily, and understanding RNA-metal ions interactions and how the later may tune the activity of the former become thus of great interest. In our study, we apply "Single Molecule FRET" to characterize the interaction of an RNA hairpin loop, known as "EBS1" (Exon Binding Site 1), with its RNA or DNA cognate. Our results reveal that these two interactions differ slightly in conformation, which is in good agreement with NMR studies performed on the same system[1]. In addition, kinetic analysis shows that the EBS1-RNA interaction displays kinetic heterogeneity in the presence of Mg^{++}, While the EBS1-DNA interaction was homogenous in the presence of K^{+} and Mg^{++}. The origin of the heterogeneity seen with EBS1-RNA interaction in the presence of Mg^{++} could be explained by a specific requirement for divalent metal ions, knowing that previous works had point out two potential binding sites for divalent metal ion in EBS1 hairpin loop [2]. Our results support the involvement of one of the two specific metal ions in determining the correct phosphodiester bond cleaved by the group II intron, an active RNA molecule to which belong EBS1 hairpin[3].

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Medicinal Chemistry Chemical Biology **MC100**

SPR as a tool for the detection of new RNA binding motifs

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RNAs play important roles in many biological processes, including disease mechanisms. A major challenge in RNA biology is understanding their regulation by RNA binding proteins (RBPs). Therefore it is important to detect new interactions and new potential targets.
The RBFOX family is an important alternative splicing factor in humans, responsible for the high diversity of protein isoforms. Previously, the SELEX method (Systematic Evolution of Ligands by Exponential Enrichment) revealed that RBFOX binds to a strict consensus sequence GCAUG [1][2], which is highly enriched in proximity to alternative splicing sites.[3]

We have developed a new SPR-based method to identify RNA sequences recognized by RBPs using libraries of RNAs. During the investigation, we identified new RNA binding motifs for RBFOX with high nM affinities. These new motifs were shown to be functional in cellular pull-down assays which indicated a new function of RBFOX independent of alternative splicing.

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