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Hydrophobic Interactions Influence the Conformational Prevalence of c-Src Tyrosine Kinase Domain

R. Boubeva, A. Cristiani, L. Pernot, R. Perozzo, L. Scapozza

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

The crystal structures of wild type c-Src kinase domain in an active and in an inactive conformation have been resolved but the molecular mechanism allowing the structural switch remains unknown. To bind Imatinib, a tyrosine kinase inhibitor targeting the inactive conformation of Abl (IC_{50} = 25 nM), c-Src undergoes a thermodynamic penalty resulting in IC_{50} value of 221 μM [1].
In this work, we aimed at the identification of key amino acid residues that are dictating the transition from the active to the inactive protein conformation to better understand the molecular basis of conformational plasticity of tyrosine kinases.
We first performed a comparative structural analysis using molecular modelling and depicted a pool of residues at the hydrophobic interface (H1-H2) of the N- and C-lobes that appears to be important for the protein conformation and motion.
We performed site-directed mutagenesis studies to mutate residues at the hydrophobic interface as well as residues contiguous to the hydrophobic interface. The mutagenesis study was also extended to the residues building another network of highly conserved hydrophobic interactions stabilizing the active kinase conformation, called the "hydrophobic spine". [2][3]
The results show that mutating the residues from the H1-H2 interface influences drastically the conformational balance of c-Src rendering the protein much more sensitive to Imatinib (up to IC_{50} = 37 nM). The X-ray studies of the complex Imatinib-c-Src mutant reveals that Imatinib is indeed bound to the inactive kinase conformation similarly to what has been observed with Abl tyrosine kinase [4].
Concluding, this study reveals that one amino acid of the hydrophobic H1-H2 interface is crucial for the conformational prevalence of c-Src.

[1] Seeliger M. et al., *Structure.* **2007**, *15*, 299-311.
[2] Azam M. et al. *Nature Structural & Molecular Biology,* **2008**, *15*, 1109-1118
[3] Kornev et al., *PNAS,* **2008**, *38*, 14377-14382
[4] Nagar et al., *Cancer Research,* **2002**, *62*, 4236-4243

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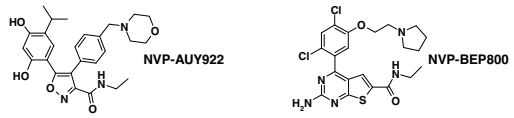
4,5-Diarylisoxazole and 2-Amino-Thienopyrimidine Hsp90 Chaperone Inhibitors as Antitumor Agents

Joseph Schoepfer[1], Patrick Chène[1], Andrew Massey[2], Paul A. Brough[2], Robert Cozens[1], Carlos García-Echeverría[1], Marc Lang[1], Mike Wood[2], Martin J. Drysdale[2] and Michael Rugaard Jensen[1]

[1]Novartis Pharma AG, 4002 Basel, Switzerland.
[2]Vernalis Ltd, Granta Park, Great Abington, Cambridge, CB1 6GB, UK.

Heat Shock Protein 90 (Hsp90) is a ubiquitously expressed molecular chaperone which plays an important role in the conformational maturation and activation of many client proteins that are implicated in oncogenesis. Therefore, Hsp90 has attracted considerable interest as a therapeutic target for anticancer drugs.[1] From the Novartis and Vernalis collaboration effort NVP-AUY922, a 4,5-diarylisoxazole derived Hsp90 inhibitor, is currently in Phase I clinical cancer trials.[2] The 2-amino-thienopyrimidine compound NVP-BEP800 was subsequently investigated as a potential orally available development candidate.[3] NVP-AUY922 and NVP-BEP800 are both potent Hsp90 inhibitors with IC_{50}s of 30 nM and 58 nM, respectively, in a Hsp90 ATP-binding site competition assay, and show antiproliferatives effects in different cancer cell lines *in vitro* and *in vivo*.



[1] M. J. Drysdale and P. A. Brough, *Curr. Top. Med. Chem.*, **2008**, *8*, 859. S. Messaoudi *et al.*, *Anti Canc. Agents Med. Chem.*, **2008**, *8*, 761.
[2] P. A. Brough *et al.*, *J. Med. Chem.* **2008**, *51*, 196. M. R. Jensen *et al.*, *Breast Cancer Res.*, **2008**, *10*, R33.
[3] P. A. Brough *et al.*, *J. Med. Chem.* **2009**, *submitted*.

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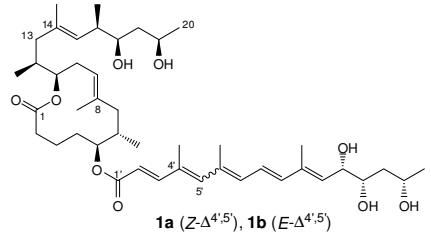
Mycolactones A and B: Total Synthesis and Generation of Antibodies

Philipp Gersbach, David Sgier, Fabian Feyen, Andrea Jantsch, Dario Neri, Karl-Heinz Altmann*

ETH Zürich, Institute of Pharmaceutical Sciences, Wolfgang-Pauli-Strasse 10, CH-8092 Zürich, Switzerland

Mycolactones A and B (**1a** and **1b**) form the major toxins of *Mycobacterium ulcerans,* which is the causative pathogen for the development of Buruli ulcer. **1a** and **1b** have immunosuppressive and cytotoxic properties, but the cellular mechanisms underlying these activities remain unknown [1].



1a (Z-$\Delta^{4',5'}$), **1b** (E-$\Delta^{4',5'}$)

Here we present a new total synthesis of mycolactones A and B, which is significantly more efficient than the one previously reported by Kishi and coworkers [2]. Key steps are a ring closing olefin metathesis to establish the *E* double bond at C8 and a modified Suzuki-Miyaura coupling to construct the C13–C14 bond. Significantly, this strategy has enabled the generation of monoclonal antibodies against substructures of **1a** and **1b** from a phage display library. These antibodies could be useful for diagnostic, but also for therapeutic applications.

[1] Simmonds, R. E.; Lali, F. V.; Smallie, T.; Small, P. L. C., Foxwell, B. M. *J. Immunol* **2009**, *182*, 2194.
[2] Song, F.; Fidanze, S.; Benowitz, A. B.; Kishi, Y. *Org. Lett.* **2002**, *4*, 647.

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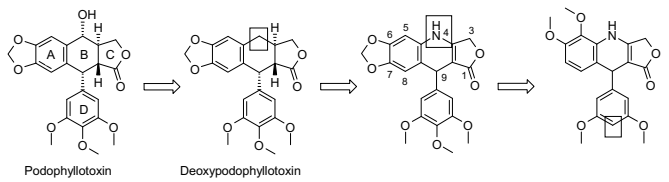
Insecticidal Heterolignans – Potent Tubuline Polymerization Inhibitors with Activity against Chewing Pests

Jens Frackenpohl[a], Isabelle Adelt[b], Horst Antonicek[b], Christian Arnold[b], Nicole Blaha[a], Sabine Hohmann[a], Elisabeth Peschel[a], Thomas Schenke[b], Robert Velten[b], Hans-Christoph Weiss[c]

[a] Bayer CropScience AG, Industriepark Höchst, G836, D-65926 Frankfurt a. Main
[b] Bayer CropScience AG, Alfred-Nobel-Strasse 50, D-40789 Monheim
[c] Currenta GmbH, Chemiepark Leverkusen, Q18, D-51368 Leverkusen

Starting from natural product podophyllotoxin substituted heterolignans were identified with promising insecticidal *in vivo* activity. The impact of substitution in each segment of the core structure was investigated in a detailed *in vivo*-SAR study, and variation of substituents in both aromatic moieties afforded promising derivatives with broad insecticidal activity against lepidopteran and coleopteran species. *In vitro* measurements supported by modeling studies indicate that heterolignans investigated in our study act as potent tubuline polymerization inhibitors interacting with the colchicine-binding site. Various unprecedented substituents have been introduced to fully explore the structure-insecticidal activity relationship. Thus, insect specific structure-activity effects were observed showing that the insecticidal SAR described herein differs from reported cytotoxicity studies.



[1] J. Frackenpohl et al., *WO 05097802*, **2005**.
[2] J. Frackenpohl et al., *Bioorg. Med. Chem.*, **2009**, *in press.*

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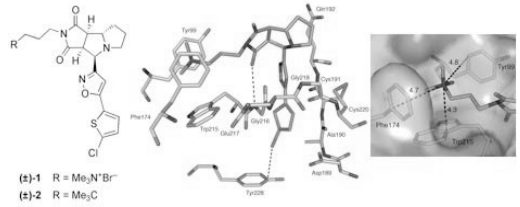
Cation–π Interactions in the S4 Pocket of Factor Xa

Laura M. Salonen[a], David W. Banner[b], François Diederich[a]

[a] ETH Zürich, HCI, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland
[b] F.Hoffmann-La Roche Ltd., PRBD-E, Molecular Structure, Bldg 65/312, 4070 Basel, Switzerland

Our group has been involved in the investigation of the cation–π interaction in factor Xa [1]. Recently, we redesigned our ligand system in order to comprehensively investigate the nature of this interaction [2].
Inhibitor (±)-**1**, bearing a quaternary ammonium ion, has a K_i value in the single-digit nanomolar range (9 nM), the *tert*-butyl derivative (±)-**2** binds less strongly (K_i = 550 nM). The X-ray crystal structure of (±)-**1** in complex with factor Xa was solved (PDB code: 2JKH, 1.25 Å) [2].



The size of the S4 pocket was explored. Finally, the dependence of the degree of N-methylation on the strength of the cation–π interaction was investigated [2].

[1] K. Schärer, M. Morgenthaler, R. Paulini, U. Obst-Sander, D. W. Banner, D. Schlatter, J. Benz, M. Stihle, F. Diederich, *Angew. Chem. Int. Ed.* **2005**, *44*, 4400–4404.
[2] L. M. Salonen, C. Bucher, D. W. Banner, W. Haap, J.-L. Mary, J. Benz, O. Kuster, P. Seiler, W. B. Schweizer, F. Diederich, *Angew. Chem. Int. Ed.* **2009**, *48*, 811–814.

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Design and Characterization of an In Vivo Active BACE Inhibitor

Rainer Machauer, Siem Veenstra, Kurt Laumen, Marina Tintelnot-Blomley, Jean-Michel Rondeau, Oliver Simic, Claudia Betschart, Kaspar Zimmermann, Paolo Paganetti, Ulf Neumann

Novartis Institutes for BioMedical Research
GDC Neuroscience, CH-4002 Basel, Switzerland

Over the last decades the pathological features of Alzheimer's disease (AD), amyloid plaques and fibrillary tangles were well characterized and the major components of the plaques identified as β-amyloid peptides (Aβ40/42). It is widely accepted that the monomers or oligomers of Aβ40/42 are neurotoxic and initiate the cascade of events responsible for the neuronal degeneration. The Aβ40/42 peptides are generated from the β-amyloid precursor protein (APP) by the sequential proteolytic action of the β- and γ-secretase enzymes. Inhibition of the membrane-bound aspartyl protease β-secretase (or BACE, β-site amyloid precursor protein cleaving enzyme) is widely considered to be one of the most promising therapeutic approaches for AD. Endoproteases like BACE achieve nanomolar binding affinities by using multiple binding interactions along the substrate backbone within the active-site cleft. For this reason inhibitors of endoproteases often have a high molecular weight (>500 Da) which usually triggers considerable efforts to optimize their pharmacokinetic properties. In contrast to inhibitors of peripheral proteases a BACE inhibitor has also to pass an additional hurdle, the blood-brain barrier. We present the results of some of our efforts in the field of BACE inhibition. Starting from the natural peptide substrate we were able to reduce this to a three amino acid lead structure. Co-crystallization of the lead with BACE and X-ray analysis led to the structure based design of novel less peptidic BACE inhibitors. This class of compounds was optimized to highly potent and cellularly active inhibitors. One of these compounds showed in vivo efficacy in a transgenic mouse model of AD after i.v. application and allowed a proof of mechanism. The morphing of the peptidic substrate to the inhibitor and first in vivo results will be discussed.

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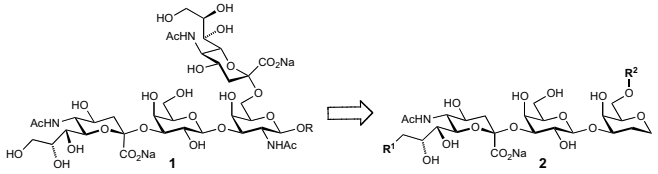
Examination of the biological role of sialic acid in gangliosides binding to the myelin-associated glycoprotein (MAG)

Oliver Schwardt[1], Heiko Gäthje[2], Angelo Vedani[1], Stefanie Mesch[1], Gan-Pan Gao[1], Sørge Kelm[2], Beat Ernst[1]*

[1] Institute of Molecular Pharmacy, Pharmacenter, University of Basel CH-4056 Basel, Switzerland
[2] University of Bremen, D-28334 Bremen, Germany

The myelin-associated glycoprotein (MAG), a member of the siglec family [1], has been identified as a potent inhibitor of neurite outgrowth of mature nerve cells in the central nervous system. The tetrasaccharide **1** was determined as main binding epitope of potent MAG antagonists like GQ1bα [2] and was therefore selected as starting point for a lead optimization program.



Modifications **2** of the core disaccharide, as well as of the α(2→3)- and the α(2→6)-linked sialic acid were synthesized and tested in a competitive binding assay and in surface plasmon resonance experiments [3]. The K_Ds of the most potent antagonists are in the low micromolar range which was confirmed by molecular modeling studies. This new class of glycomimetics will allow to validate the role of MAG in the axon regeneration process.

[1] Crocker, P.R. *Curr. Opin. Struct. Biol.* **2002**, *12*, 609.
[2] Bhunia, A.; Schwardt, O.; Gäthje, H.; Gao, G.-P.; Kelm, S.; Benie, A. J.; Hricovini, M.; Peters, T.; Ernst, B. *ChemBioChem* **2008**, *9*, 2941.
[3] Schwardt, O.; Gäthje, H.; Vedani, A.; Mesch, S.; Gao, G.-P.; Spreafico, M.; von Orelli, J.; Kelm, S.; Ernst, B. *J. Med. Chem.* **2009**, *52*, 989.

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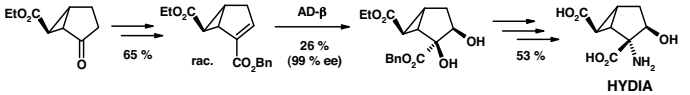
Asymmetric Synthesis and Receptor Pharmacology of the Group II mGlu Receptor Ligand (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid – HYDIA

Thomas J. Woltering,* Linda Lundström, Geo Adam, Philipp Huguenin, Jürgen Wichmann, Edilio Borroni, Frédéric Knoflach, Silvia Gatti and Bernd Kuhn

Pharmaceutical Division, Discovery Research CNS & Medicinal Chemistry, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland

The asymmetric synthesis and receptor pharmacology of (1*S*,2*R*,3*R*,5*R*,6*S*)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (HYDIA) is described. The key step of the synthesis utilizes Sharpless' asymmetric dihydroxylation (AD-β) for the kinetic resolution of a bicyclic racemic precursor olefin. In contrast to the bicyclic glutamate analogue LY354740, which is a potent and selective agonist for the group II metabotropic glutamate receptors (mGluRs), this new conformationally restricted and also hydroxylated glutamate analogue is a potent and selective antagonist for the group II mGluRs.[1]



We also synthesized ^{3}H-HYDIA by which we could determine its binding to mGlu2 with the aid of mutagenesis studies and computational modelling, revealing that HYDIA is proposed to bind in an open conformation model of mGlu2.[2]

[1] T. J. Woltering, G. Adam, P. Huguenin, J. Wichmann, S. Kolczewski, S. Gatti, A. Bourson, J. N. C. Kew, G. Richards, J. A. Kemp, V. Mutel, F. Knoflach, *ChemMedChem* **2008**, *3*, 323.
[2] L. Lundström, B. Kuhn, J. Beck, E. Borroni, J. G. Wettstein, Thomas J. Woltering, S. Gatti, *ChemMedChem* **2009**, *4*, *accepted*

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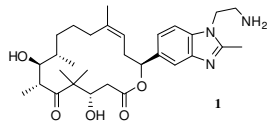
Total Synthesis of New Functionalized Epothilone Analogs for Prodrug Design and Tumor Targeting

S. Anthoine Dietrich, J. Gertsch, K.-H. Altmann

Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, HCI, CH-8093 Zurich, Switzerland

Epothilones are microtubule-stabilizing natural products which exhibit strong antiproliferative effects *in vitro* and potent antitumor activity *in vivo*, including tumor growth inhibition in multi-drug resistant human tumor models.[1] Epothilones have served as successful lead structures for anticancer drug discovery and several epothilone-derived agents have entered clinical trials in humans. However, the therapeutic utility of epothilones would benefit greatly from an increase in their selectivity for tumor cells, which would reduce side effects and widen their therapeutic window. In this context we have devised and synthesized novel epothilone analog **1**, with the goal of using the newly introduced primary amino group as an attachment site for various tumor-targeting moieties, such as folic acid[2] or high molecular weight PEG[3], which could be conjugated to core structure **1** either irreversibly or through an enzymatically cleavable linker.
In this presentation we will discuss the total synthesis of epothilone analog **1** and of some selected tumor-targeted derivatives. The in vitro biological activity of these new epothilone analogs and conjugates will be discussed with respect to their interactions with the tubulin/microtubule system and the inhibition of human cancer cell proliferation.



1

[1] Goodin, S.; Kane, M. P.; Rubin, E. H. *J. Clin. Onc.* **2004**, *22(10)*, 2015;
[2] Leamon, C. P.; Reddy, J. A. *Adv. Drug Delivery. Rev.* **2004**, *56*, 1127;
[3] Maeda, H.; Greish, K.; Fang, J. *Adv. Polym. Sci.* **2006**, *193*, 103.

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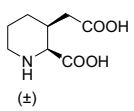
Synthesis and biological evaluation of novel NMDA receptor and glutamate transporter ligands guided by virtual screening of the chemical universe database GDB

Erika Luethi, Kong Thong Nguyen, Jean-Louis Reymond*

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

GDB is an exhaustive library of molecules up to 11 C, N, O, F atoms possible considering simple chemical stability and synthetic feasibility rules [1]. For the discovery of novel bioactive small molecules, virtual hit libraries for the NMDA receptor and the glutamate transporter EAAT2 were generated from GDB using docking. These targets are of growing importance in medicine and biology due to their implication in many neurodegenerative diseases [2][3].
Herein we present the synthesis and biological evaluation of yet unknown but readily feasible molecules from these virtual hit libraries, e.g. kainic acid analogue **1**. Among these compounds, one inhibitor of NMDA receptor glycine site (2 [4]) and blockers of EAAT2 were identified.



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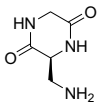

2

[1] T. Fink, H. Bruggesser, J.-L. Reymond, *Angew. Chem. Int. Ed.* **2005**, *44*, 1504.
[2] E. A. Waxman, D. R. Lynch, *Neuroscientist* **2005**, *11*, 37.
[3] N. J. Maragakis, J. D. Rothstein, *Neurobiology of Disease* 2004, *15*, 461.
[4] K. T. Nguyen, E. Luethi, S. Syed, S. Urwyler, S. Bertrand, D. Bertrand, J.-L. Reymond, *Bioorg. Med. Chem. Lett.* **2009**, http://dx.doi.org/10.1016/j.bmcl.2009.04.021.

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Molecular Recognition – A drug designers' guide of non-covalent protein-ligand interactions

Caterina Bissantz

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

Successful structure-based drug design requires a thorough understanding of typical geometries and strengths of non-covalent protein-ligand interactions. One possibility to derive such knowledge is by statistical analysis of high-resolution crystal structures of small molecules and protein-ligand complexes.
In this work, we derived typical geometries of non-covalent interactions by analysis of the histograms of their geometric descriptors which were obtained by searches of entries of the Cambridge Structural Database (CSD) [1].
The initial searches were carried out on small molecule crystal structures (CSD) and not on protein-ligand crystal structures. The reason for this is the assumed higher rate of errors in the coordinates of protein-ligand complexes compared to small molecule structures. However, in the second step, the CSD values were compared with the geometries observed in protein-ligand complexes to validate their relevance for drug design.
We then calculated the frequency of each individual interaction as the ratio between the number of entries where an interaction is observed in its typical geometry and the number of those entries where this interaction could be formed due to the presence of the required functional groups. This allowed a simple ranking of the non-covalent interactions according to their observed frequencies.

[1] Allen, F. H. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *B58*, 380-388.

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The H1 Domain of the Asialoglycoprotein Receptor: Towards a Structural Understanding of Receptor-Mediated Endocytosis

Brian Cutting[1], Karin Johansson[1], Helena Kovacs[2], Said Rabbani[1] and Beat Ernst[1]

[1] University of Basel, Institute of Molecular Pharmacy, Pharmacenter, CH-4056 Basel, Switzerland
[2] Bruker Biospin AG, CH-8117 Fällanden, Switzerland

The asialoglycoprotein receptor (ASGP-R) is a C-type lectin located on hepatocytes. The ASGP-R consists of two homologous subunits, designated H1 and H2, which form a non-covalent heterooligomeric complex. Its main function is to maintain serum glycoprotein homeostasis through the endocytosis of asialoglycoproteins, which lack a terminal sialic acid and present galactose residues on their glycan structure. Following internalization via clathrin-coated pits, the endocytosed glycoproteins are released from ASGP-R in the lower pH of the endosome. Our efforts in understanding the mechanism of ASGP-R mediated endocytosis focus upon H1, for which crystallographic data exists.

Although both Gal and Gal*N*Ac bind to H1, Gal*N*Ac does so with an order of magnitude greater affinity. Herein, the preference of H1 for Gal*N*Ac is demonstrated by the pharmacophore identified by saturation transfer difference NMR. Through an HSQC titration of H1, isotopically enriched in ^{15}N and ^{13}C, with Gal*N*Ac, the binding is shown to be specific and the K_D determined. The key residues in H1 responsible for binding Gal*N*Ac were identified through three-dimensional NMR experiments of the labeled receptor.

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11βHSD1 Inhibitors for Type 2 Diabetes

Alexander Mayweg, Kurt Amrein, Daniel Hunziker, Bernd Kuhn, Werner Neidhart, Michael Otteneder, Armin Ruf, Stefanie Tozzo, Philippe Verry.

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

11β-HSD1 converts inactive cortisone into the active glucocorticoid, cortisol, a natural antagonist of insulin action. Human clinical studies have suggested an active role for cortisol in the pathogenesis of human type 2 diabetes, and recent data from preclinical studies have revealed that 11β-HSD1 and local glucocorticoid production play a critical role in mediating the initiation of insulin resistance and progression to diabetes. Therefore, selective inhibitors of 11β-HSD1 may provide a new class of drugs to treat type 2 diabetes as well as conditions often associated with this disease, such as dyslipidemia, atherosclerosis, and coronary heart disease. Lead structures for the HSD1 program were generated using a combined approach of HTS, hits, X-ray and molecular modeling. Low nanomolar and selective inhibitors were designed which were optimized for physicochemical properties.

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Identification of a member of a novel galactosyltransferase family unravels the molecular basis of the nematotoxicity of galectin CGL2

Alexander Titz[1], Alex Butschi[2], Katharina Paschinger[3], Katharina Nöbauer[4], Thierry Hennet[5], Bernard Henrissat[6], Michael Hengartner[2], Iain B.H. Wilson[3], Markus Künzler[1], and Markus Aebi[1]

[1] Institute of Microbiology, ETH Zürich; [2] Institute of Molecular Biology, University of Zürich; [3] Department of Chemistry, University of Natural Resources and Applied Life Sciences, Vienna; [4] VetOMICS, University of Veterinary Medicine, Vienna; [5] Institute of Physiology, University of Zürich; [6] CNRS and Universités Aix-Marseille I & II;

Galectin CGL2 from the fungus *Coprinopsis cinerea* displays toxicity towards the model nematode *Caenorhabditis elegans* and thus may be part of a lectin-mediated defense system of fungi against predators and parasites. In a genetic screen, several CGL2-resistant *C. elegans* mutant strains were isolated. All identified mutations were located in genes affecting *C. elegans N*-glycan core modification. Besides a mutation in the *fut-8* gene encoding the core alpha-1,6-fucosyltransferase, a mutation in a putative galactosyltransferase gene was identified. Comparative *N*-glycome analysis of the nematode mutants indicated the loss of a hexose linked to the alpha-1,6-linked core fucose in *N*-glycans of the putative galactosyltransferase mutant strain. Heterologous expression and enzymatic characterisation of the putative galactosyltransferase confirmed the function of the enzyme. High selectivity for the 1,6- over 1,3-linked *N*-glycan core fucosides was determined together with additional structural requirements for enzyme activity. The data suggests the analyzed galactosyltransferase to be responsible for the synthesis of core galactose epitopes in *C. elegans N*-glycans and that recognition of these glycan epitopes mediates the nematotoxicity of CGL2.
Classification of the protein sequence shows that this enzyme constitutes a novel family of glycosyltransferases. Interestingly, its closest homolog is found in a human pathogen. Consequently, the knowledge gained from our studies might lead to vaccines or functional inhibitors to fight this pathogen.

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Determination of the lipophilicity of Cyclosporin A by UHPLC

Amandine Guillot, Yveline Henchoz, Davy Guillarme, Jean Luc Veuthey, Pierre-Alain Carrupt, Sophie Martel

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

Lipophilicity, a key parameter in the study of pharmacokinetic properties of new chemical entities (NCE), has to be evaluated for a large number of compounds in the early stages of drug discovery. Therefore the development of high throughput methods to determine partition coefficients is an important challenge in pharmaceutical research. In this context, RP-LC methods, based on correlation between lipophilicity and retention factors, have been largely used for the determination of log P_{oct} of neutral, acidic and basic compounds presenting moderate lipophilicity ($0<\log P_{oct}<5$). However, these methods remain limited for highly lipophilic compounds due to high analysis time. Recently, a method based on ultra-high pressure liquid chromatography (UHPLC) has permitted the determination of partition coefficients with a drastic decrease in analysis time [1]. Moreover this technique appears to be a promising way to determine high log P_{oct} values. Different stationary phases and experimental conditions were therefore tested on highly lipophilic compounds (log P up to 8) [2]. The two best methods were then applied to a series of cyclopeptides and especially to the lipophilic Cyclosporin A (CsA) which has shown different lipophilicity behaviours depending on the method used.

References

[1] Henchoz Y., Guillarme D., Rudaz S., Veuthey J.L., Carrupt P.A., *J. Med. Chem*, **2008**, *51*, 396.
[2] Guillot A., Henchoz Y., Moccand C., Guillarme D, Veuthey J.L., Carrupt P.A, Martel S., *Chemistry & Biodiversity,* **2009,** *in press*.

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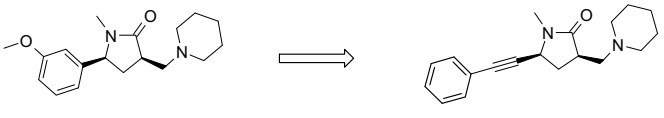
Gamma-Lactams – A Novel Scaffold for Highly Potent and Selective α7 Nicotinic Acetylcholine Receptor Agonists

Albert Enz[a], Dominik Feuerbach[a], Mathias U. Frederiksen[b], Conrad Gentsch[a], Konstanze Hurth[b], Werner Müller[b], Joachim Nozulak[b], Bernard L. Roy[b]

[a]Novartis Institutes for Biomedical Research, Neuroscience Disease Area, 4002 Basel, Switzerland; [b]Novartis Institutes for Biomedical Research, Global Discovery Chemistry-Neuroscience, 4002 Basel, Switzerland

A novel class of α7 nicotinic acetylcholine receptor (nAChR) agonists has been discovered through high-throughput screening. The *cis* γ-lactam scaffold has been optimized to reveal highly potent and selective α7 nAChR agonists with *in vitro* activity and selectivity and with good brain penetration in mice.



HTS hit
pEC_{50} = 5.9
Emax = 128

Optimized γ-Lactam
α7 pEC_{50} = 8.0
E_{max} = 104
α3b4 Selectivity ratio 126
α4b2 Selectivity ratio 820
α1β1γδ Selectivity ratio 1299
$5HT_3$ Selectivity ratio 126

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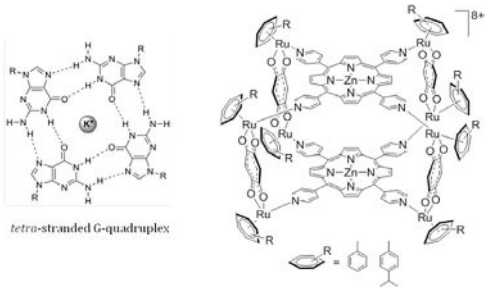
Supramolecular Cubes as Selective Quadruplex DNA Binders.

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

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

One of the main challenges in cancer chemotherapy is developing drugs that are selective towards cancer cells in order to reduce the general toxicity, resistance mechanisms and consequently the side effects of the treatment. One such targeting method involves telomerase inhibition by stabilization of G-quadruplexes. Conventional G-quadruplex binders are planar molecules with large π-aromatic surfaces and positive charges.[1]



Recently, we have been involved in the development of new metal-based complexes as telomerase inhibitors.[2] Our strategy is to exploit supramolecular self-assembly to generate new quadruplex binders. The interactions of these supramolecular assemblies with duplex and human telomeric quadruplex DNA are presented. The results show that these ruthenium octacationic coordination cubes are excellent quadruplex DNA stabilizers with a high degree of selectivity for quadruplex over duplex DNA.

[1] Neidle, S., Balasubramanian, S., *Quadruplex Nucleic Acids,* RSC Publishing, Cambridge, **2006**.
[2] Barry, N.P.E., Abd Nurul, N., Vilar, R., Therrien, B. *in preparation*.

73

In vitro and *in vivo* studies of new photoluminescent oxygen sensors for non-invasive intravascular pO_2 measurements

Patrycja Nowak-Sliwinska, Hubert van den Bergh, Georges Wagnières

Swiss Federal Institute of Technology, EPFL, Lausanne, Switzerland

The rate of oxygen consumption during PDT plays an important role for the determination of the optimal therapeutic dose. One of the methods to measure the tissular oxygen partial pressure (pO_2) is based on the use of luminophores presenting an oxygen-dependent quenching of their phosphorescence. The time-resolved luminescence spectroscopy of palladium (PdTCPP) and ruthenium (RuDPP) porphyrin complexes exhibit a strong sensitivity to the pO_2. Unfortunately, they are phototoxic and leak rapidly from the vessels.

Therefore, this research aimed at developing of biocompatible and non-phototoxic oxygen sensors based on PdTCPP and RuDPP complexes incorporated into oxygen permeable nanoparticles (polysaccharide-based) or copolymer vesicles to perform non-invasive *in situ* and *in vivo* measurements of the pO_2. *In vitro* and *in vivo* studies were performed with an optical fiber-based time-resolved spectrophotometer. *In vivo* studies were conducted after intravenous injection of the pO_2 probes - loaded nanovectors in the chick's embryo chorioallantoic membrane (CAM) model observed with an epi-fluorescence microscope under different oxygenation and PDT conditions.

In vitro studies showed that the incorporation of the pO_2 probes in nanovectors induce a diminished sensitivity to oxygen by less than one order of magnitude. However, biocompatibility studies demonstrated that the oxygen probe luminescence tends to be heterogeneous and to induce 'clumping tendency' resulting in a more or less decreased viability of the embryos. Our *in vivo* experiments conducted under low oxygenation conditions resulted in a strong enhancement of the luminescence thus confirming previous *in vitro* studies.

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Discovery of Novel Triazole Nucleoside Analogues as Antiviral and Anticancer Candidates

Yi Xia[1], Jinqiao Wan[2], Yang Liu[2], Menghua Wang[2], Palma Rocchi[3], Fanqi Qu[2], Johan Neyts[4], Juan L. Iovanna[3], Ling Peng[1*]

[1]CNRS UMR 3118, 163, avenue de Luminy, 13288 Marseille, France; [2]College of Chemistry and Molecular Sciences, Wuhan University, 430072 Wuhan, P. R. China; [3]INSERM U624, 163, avenue de Luminy, 13288 Marseille, France; [4]Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Synthetic nucleoside analogs are of considerable importance in the search for new structural leads with antiviral and anticancer activity. In our ongoing research on triazole compounds, we have developed structurally novel triazole nucleosides in an attempt to identify new antiviral and anticancer drug candidates. Among these novel ribonucleoside, compound **1** (Figure 1) can inhibit hepatitis C virus (HCV) replication efficiently [1][2], whereas compound **2** (Figure 1) demonstrated potent apoptosis-induced anti-proliferative activity against pancreatic cancer cells both *in vitro* and *in vivo*, with no adverse effect [1][3]. These aryltriazole compounds therefore constitute promising leads in the search for new antiviral and anticancer candidates.

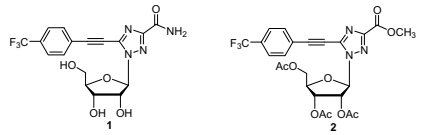

Figure 1: Triazole nucleosides with selective antiviral (**1**) and anticancer (**2**) activity.

[1] J. Q. Wan, Y. Xia, Y. Liu, M. H. Wang, P. Rocchi, J. H. Yao, F. Q. Qu, J. Neyts, J. L. Iovanna, L. Peng, *J. Med. Chem.* **2009**, *52*, 1144.
[2] L. Peng, F. Q. Qu, R. Z. Zhu, J. Neyts. Patent PCT/BE2008/000059.
[3] Y. Xia, J. Q. Wan, P. Rocchi, F. Q. Qu, J. L. Iovana, L. Peng, Patent EU 08 155481.8.

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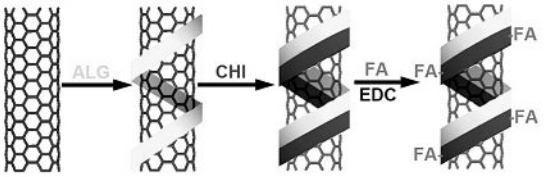
Double Polysaccharide layers coated Single Wall Carbon Nanotubes for Targeted Delivery of Doxorubicin into HeLa Cells

Zhang Xiaoke, Meng Lingjie, Lu Qinghua

School of Chemistry and Chemical Technology, Shanghai Jiaotong University, 200240, Shanghai, P.R. China.

Single wall carbon nanotubes (SWCNTs) wrapped by positively charged chitosan (CHI) and negatively charged alginate sodium (ALG) have been successfully prepared via a layer-by-layer assembly process. The obtained SWCNT-polysaccharide complex, termed as CHI/ALG-SWCNTs can load doxorubicin (DOX) via π-π stacking and electrostatic interaction at pH 7.4, forming new complex containing DOX (termed as DOX-CHI/ALG-SWCNTs). The DOX-CHI/ALG-SWCNTs can release DOX at pH 5.5 (lysosomal pH). After bounding folic acid (FA), a targeted agent for many tumors, onto CHI/ALG-SWCNTs by amidation reaction, the new FA functionalized complex termed as FA-CHI/ALG-SWCNTs can targetedly deliver DOX into the lysosomes of HeLa cells with much higher efficiency compared to the free DOX and CHI/ALG-SWCNTs, and then gradually release DOX at lysosomal pH to bind with nuclear DNA and inhibit the cellular proliferation.



Scheme 1 Modification of SWCNTs with ALG, CHI and FA.

[1] L. Li, L. Meng, X. Zhang, et al., *J. Mater. Chem.* DOI: 10.1039/b823322e.
[2] J. Liu, A. G. Rinzler, H. J. Dai, et. al., *Science* **1998**, 280, 1253.

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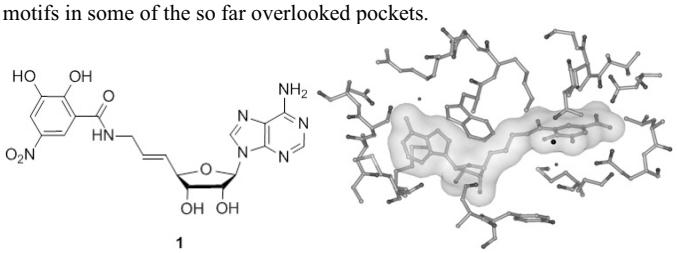
Novel Bisubstrate Inhibitors of Catechol-*O*-Methyltransferase (COMT)

Manuel Ellermann[a], Ralph Paulini[a], Edilio Borroni[b], Gerhard Zürcher[b], Roland Jakob-Roetne[b], François Diederich*[a]

[a]Laboratorium für Organische Chemie, ETH Zürich, Wolfgang-Pauli-Str. 10, 8093 Zürich
[b]Pharma Division, Präklinische Forschung, F. Hoffman-La Roche AG, Grenzacherstr. 124, 4070 Basel

Catechol-*O*-Methyltransferase (COMT) is one of the key enzymes involved in catecholamine catabolism. Therefore it is a valuable target for the treatment of CNS disorders such as Parkinson's disease [1]. Highly potent bisubstrate inhibitors (e.g. **1**, right: X-ray crystal structure with COMT) have been developed by *de novo* design [2], however their interactions, in some important binding sites, remains unexplored [3]. In ongoing work we have synthesized novel bisubstrate inhibitors in order to investigate the binding motifs in some of the so far overlooked pockets.



1

[1] P. T. Männistö, S. Kaakola, *Pharmacol. Rev.* **1999**, 51, 593-628.
[2] C. Lerner, A. Ruf, V. Gramlich, B. Masjost, G. Zürcher, R. Jakob-Roetne, E. Borroni, F. Diederich, *Ang. Chem. Int. Ed.* **2001**, 40, 4040-4042.
[3] R. Paulini, C. Trindler, C. Lerner, L. Brändli, W. B. Schweizer, R. Jakob-Roetne, G. Zürcher, E. Borroni, F. Diederich, *ChemMedChem* **2006**, 1, 340-357.

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New Silver-coated surfaces for self-protecting implants

Priscilla. S. Brunetto, Tünde Vig Slenters, Katharina. M. Fromm*

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

The development of new bioactive surfaces represents a solution to a medical problem: bacterial adhesion on surfaces, mainly by S*taphylococci*. The resultant biofilm is resistant to aggressive pharmacological agents as well as host defences [1].
Silver compounds are known to possess antimicrobial properties and the current revival of silver chemistry in this context initiated us to use coordination polymer compounds with Ag^+ [2-5] for coating purposes and for their application as antimicrobial compounds.
Having control over the polymorph formation, and thus structure and light stability, we were able to deposit Ag-coordination polymer networks on surfaces such as gold and titanium. The so-coated surfaces were exposed to bacteria in different assays [6] and tested for their biocompatibility for soft- and hard-tissue integration. These different *in vitro* tests have turned out to be very promising for further investigations and use as antimicrobial compounds. This may be an efficient solution conquering bacterial adhesion and biofilm.

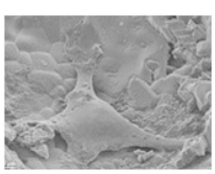
[1] B. Jose, V. Antoci, A. R. Zeiger, E. Wickstrom, N. J. Hickok, C*hem. & Biol.*. **2005**, *12*, 1041.
[2] A. Y. Robin, J. L. Sague Doimeadios, A. Neels, T. Vig Slenters, K. M. Fromm. *Inorg. Chim. Acta*. **2007**, *360*, 212.
[3] A. Y. Robin, K. M. Fromm. *Coord. Chem. Rev*. **2006**, *250*, 2127.
[4] A. Y. Robin, J. L. Sague, K. M. Fromm, Cryst. Eng. Comm. **2006**, *8*, 403.
[5] P. S. Brunetto, K. M. Fromm. *Chimia*. **2008**, *62*, 249.
[6] T. Vig Slenters, I. Hauser-Gerspach, A. U. Daniels, K. M. Fromm. *J. Mat. Chem.* **2008**, *18*, 5359.

78

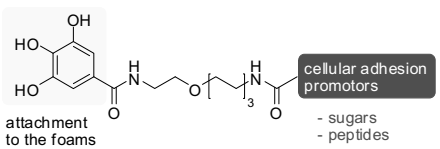
Functionalization of particle-stabilized foams for bio-applications

Françoise Borcard,[1] Hélène Fiaux,[1] Franziska Krauss Juillerat,[2] Sandrine Gerber-Lemaire,[1] Lucienne Juillerat-Jeanneret[3] and Urs Gonzenbach[2]
[1] *ISIC, BCH, EPFL, CH-1015 Lausanne, Switzerland*
[2] *Department of Materials, ETH-Zürich, Zürich, Switzerland*
[3] *University Institute of Pathology, CHUV, Lausanne, Switzerland*

Improved biological and mechanical functionality of musculoskeletal tissue-engineered constructs is required for clinical application. As vascular arrangement precedes and dictates the architecture of the new bone, preconstruction of an appropriate vascular network in a scaffold displaying pore size and surface architecture conductive for the stabilization and maintenance of functional vessels is a prerequisite to improved bone substitutes in which the vascular network must be prepared first. Following the recent development of new particle-stabilized foams [1] that allow the proliferation and colonization of human endothelial cells (HCEC) within the porous material,[2] we present here the synthesis of gallate derivatives containing cellular adhesion promoting entities for their chemical functionalization. Preliminary biological evaluation of the resulting conjugates indicates their attachment to the foams.



HCEC cell inside a foam pore



[1] a) Gonzenbach, U. T.; Studart, A. R.; Tervoort, E.; Gauckler, L. J. *Angew. Chem. Int. Ed.* **2006**, *45*, 3526. b) Krauss Juillerat, F.; Elser, P.; Gonzenbach, U. T.; Studart, A. R.; Gauckler, L. J. *J. Europ. Ceram. Soc*. **2009**, submitted.
[2] Krauss Juillerat, F.; Gauckler, L. J.; Scaletta, C.; Gerber-Lemaire, S; Applegate. L. A.; Juillerat-Jeanneret, L.; Gonzenbach, U., submitted.

79

Identification of a new scaffold for $GABA_A$ receptors: a preliminary SAR study with piperamides in a black pepper extract

Janine Zaugg[1], Igor Baburin[2], Steffen Hering[2], Matthias Hamburger[1]

[1]University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland
[2]University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Pharmacotherapy with substances acting at the $GABA_A$ receptor, such as benzodiazepines and non-benzodiazepines, is accompanied by well-known side effects resulting from insufficient target selectivity for different $GABA_A$ receptor subtypes. Highly selective $GABA_A$ receptor ligands are therefore an unmet medical need [1]. In a program for discovery of new scaffolds for $GABA_A$ receptor modulating compounds, a library of 880 plant and fungal extracts was screened in a functional assay using *Xenopus laevis* oocytes which transiently express recombinant $GABA_A$ receptors. An ethyl acetate extract of black pepper (*Piper nigrum* L., Piperaceae) fruits significantly potentiated GABA induced chloride-ion current through $GABA_A$ receptors of defined subunit composition ($\alpha_1\beta_2\gamma_{2S}$). A combination of HPLC based activity profiling [2], LC-PDA-TOFMS and offline microprobe NMR analysis allowed rapid identification of the active compounds with mg-amounts of extract. The major active compound was identified as piperine, while dihydropiperine showed less activity. Another 11 structurally related, weakly active or inactive amides were also characterized. Structural features critical for $GABA_A$ receptor agonistic activity of the piperine scaffold could be identified by the combination of structural and pharmacological data of this compound series.

[1] Whiting, P.J., *Curr. Opin. Pharmacol.* **2006**, *6*, 24.
[2] Hamburger, M., Potterat, O., *Curr. Org. Chem.* **2006**, *10*, 899.

80

Mimetics of sLe^x as P-selectin antagonists

Florian Binder , Katrin Lemme, Oliver Schwardt, Said Rabbani, Beat Ernst

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

The interaction of E-, P- and L-selectin with their natural carbohydrate ligands has been shown to mediate the initial step of the recruitment of leukocytes to sites of inflammation. Therefore, selectins play a crucial role in many physiological processes and disease states. The inhibition of the leukocyte-endothelial cell adhesion process offers a potent therapeutic approach in cases where excessive recruitment of leukocytes is involved, as in stroke, reperfusion injury, psoriasis, asthma and rheumatoid arthritis [1].
The common binding epitope of all physiological selectin ligands is the tetrasaccharide sialyl Lewis^x, which makes it a lead structure for the design of selectin antagonists [2].
In order to enhance the affinity towards P-selectin, the core of sLe^x is rigidified by replacement of Glc*N*Ac with various mimics, a concept that has already been applied successfully in the case of E-selectin antagonists [3].

[1] Vestweber, D.; Blanks, J. E.; *Physiol. Rev.* **1999**, *79*, 181-213.
[2] Kaila, N.; Thomas, B.E.; *Med. Res. Rev.* **2002**, *22*, 566-601.
[3] Thoma, G.; Magnani, J. L.; Patton, J. T.; Ernst, B.; Jahnke, W.; *Angew. Chem. Int. Ed.* **2001**, *40*, 1941-1945.

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Computational Study of the Enzymatic Reaction Mechanism in Dengue Methyltransferase

Tobias Schmidt, Torsten Schwede, Markus Meuwly

Swiss Institute of Bioinformatics, Basel,
Department of Chemistry & Biozentrum, University of Basel,
Klingelbergstrasse 50/80, CH-4056 Basel, Switzerland

Dengue fever is a mosquito-borne viral infectious disease predominantly prevalent in tropical regions with annually 50−100 million cases and around 25000 deaths worldwide. It is one of the most important emerging infectious diseases in many areas of the world. Currently, no vaccines or specific drug treatments are available.[1]
The dengue virus is a single-stranded, positive sense RNA virus belonging to the *Flavivirus* genus of the *Flaviviridae* family. The 5' end of the dengue genome contains a type 1 cap structure which is essential for viral replication by enhancing RNA stability and increasing translation efficiency.[2] During RNA cap formation, a viral methyltransferase (NS5MTase) is required for the transfer of a methyl group to the nascent viral RNA cap structure. Hence, this MTase is an attractive target for drug discovery.[3,4]
The detailed enzymatic reaction mechanism of the methyl transfer from S-adenosyl-L-methionine to the viral RNA cap structure, catalyzed by NS5MTase, has not been elucidated so far. Thus, in this work, we investigate possible reaction pathways at an atomistic level, using computer simulations. We aim at a better understanding of the molecular basis of this disease related enzymatic function, which significantly benefits rational drug discovery and could ultimately lead to highly active transition-state analogue inhibitors.

[1] D.J. Gubler, *Novartis Found. Symp.*, **2006**, *277*, 3.
[2] G.R. Cleaves *et al.*, *Virology*, **1979**, *96*, 159.
[3] M.P. Egloff *et al.*, *Embo Journal*, **2002**, *21*, 2757.
[4] M. Podvinec *et al.*, *in preparation.*

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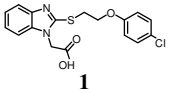
THIOBENZIMIDAZOLE-1-ACETIC ACIDS AS CRTH2 ANTAGONISTS

J. Pothier, A. Fecher, M. Gude, K. Hilpert, M. A. Riederer, X. Leroy, O. Peter, H. Kletzl, C. Gnerre, R. Bravo, S. Delahaye, L. Piali, S. Froidevaux, H. Fretz.

Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland

The human chemoattractant receptor-homologous molecule expressed on T-helper 2 cells (hCRTH2) is expressed on eosinophils, basophils, and T-helper 2 lymphocytes. The CRTH2 receptor belongs to the G-protein coupled receptor family. Upon its activation by prostaglandin D2 (PGD2) it plays a role in the chemotactic recruitment of granulocytes and Th2 cells to inflammation sites. Therefore, CRTH2 antagonists are expected to be valuable for the treatment of allergic inflammatory disorders [1][2].

High-throughput screening of our in-house compound collection for hCRTH2 antagonists by means of a FLIPR assay provided thiobenzimidazole-1-acetic acid **1** as a hit, antagonizing the hCRTH2 receptor with IC50 = 6.58 μM.



1

A straightforward synthetic route to analogues of **1** has been devised and a detailed structure activity relationship (SAR) could be established. In addition, pharmacokinetic results will be presented.

[1] Kostenis, E.; Ulven, T., *Trends Mol. Med.* **2006**, *12*, 148-158.
[2] Pettipher, R. et al., *Nat. Rev. Drug Discovery* **2007**, *6*, 313-325.

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Development of a new artificial membrane to predict the passive permeation through the blood-brain barrier using PAMPA

Céline Passeleu, Julien Boccard, Serge Rudaz, Pierre Alain Carrupt, Sophie Martel

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne; quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland

Inappropriate pharmacokinetic (PK) has been recognized as being one of the major factors leading to the withdrawal of new chemical entities (NCEs) from drug development. Therefore, a large number of compounds has to be screened before matching one drug candidate disclosing good ADMET (absorption, distribution, metabolism, elimination, toxicity) properties during the early stage of drug discovery. *In vitro* high throughput methods thus become tools of choice to assess compounds PK properties and in particular their ability to penetrate biological membranes such as the blood-brain barrier (BBB). Parallel artificial membrane permeability assay (PAMPA) is a high throughput technique developed to predict passive permeability through biological membranes, where a donor and an acceptor compartments are separated by a liquid artificial membrane. Depending on the nature of this membrane, different biological barriers can be targeted [1]. This technique has been already applied to BBB penetration studies using phospholipids allowing the ranking of compounds in two classes: compounds passively transported (CNS+) or not transported (CNS-) into the brain [2].
In this study, a membrane composed of octanol, ortho-nitrophenyloctylether (o-NPOE) and hexadecane has been evaluated and optimized to predict the passive permeation through the BBB using the PAMPA technique and avoiding the well-known drawbacks of the biological material.

[1] Y. Henchoz, B. Bard, D. Guillarme, P.-A. Carrupt, J.-L. Veuthez, S. Martel. *Anal. and Bioanal. Chem.* No pp. yet given. **2009**
[2] L.Di, E.H.Kerns, K.Fan, O.J.McConnell, G.T.carter, *Eur. J. Med. Chem.* **2003**, *38*, 223-232

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Synthesis and Evaluation of *N*-(3-Pyridyl)benzamides as Potent and Selective Inhibitors of Aldosterone Synthase (CYP11B2)

Carsten A. Vock, Marieke Hafner, Dominic Ammann, Michael Zender, Christina Ries, and Rolf W. Hartmann

FR 8.2 – Pharmaceutical and Medicinal Chemistry, Saarland University, D-66041 Saarbrücken, Germany

Aldosterone synthase (CYP11B2) is the key enzyme in the human biosynthesis of mineralocorticoids, catalyzing the three-step interconversion of 11-desoxycorticosteron to aldosterone via corticosterone and 18-hydroxycorticosterone.[1] Due to its pivotal role, CYP11B2 is claimed as useful target for the treatment of hyperaldosteronism, myocardial fibrosis and congestive heart failure.[2]

Recently, strong and successful efforts have been made to find highly potent and selective inhibitors of aldosterone synthase.[3] Herein, we report on synthesis and biological evaluation of *N*-(3-Pyridyl)benzamides as selective inhibitors of CYP11B2. Selectivity data - determined in our established *in-house* cellular-based test system using V79 chinese hamster cells stably transfected with either CYP11B2 or the highly homologous CYP11B1 enzyme – as well as additional activity data with other key enzymes of human steroid biosynthesis (CYP17, CYP19) will be presented.

[1] M. Bureik, M. Lisurek, R. Bernhardt, *Biol. Chem.* **2002**, *383*, 1537.
[2] R. W. Hartmann, U. Müller, P. B. Ehmer, *Eur. J. Med. Chem.* **2003**, *38*, 363.
[3] a) S. Lucas, R. Heim, C. Ries, K. E. Schewe, B. Birk, R. W. Hartmann, *J. Med. Chem.* **2008**, *51*, 8077; b) Q.-Y. Hu, G. M. Ksander (Novartis AG), **WO200807862**; c) P. Herold, R. Mah, S. Stutz, V. Tschinke, A. Stojanovic, N. Jotterand, B. Bennacer (Speedel Experimenta AG), **WO2008119744**.

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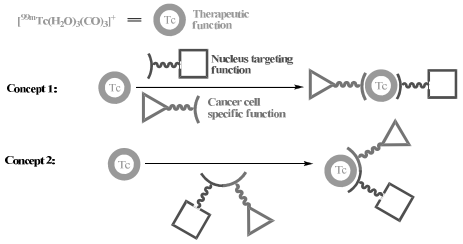
Nuclear Targeting With Intercalators

Karel Zelenka[1], Lubor Borsig[2], Roger Alberto[1]

[1]Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[2]Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

In nuclear medicine, various radionuclides are applied in diagnosis (^{99m}Tc, ^{18}F) and therapy (^{131}I, ^{153}Sm etc.). ^{99m}Tc emits, besides γ-emission, about 4 low energy Auger electrons (plus IC electrons) with very short range but, with high LET. Our aim is to specifically target cell nucleus (DNA)[1] as the most sensitive molecule in cells crucial for survival. The Auger electrons of ^{99m}Tc will cause, once incorporated into the cell nucleus, single- or double-strand break of DNA[2], thereby inducing cell death by apoptosis or necrosis. We explore a "building block approach" which allows a systematic replacement of all parts of the trifunctional molecule, aiming "ultimate" cell specific nuclear targeting complexes. The building block principle is given in scheme for the two different concepts we are investigating.



[1] Agorastos, N.; Borsig, L.; Renard, A.; Antoni, Ph.; Viola, G.; Spingler, B.; Kurz, Ph.; Alberto, R., *Chem. Eur. J.*, **2007**, *13,* 3842-3852.
[2] Häfliger, P.; Agorastos, N.; Spingler, B.; Georgiev, O.; Viola, G.; Alberto, R. *ChemBioChem* **2005**, *6*, 414-421.

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In vitro properties of PET imaging radiotracers

E. Briard[1a], D. Orain[1a], S. D. Kraemer[2], M. Honer[2], P. Maguire[1b], S. Ametamey[2], P. A. Schubiger[2], Y. Auberson[1a]

1) Novartis Institutes for BioMedical Research, (1a: Global Discovery Chemistry, 1b: BioMarker Development), 4002 Basel, Switzerland
2) ETH, Radiopharmaceutical Science, Institute of Pharmaceutical Sciences , 8093 Zurich, Switzerland

The development of novel radiotracers for Positron Emission Tomography (PET) remains limited by our ability to predict successful candidates. These must often be developed all the way to non-human primate PET studies before their clinical fitness is properly evaluated. The identification of *in vitro* properties that can predict the potential of a radiotracer candidate before radiosynthesis would reduce costs and speed up tracer optimization and development considerably.

High non-specific binding is a common reason for failure of PET tracer candidates. A high non-specific signal generally results from binding of the tracer to proteins in tissue, this binding is non-saturable. However in PET imaging any non-displaceable signal will reduce the overall useful signal, this could also come from specific off-target binding. The signal to noise will also be reduced by low *in vivo* binding to target, a significant background signal from radiometabolite(s), or a combination thereof. *In vivo*, for reversibly binding radiotracers, the detection of specifically bound ligand (B) is related to the affinity (K_D) for the binding site relative to the concentration of that binding site (B_{max}) and the concentration of free ligand (F).

$$\frac{B}{F} \approx \frac{B_{\max}}{K_D} \qquad K_D = \frac{k_{off}}{k_{on}}$$ where B, F, B_{avail} and K_D are molar

We discuss here how *in vitro* characteristics such as k_{on}, k_{off}, and non-specific binding (determined in cell culture or with rat brain homogenate) correlate with the outcome of *in vivo* studies. The characteristics of ligands which failed *in vivo* due to non-specific binding will be compared with successful tracers.

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Antioxidant profiling of new chemical entities from synthetic and natural origin

Delphine Cressend, Marianne Reist, Pierre-Alain Carrupt

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

Antioxidant compounds have become essential to prevent diseases partly induced by oxidative stress, such as cancer or neurodegenerative diseases (e.g. Alzheimer, Parkinson). To further understand and characterize their antioxidant properties, the radical scavenging activity of a large set of reference antioxidants and synthetic compounds was tested against three different radicals by four 96-well microplate assays. The antioxidant activities were ranked by cluster analysis in order to define the antioxidant profile of each compound.

The first assay was realised with a protein, the alkaline phosphatase (ALP) hydrolyzing the 4-methylumbelliferyl phosphate (MUP) to a fluorescent substrate, the 4-methylumbelliferone (MU). The marker of oxidative damages was monitored by decrease of ALP's catalytic activity induced by peroxyl radicals generated by the 2,2'-azobis-(2-methlpropionamidine) dihydrochloride (AAPH). The second assay, based on the oxygen radical absorbance capacity (ORAC) was still carried out with peroxyl radicals, generated by AAPH. The marker of oxidative damages was monitored by the fluorescence decrease of fluorescein. Both last ones were spectrophotometric assays, the effectiveness of scavenging activity being monitored by, respectively, the absorbance decrease at 755 nm for 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet-}$) and at 515 nm for 2,2-diphenylpicrylhydrazyl radical ($DPPH^{\bullet}$).

From the cluster analysis, several antioxidant groups have been constituted and the similarity of the antioxidant profile of each group compared with the antioxidant profile of reference compounds. Thus for new chemical entities from synthetic or natural origin, the position in the antioxidant space with respect to the one of reference compounds can be established.

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Assembly of a Large Database of Virtual Molecules

Lorenz Blum, Jean-Louis Reymond*

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

Fragment based drug discovery has become more and more important in the field of pharmaceutical research. Out of this trend emerges the need for new virtual libraries with novel fragment types. Usually libraries are created by taking subsets of databases, by combining building blocks or modification of known scaffolds. However, these methods are not well-suited to discover completely new structural types. We now report GDB-13, based on an earlier published, exhaustive enumeration approach [1][2]. The database contains small organic molecules up to 13 atoms of C, N, O, S and Cl. With 977'468'314 structures, it is the largest small organic molecule database to date. The distribution of descriptor values shows that almost all molecules (99.9 %) fulfill drug-like criteria. The average molecular weight is 179.9 ± 8.3 Da, the logP is 0.01 ± 1.24 and the rotatable bond count is 1.5 ± 1.5, which lies indeed well within the desirable range for bioactive fragments.
GDB-13 contains a plethora of structures not present in databases of already existing compounds such as ZINC, ACX or PubChem, and can be of great usefulness in the search for new bioactive fragments [3].

[1] T. Fink, H. Bruggesser, J.-L. Reymond, *Angew. Chem., Int. Ed.* **2005**, *44*, 1504.
[2] T. Fink, J.-L. Reymond, *J. Chem. Inf. Model.* **2007**, *47*, 342.
[3] K. T. Nguyen, et al., *ChemMedChem.* **2008**, *3,* 1520

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Virtual screening by self-organising maps (SOM) with Lipinski's *rule of '5'*

Ruud van Deursen & Jean-Louis Reymond*

University of Berne, Department of Chemistry & Biochemistry, Freiestrasse 3, 3012 Bern, vandeurs@ioc.unibe.ch

Recently we introduced CST as a structure generator algorithm.[1] CST was used to create an *in Silico* database of ~2 Mio. structures spanning the intermediates between four AMPA-*R* ligands, AMPA, CNQX, Kainic acid and Glutamic acid, in search for new ligands for this important CNS-target. Here we present the virtual screening results by combining protein ligand-docking and a 200x200 neurons self-organising map trained with Lipinski's *rule of 5* descriptor values as input.[2] The results are shown for the *in Silico* generated database and the ZINC database.[3]

[1] R van Deursen, J-L Reymond, *ChemMedChem*, **2007**, *2*, 636-640.
[2] CA Lipinski, F Lombardo, BW Dominy, PJ Feeney, *Adv. Drug Deliv. Rev.*, **1997**, *23*, 3-25.
[3] JJ Irwin, B Shoichet, *J. Chem. Inf. Model.*, **2005**, *45*, 177-182.

90

NMR Studies on the Physical Chemistry of Porphyrinic Photosensitizers and their Membrane Interactions

Mattia Marzorati, Martina Vermathen and Peter Bigler

University of Berne - NMR-Laboratory
Freiestrasse 3, 3012 Bern, Switzerland

Photodynamic therapy (PDT) is a well known method for the treatment of several diseases and is currently applied in medical fields like oncology, dermatology and cosmetic surgery [1]. In this therapy, a key role is carried out by the photosensitizer (PS), typically a porphyrinic compound, which after uploading in the diseased cells can stimulate cell death if excited by light of a particular wavelength. The interactions between membranes and PSs are important mechanisms which are still not well understood.
In our last papers we demonstrated that NMR spectroscopy can be efficiently used for understanding the distribution process of different porphyrinic compounds into phospholipid bilayer vesicles serving as model membranes. In particular, we studied the behavior of 3 chlorin derivatives: chlorin e6, mono-l-aspartyl-chlorin e6 and rhodin G7 [2][3].
In this study, we extend our research to a series of naturally derived porphyrins like Hematoporphyrin IX, Protoporphyrin IX, Deuteroporphyrin IX and some of their commercially available derivatives. Preliminary data on the aggregation behavior of these compounds in buffered saline solution and in organic solvents were obtained from ^{1}H-NMR carried out at different temperatures analyzing changes in relaxation time, chemical shift and line width of the porphyrin signals. Secondly, the interactions of these PSs with model membranes, i.e. large unilamellar phospholipid vesicles with an average diameter of 100 nm, were probed by ^{1}H-NMR, studying mainly chemical shift changes of the phospholipid signals as function of time.

[1] Hamblin M., Mroz P., Advances in Photodynamic Therapy, **2008**.
[2] Vermathen M. et al, *Langmuir* **2008**, *24*, 12521.
[3] Vermathen M et al, paper in preparation.

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Stereoselective block of hERG1 channel by bupivacaine scrutinized at molecular level.

L. Sintra Grilo (1, 2), A. Daina (1), P.-A. Carrupt (1), H. Abriel (2)

(1) School of Pharmaceutical Sciences, UniGe, UniL. (2) Department of Clinical Research, UniBe.

In the heart, the hERG1 voltage-gated K^+ channel mediates the I_{Kr} current, which is crucial for the duration of cardiac action potential. Undesired block of the channel may prolong the QT interval with increased risk of malignant ventricular arrhythmias [1]. Although the molecular determinants of hERG1 block have been studied thoroughly, stereoselectivity has been poorly studied. (S)-bupivacaine was the first drug reported to have higher affinity for hERG1 than its enantiomer [2].
This study aims at understanding the principles underlying the stereoselectivity of bupivacaine block with the help of both electrophysiology experiments and molecular modeling simulations. Patch-clamp recordings using cells expressing hERG1 confirmed that (S)-bupivacaine blocked the wild-type (WT) channel more potently than (R)-form. Stereoselectivity was reversed in mutant F656A and abolished in Y652A. Putative binding modes of (S)- and (R)-bupivacaine inside an open form model of hERG1 channel [3] were predicted by docking simulations, allowing a clear depiction of ligand-protein interactions. Estimated binding energies for both enantiomers in WT and mutants Y652A/F656A are in line with electrophysiology measurements.
These results may be considered as a confirmation at the molecular level of bupivacaine stereoselective behavior towards hERG1. Moreover this information lays the foundations for a structural guideline to filter out potentially cardiotoxic drug candidates *in silico*.

[1] Rossenbacker T. and Priori S., *Rev Esp Cardiol*. **2007**, *60*, 675-682.
[2] Gonzalez T. *et al., Brit J Pharmacol*. **2002**, *137(8)*, 1269-79.
[3] Farid R. *et al*., *Bioorgan Med Chem*, **2006**, *14*, 3160-3173.

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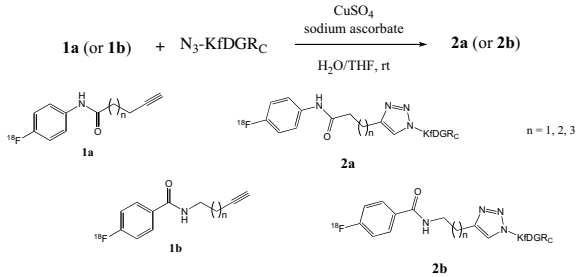
^{18}F-labeling of peptides via Click Chemistry for Positron Emission Tomography (PET) imaging of tumor integrin $\alpha_v\beta_3$ and/or $\alpha_5\beta_1$ expression

A. Monaco[1,2], L.Scapozza[2] and Y. Seimbille[1]

[1]Cyclotron Unit, University Hospital of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva;
[2] School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, 30 quai Ernest Ansermet, 1211 Geneva

Angiogenesis plays a pivotale role in tumor growth and proliferation and, as result, chosen as therapeutic target despite the lack of established methods to predict tumor response to antiangiogenic drugs. We report here a series of ^{18}F-labeled RGDfK$_C$ peptides as molecular probes for PET imaging of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin-receptors. Two series of alkyne containing prosthetic groups (**1a** and **1b**) have been prepared and subsequently attached to an azido-RGDfK$_C$ peptide through Cu(I)-catalyzed Huisgen cycloaddition.



The click reaction was carried out under mild conditions, with good yields and times practical for preparation of ^{18}F-labeled radiopharmaceuticals. Biological evaluation of the new $\alpha_v\beta_3$ and/or $\alpha_5\beta_1$ antagonists is underway.

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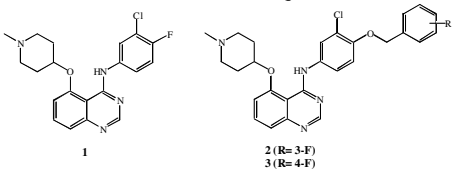
Synthesis of EGFR/HER-2 Inhibitors as Molecular Probes for Positron Emission Tomography (PET) Imaging of Cancer

S. R. Pimple[1, 2], L. Scapozza[2] and Y. Seimbille[1].

[1]Cyclotron Unit, University Hospital of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland.
[2]School of Pharmaceutical Sciences, University of Geneva & University of Lausanne, 30 Quai Ernest Ansermet, 1211 Geneva, Switzerland.

Identification of subset of cancer patients that might benefit from tyrosine kinase inhibitors remains a major challenge. Therefore, we herein report multistep synthesis of 5-substitued-anilinoquinazolines, as well as their ^{18}fluorine-labeled analogs, as potential PET tracers to predict EGFR/HER-2 targeted therapy. The nonradioactive compounds were synthesized starting from 5-fluoroquinazolone by introducing the N-methyl-piperidinyl group at position 5, followed by conversion of the quinazolone into 4-chloro- or 4-thiomethyl-quinazoline, and subsequent attachment of aniline or substituted benzyloxyanilines. The synthesis of ^{18}fluorine-labeled analogs involved preparation of ^{18}F-labeled monocyclic or bicyclic anilines followed by condensation with the 4-chloroquinazoline intermediate.



The biological evaluation of these compounds using cancer cell lines harboring varying degrees of EGFR and HER-2 expression or mutational status is underway.

[1] Seimbille, Y. et al; *J. Label. Compd. Radiopharm.*, **2005**, *48*, 829-843.

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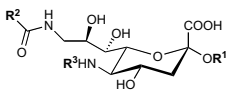
Synthesis and Biological Evaluation of Siglec-2 Antagonists

Stefanie Mesch[a], Hendrik Koliwer-Brandl[b], Oliver Schwardt[a], Soerge Kelm[b], Beat Ernst[a]

[a]Institute of Molecular Pharmacy, Pharmacenter, University of Basel, CH-4056 Basel, Switzerland
[b]Department of Physiological Biochemistry, University of Bremen, D-28334 Bremen, Germany

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are mainly expressed in the immune system. Silgec-2, also known as CD22, is a B-cell specific transmembrane protein, being involved in B-cell regulation such as cellular activation thresholds and survival [1]. Consequently, CD22 modulates B-cell immune response, prevents autoimmunity and controls homing of recirculation of B-cells back to the bone marrow. Furthermore, it is supposed that binding of CD22 to glycoprotein ligands on T-cells can modulate T-cell signaling [2]. These properties make it an interesting target for medicinal chemistry. As reported earlier, CD22 binds with high affinity to sialic acid derivative **1** [3]. Here we report the synthesis and biological evaluation of a small library of optimized CD22-ligands, based on Neu5Ac derivative **1**.



1 R^1 = Me, R^2 = Biphenyl, R^3 = Ac

[1] Crocker, P. R., Paulson, J. C., Varki, A. *Nat. Rev. Immunol.* **2007**, *7*, 255.
[2] Tuscano, J. P., Engel, T. F., Kehrl, J. H. *Blood* **1996**, *87*, 4723.
[3] Kelm, S., Gerlach, J., Brossmer, R., Danzer, C., Nitschke, L. *J. Exp. Med.* **2002**, *195*, 9, 1207.

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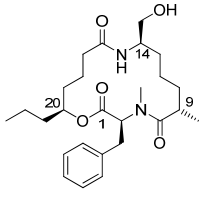
Total Synthesis and Structure Elucidation of Haliclamide

Bernhard Pfeiffer, Sandra Gisler, Luzi Braduner, Ursula Senft, Karl-Heinz Altmann*

Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich

Marine sponges are well known producers of bioactive natural products. In 2001 Randazzo *et al.* [1] reported the isolation and structure elucidation of a new secondary metabolite from *Halicloma sp.*, a marine sponge from Vanuatu, and termed it haliclamide (**1**). Haliclamide was shown to exhibit *in vitro* antitumor activity against the human bronchopulmonary non-small-cell-lung-carcinoma cell line NSCLC-N6 with an IC_{50}-value of 4 µg/ml. The structure of **1** showed a 16-membered cyclic depsipeptide with similarities to other bioactive depsipeptides such as *e.g.* spongidepsin, jasplakinolide, chondramide C or the very potent cytotoxic doliculide. Randazzo *et al.* however could not determine the absolute configuration of the stereocenters at C9 and C20. In an effort to identify the absolute configuration of these stereocenters we synthesized the four possible diastereoisomers of haliclamide in a convergent approach. Comparison of the resulting ^{1}H- and ^{13}C-NMR spectra with those reported for natural haliclamide, the structure of the natural product could be established as **1**. The cytotoxic, antifungal and antibiotic activity of **1** and it diastereoisomers were investigated.



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[1] A. Randazzo, C. Debitus, L. Gomez-Paloma, *Tetrahedron* **2001**, *57*, 4443.

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Influence of Mutations on the Stablility of Insulin Dimer

Lixian Zhang, Markus Meuwly

Chemistry Department, University of Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland

Insulin, a 5.8 kDa dual-chain hormone, is a small protein that plays an eminent role in the hormonal control of metabolism. Before binding to its transmembrane insulin receptor, insulin must dissociate from its hexameric storage form through an intermediate dimer state to the bioactive monomer. On the other hand, the native insulin monomer is an active form that tends to aggregate to form dimers and hexamers in solution. The dissociation of the hexamer is achieved with different mutations in the insulin peptide chain. We created the mutations at those positions which play an important role in the dimerization process computationally. The molecular dynamic has been performed to explore the effects of these mutations of the insulin dimer. Different trajectories and analysis have been carried out. The preliminary MD study shows that the AlaB24 and GlyB24 mutant dimers are less stable than the native dimer.[1] The influence caused by these different mutants leads to important insights and suggests which mutations make the insulin monomer less aggregation and keep it as the native form to treat diabetes mellitus.

[1] Zoete, V.; Meuwly, M. *J Comput Chem.* **2006**, *27*, 1843.

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

Creus, M., Zimbron, J.M., Sardo A., Wohlschlager T., Ward T.R.

Department of Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

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

Here we describe that variants of metal complexes embedded within a protein scaffold modulate the DNA-binding activity *in vitro.* We used streptavidin as a 'targeting host-protein' and a biotinylated metal complex as a 'non-selective drug'.

We present evidence that the incorporation of the well-defined protein environment of streptavidin around a DNA-binding complex increases selectivity toward telomeric DNA through second coordination interactions. The nature of the complex as well as defined mutations around the 'active site' of the protein-host influenced target-recognition and binding.

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

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Insights into the Dynamics of Type I and Type II Thymidine Kinases

Yvonne Westermaier, Leonardo Scapozza

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

We aim at shedding light on the dynamic properties of thymidine kinases (TKs) to better define their substrate preferences and improve substrate affinity. This is of interest in enzyme-prodrug activating gene therapy, where TK activates a therapeutically efficient nucleoside, which acts as DNA chain terminator when triphophorylated. These nucleosides can be radiolabeled and used as Positron Emission Tomography tracers to monitor the enzyme's activity *in situ*, because once in the cells, they remain trapped due to their negative charge.
In our work, we distinguish between type I and type II TKs, because they differ in cellular location, length and substrate acceptance. We investigated the dynamics of protein-ligand interaction for both TK types with several Molecular Dynamics (MD) techniques using NAMD2.6 [1]. Classical MD was used to assess the enzyme stability and principal component analysis to identify and group the most pronounced movements. As large conformational changes involving the transition between a closed, substrate bound TK and an open apo form cannot be simulated with conventional MD techniques, opening was achieved with Steered MD (SMD). Possible substrate egress routes were then identified using random acceleration MD [2]. The biologically most sound exit direction (pointing towards the ATP binding site) served as substrate pulling direction out of TK-substrate complexes. We correlate the substrate extraction work with experimentally determined affinity data and extrapolate potential binding affinities of fluorinated analogues. Finally, different substrate preferences are rationalized based on structural evidence and dynamics.

[1] JC Phillips et al. *J Comput Chem* **2005**, 26, 1781.
[2] SK Lüdemann et al. *J Mol Biol* **2000**, 303, 797.

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Improved enzymatic protocols for the synthesis of acylated acyl carrier proteins of *P. falciparum*

Leonardo Lauciello, Gabriela Lack, Leonardo Scapozza, Remo Perozzo

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

Acylated acyl carrier proteins (acyl-ACPs) are the natural substrates of the enzymes involved in the type-II fatty acid biosynthesis (FAS-II). The most currently used method to produce acyl-ACPs involves the transfer of a phosphopantetheine moiety from CoA to ACP by E. coli holo-ACP synthase (EcACPS) and the subsequent thioesterification of a fatty acid to the terminal sulfhydryl group of the phosphopantetheine by the E. coli acyl-ACP synthase (EcAAS).[1] Both enzymes are known to accept ACPs from other organisms, including *P. falciparum* ACP (PfACP). Alternatively, EcACPS may even be used for the direct transfer of acylated phosphopantetheine moieties from their corresponding CoA derivatives to apo-ACP.[2]
In this work we investigated the potential and limits of the two methods to synthesize and purify preparative amounts of the natural substrates of *P. falciparum* FAS-II. We found EcAAS activity to be independent from modifications at the β-position, readily accepting as substrates fatty acids with chain lengths starting from C8 to C20. EcACPS accepts very efficiently acyl-CoAs with chain lengths up to C16, while a decrease in activity was observed with the use of longer chain (C18 to C20) acyl-CoAs.

[1] Rock, C. O.; Garwin, J. L.; Cronan, J. E., Jr. *Methods Enzymol.* **1981**, *72*, 397-403.

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[2] Carreras, C. W.; Gehring, A. M.; Walsh, C. T.; Khosla, C. *Biochemistry* **1997**, *36*, 11757-11761

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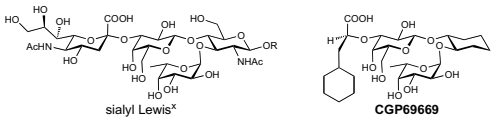
Synthesis, Development and Optimization of E-selectin Ligands Guided by NMR and Biacore Studies

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

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

The selectins play a key role in the body's defense mechanism against inflammation [1]. They are responsible for the initial steps of the inflammatory response (tethering and rolling of leukocytes on endothelial cells), which are a prerequisite for the extravasation of leukocytes into inflamed tissue. However, excessive tissue infiltration can lead to acute or chronic reactions, as observed in reperfusion injuries, stroke or rheumatoid arthritis [2]. Therefore, the antagonism of selectins is regarded as a valuable pharmaceutical goal.
Since all physiological ligands of the selectins contain the sialyl Lewis^x motif [3], this tetrasaccharide was chosen as a starting point in the search for E-selectin antagonists.
Based on the lead **CGP69669** [4], several high-affinity antagonists were developed.



[1] *Physiology and Pathophysiology of Leukocyte Adhesion*; Granger, D. N., Schmid-Schönbein, G. W., Eds.; Oxford University Press: New York, 1995.
[2] Mousa, S. A. *Drugs Fut.* **1996**, *21*, 283.
[3] Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, 91, 7390.
[4] Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, *3*, 1571.

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Design and SAR of azacarbazole derivatives: a novel class of potent inhibitors of anaplastic lymphoma kinase (ALK)

[1]Shaheen Ahmed, [2]Cedric Schneider, [3]Luca Mologni, [2]Peter Goekjian, [3]Carlo Gambacorti-Passerini, [1]Leonardo Scapozza

[1]University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland
[2]University of Lyon, France
[3]University of Milano-Bicocca, Italy

Anaplastic lymphoma kinase (ALK) is a receptor-type protein tyrosine kinase that is expressed preferentially in neurons of the central and peripheral nervous systems at late embryonic stages. Oncogenic ALK fusion proteins (NPM/ALK and associated variants) are expressed in about 60% of anaplastic large cell lymphomas (ALCLs) but are absent in normal tissues [1]. Furthermore, recent studies show that ALK fusion proteins are also involved in certain forms of neuroblastoma, breast and lung cancer [2,3]. NPM/ALK therefore represents a promising target for the development of anticancer agents.
As, to date, no crystal structure of ALK is available, several homology models were built [4], representing different activation states of the ALK kinase domain. *De novo* design and virtual screening was performed on these models and several scaffolds for potential ALK inhibitors have been detected and were further derivatized. Binding mode hypotheses for these compounds could be established and validated by means of MD simulations. A next generation of this novel compound class, which show promising potency on enzyme-based and cell assays, are currently being developed upon the SAR studies presented here.

[1] SW Morris et al. *Science,* **1994**, 263, 1281.
[2] M Soda et al. *Nature,* **2007***,* 448, 561.
[3] R Chiarle et al. *Nat Rev Cancer*, **2008**, 8(1), 11.
[4] RH Gunby et al. *J Med Chem,* **2006**, 49(19), 5759.

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Identifying new targets for existing drugs using an optimized yeast three-hybrid system

Christopher Chidley, Evelyne Muller and Kai Johnsson

Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne

In biomedical research, the identification of the protein targets of a small molecule drug is a critical and difficult challenge. Furthermore, the discovery of unknown protein targets of approved drugs can lead to the explanation of drug side effects or to the discovery of potential new therapeutic uses.
In this work, we shall present an optimized yeast three-hybrid system for the identification of small molecule-protein interactions. In the yeast three-hybrid system, the interaction of a small molecule with a protein is detected by linking their association to the transcriptional activation of a reporter gene. This allows the screening of large protein libraries towards the identification of small molecule-protein interactions.
Specifically, we will show how the optimization of the system leads to a better sensitivity of the interactions that can be detected together with a reduced number of false positives that arise during protein library screenings. In a second part, we shall show the screening results of about ten drug derivatives against several protein libraries. The outcome of the screenings illustrates how the system can be efficiently used for the discovery of the protein targets for small molecule drugs.

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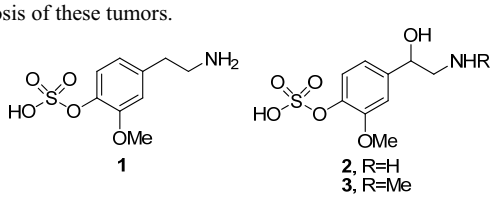
Synthesis of sulfoconjugated metanephrines and application to the diagnosis of neuroendocrinal tumors

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

Pheochromocytomas and paragangliomas are rare, heterogeneous tumors of the chromaffin cells, which produce and secrete the vasoconstrictors catecholamines. These tumors are potentially lethal if not diagnosed and treated appropriately.[1] The biological diagnosis actually relies on the identification of excessive secretion of free metanephrines in plasma.[2] Nevertheless, the simultaneous measurement of free and sulfoconjugated metanephrines would allow the development of a more sensitive and specific measurement procedure for the diagnosis of pheochromocytomas. The aim of this work was to develop efficient synthesis pathways of derivates of type **1**-**3** of sulfoconjugated metanephrines that will be used as references for the calibration of a LC-MS based measurement system for the diagnosis of these tumors.



[1] Manger, W.M. *Ann. N. Y. Acad. Sci.* **2006**, *1073*, 1-20.
[2] a) Mornex, R.; Peyrin, L.; Pagliari, R.; Cottet-Emard, J.- M. *Horm. Res.* **1991**, *36*, 220-226. b) Eisenhofer, G.; Siegert, G.; Kotzerke, J.; Bornstein, S. R.; Pacak, K. *Horm. Met. Res.* **2008**, *40*, 329-337. c) d'Herbomez, M.; Rouaix, N.; Bauters, C.; Wémeau, J.- L. *Presse Med.* **2009**, doi: 10.1016/j.lpm.2008.08.011.

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Fluorinated 9H-Xanthene-9-carboxylic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhancers

Eric Vieira[a], Jörg Huwyler[b], Synèse Jolidon[a], Frédéric Knoflach[a], Vincent Mutel[c] and Juergen Wichmann[a]

a: Pharma Division, Discovery Research, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland. b: Present address: Institute of Pharma Technology, University of Applied Sciences, Northwestern Switzerland / Gründenstrasse 40, CH-4132 Muttenz / Switzerland. c: Present address: Addex Pharmaceuticals,12 Chemin Des Aulx, CH-1228 Plan Les Ouates/ Switzerland

L-glutamate, the major excitatory amino acid neurotransmitter in the central nervous system, binds to and activates several classes of receptors which are divided into two groups termed ionotropic (iGluR) and metabotropic glutamate receptors (mGluR). The latter family comprises eight subtypes of G-protein coupled receptors (GPCRs), grouped according to pharmacology and coupling to second messengers. A role for group I mGlu receptor activation has been proposed in physiological processes including pain perception, learning and memory, as well as in certain psychiatric and neurological disorders. Positive allosteric modulators (PAMs or enhancers) of the mGluR5 receptor have gained recent interest due to their possible use for the treatment of schizophrenia. Although both mGluR1 and mGluR5 belong to group 1 mGluR's, the expression patterns of these receptors in the brain are quite different, mGluR1 expression being for example much higher in cerebellar Purkinje cells. Thus mGluR1 PAMs could be of interest in indications where reduced mGluR1 function in this brain area leads to motor impairment (cerebellar ataxia). The synthesis of a new class of selectively fluorinated small molecule mGluR1 enhancers with improved pharmacokinetic properties as pharmacological tools for the study of the physiological roles mediated by mGlu1 receptors is presented.

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Structural and functional studies of the varO variant of *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1)

Alexandre Juillerat, Sébastien Igonet, Micheline Guillotte, Inès Vigan-Womas, Stéphane Gangnard, Grazyna Faure, Bruno Baron, Bertrand Raynal, Odile Mercereau-Puijalon and Graham A. Bentley

Institut Pasteur, 25-28 rue du Dr. Roux, F-75015 Paris, France

Plasmodium falciparum strains causing rosetting and auto-agglutination are virulence phenotypes associated with severe malaria in African children. Rosetting is directly mediated by the N-terminal DBL1α domain of PfEMP1 in three *P. falciparum* laboratory strains: FCR3S1.2, R29 and, more recently, varO [1]. Two possible intervention strategies against severe malaria require either the prevention of rosette and auto-agglutination formation by vaccination or the disruption of such cellular clusters with soluble inhibitory drugs. It has been previously shown that PfEMP1 molecule bind to multiple receptors, including polysaccharides and that sulfated polysaccharides with inhibitory capacity can be considered as anti-adhesion drugs. Insight of the structures of saccharide-binding domains would allow the developpement of such inhibitor compounds. With the objective of analysing cytoadherence by the varO parasites in functional, serological and structural studies, we have expressed the DBL1α_1 domains using the *E. coli* expression systems and performed a biochemical and biophysical study of the recombinant N-terminal domain. The ability of the recombinant domains to bind to heparin and other sulphated glycans was analyzed by Biacore and shown to correlate with the capacity to disrupt the rosettes in parasite cultures. These results will provide a basis for developing vaccine candidates and inhibitors to rosetting and auto-agglutination of infected erythrocytes.

[1] I. Vigan-Womas, M. Guillotte, C. Le Scanf, S. Igone, S. Petres, A. Juillerat, C. Badaut, F. Nato, A. Schneider A, A. Lavergn, H. Contamin, A. Tall, L. Baril, G.A. Bentley, O. Mercereau-Puijalon, *Infect Immun,* **2008**, *76*, 5565.