Medicinal Chemistry **49**

Novel pyrazolidine-3,5-dione derivatives are $P2Y_{12}$ receptor antagonists and inhibit ADP-triggered blood platelet aggregation

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G protein-coupled receptor (GPCR) $P2Y_{12}$, a clinically relevant platelet receptor for adenosine diphosphate (ADP), is involved in the ADP-induced blood platelet aggregation. Upon ADP activation, $P2Y_{12}$ signals through Gi_2, inhibiting Gi_2 mediated adenylyl cyclase activity. This $P2Y_{12}$ receptor signaling is responsible for the amplification of platelet aggregation, the potentiation of platelet secretion and the constitution of stable aggregates, leading to thrombus formation. Highly active metabolites of the thienopyridine based antiplatelet drugs clopidogrel, ticlopidine and CS-747 are considered to irreversibly block the $P2Y_{12}$ receptor. Furthermore, various nucleotide or nucleoside based compounds are known to act as ADP competitive $P2Y_{12}$ antagonists and as such inhibit ADP-induced platelet aggregation. Nevertheless, the $P2Y_{12}$ receptor remains a key target for new antithrombotic drugs.

Screening of our in-house compound collection by means of an *in vitro* fluorescent imaging plate reader (FLIPR™) assay delivered a pyrazolidine-3,5-dione derivative as hit compound.

Herein, we wish to report the discovery and optimization of 4-benzylidene-pyrazolidine-3,5-dione analogues towards novel, potent and selective $P2Y_{12}$ receptor antagonists, inhibiting platelet aggregation induced by the agonists ADP or 2-MeSADP in a reversible and concentration dependent manner.

Medicinal Chemistry **50**

VEGFR2 Kinase Inhibitors for Antiangiogenic Therapy in Cancer

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Drugs targeted towards protein kinases have proved to be effective in clinical settings for the treatment of cancer, and thus vindicated protein kinases as drugable targets for the discovery of new therapeutic agents.
Since most cancers are at least in part dependent upon the development of a tunour vasculature, targeting angiogenesis via inhibition of vascular endothelial growth factor (VEGF) receptor signaling is an attractive goal in this area.
The first agent to show clinical benefit in this respect was Arvastin, a monoclonal antibody directed at VEGF itself, which prolongs survival of patients suffering from colon cancer. The VEGF receptor (VEGFR) initiates intracellular signaling via its tyrosine kinase activity , and a first generation VEGFR inhibitor, valantanib, having shown promise in Phase 2 studies, is currently in phase 3 clinical trials. The prospects for angiogenesis inhibitors in cancer as well as other inflammatory and proliferative diseases has stimulated considerable pharmaceutical research for second generation agents.
Some of the latest advances in this area will be discussed.

Medicinal Chemistry **51**

Molecular Recognition Studies in the Active Site of Thrombin

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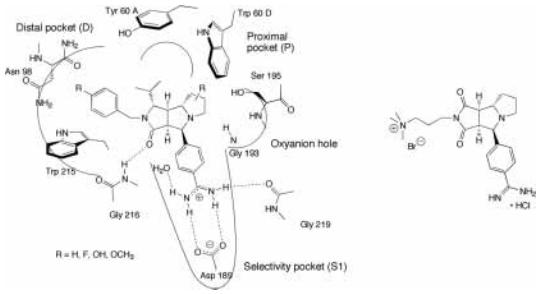
The reversible binding between an enzyme and its substrate relies on the formation of various kinds of non covalent interactions. The discovery of a so far underestimated type of orthogonal multipolar interaction during a fluorine scan on a tricyclic thrombin inhibitor [1] encouraged us to further investigate related interactions of fluorine, hydroxy and methoxy substituents, respectively, in the oxyanion hole as well as in position 4 of the phenyl ring reaching into the D-pocket of the active site (figure left). Moreover, our aim was to explore the possible formation of cation-pi attractions between the indole ring of a tryptophane residue and a quaternary ammonium, linked to the tricyclic scaffold of the inhibitor, directed towards it (figure right) [2].



[1] J. A. Olsen, D. W. Banner, P. Seiler, B. Wagner, T. Tschopp, U. Obst-Sander, M. Kansy, K. Müller, F. Diederich, *ChemBioChem* **2004**, *5*, 666-675.
[2] K. Schärer, M. Morgenthaler, P. Seiler, U. Obst-Sander, D. W. Banner, T. Tschopp, F. Diederich, *Angew. Chem. Int. Ed.*, in press.

Medicinal Chemistry **52**

Exploring The Binding Mode Of Sialic Acid Derivatives As Myelin-associated Glycoprotein (MAG) Ligands

Daniel Strasser,[a] Sachin Shelke,[a] Brian Cutting,[a] Michele Porro,[a] Gan-Pan Gao,[a] Tamara Viseruna,[a] Oliver Schwardt,[a] Said Rabbani,[a] Heiko Gäthje,[b] Soerge Kelm,[b] Beat Ernst[a]

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The myelin-associated glycoprotein (MAG), a member of the siglec family, has been identified as a potent inhibitor of neurite outgrowth of mature nerve cells in the CNS.[1] Molecules that block such inhibitor proteins have the potential to enhance axon regeneration and functional recovery.
Several gangliosides like GD1a, GT1b or GQ1bα have been shown to be potent ligands of MAG in *in vitro* binding assays.[2] In SAR studies, the α(2-3)- and α(2-6)-linked sialic acid moieties of these gangliosides were identified to be the important elements for binding. Finally, with sialic acid derivatives binding affinity could be further improved by more than a factor 1000.
In order to understand the increase in binding affinity, several sialic acid based derivatives were analyzed more in detail by biosensor analysis, STD-NMR and molecular modelling. By using a set of structurally diverse ligands, important elements interacting with MAG and the kinetic properties of their interactions could be determined.

[1] A.A. Vyas, H.V. Patel, S.E. Fromholt, M. Heffer-Lauc, K.A. Vyas, J. Dang, M. Schachner, R.L. Schnaar *PNAS* **2002**, *99*, 8412.
[2] B.E. Collins, H. Ito, N. Sawada, H. Ishida, M. Kiso, R.L. Schnaar *J. Biol. Chem.* **1999**, *274*, 37637.

Medicinal Chemistry **53**

PPARα/γ Co-Agonists: Combining Antidiabetic and Hypolipidemic Activity

U. Grether, A. Bénardeau, J. Benz, A. Binggeli, B. Bittner, M. Böhringer, A. Flament, H. Hilpert, G. Hirth, H. Isel, B. Kuhn, H. P. Märki, M. Meyer, P. Mohr, K. Püntener, S. Raab, F. Ricklin, A. Ruf, U. Sprecher, P. Verry, N. Wyttenbach

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

Type 2 diabetes is a metabolic disease characterized by insulin resistance and hyperglycaemia which is often accompanied by hyperlipidemia. Two classes of compounds were empirically discovered decades ago, known as the thiazolidinediones (TZDs) and the fibrates. While the TZDs lower blood glucose as well as insulin levels and improve insulin sensitivity, the fibrates are effective at lowering serum triglycerides and raising HDL cholersterol levels.

Recently, the ligand-dependent transcription factors Peroxisome Proliferator Activated Receptor-γ (PPARγ) and -α (PPARα) were identified as being the primary molecular targets for the antidiabetic TZDs and the lipid lowering fibrates, respectively. This has provided new opportunities for the treatment of type 2 diabetes, since the profile of a PPARα/γ co-agonist appears well-suited for addressing both hyperglycaemia as well as the enhanced cardio-vascular risk of diabetic patients.

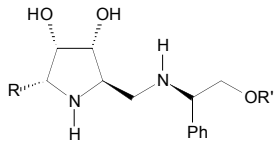
Through combining the putative pharmacophores of known PPARγ- and α-selective agents within a single molecule, novel α-alkoxyphenylpropionic acids were identified as a new class of PPARα/γ co-agonists. The optimization of the relative PPARα/γ/δ potencies supported by X-ray co-crystal structures and molecular modelling as well as the profiling of advanced compounds in animal models of type 2 diabetes and dyslipidemia will be presented.

Medicinal Chemistry **54**

Pyrrolidine derivatives as new inhibitors of α-mannosidases and growth inhibitors of human cancer cells

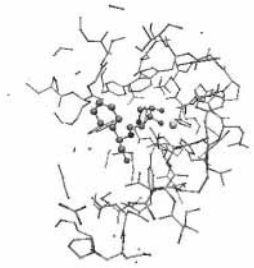
Helene.Fiaux,[1] Doug Kuntz,[2] Pierre Vogel,[1] David Rose,[2] Sandrine Gerber-Lemaire[1] and Lucienne Juillerat Jeanneret[3]
[1] *LGSA, EPFL, BCH,CH-1015 Lausanne, Switzerland*
[2] *Ontario Cancer Institute, Toronto, Canada*
[3] *University Institute of Pathology, CHUV, Lausanne, switzerland*

The specific inhibition of *N*-linked glycoprotein-processing α-mannosidases may provide a useful anti-cancer strategy.[1] Based on a combinatorial method for the discovery of glycosidase inhibitors[2], we prepared a new family of pyrrolidine derivatives. These compounds demonstrated potent inhibitory activity on α-mannosidase from jack bean and were able to inhibit the growth of melanoma and glioblasoma cells.[3]



R = H, OH, Me, CH_2OH

R' = H, inhibition of α-mannosidase from jack bean
Ki = 2.3 to 0.135 μM
R' = C(O)Ar, inhibition of human cancer cells
Melanoma and glioblastoma cells
LC_{50} = 100-200 μM



R,R = H, inhibitor in the active site of Golgi α-mannosidase II

[1] White, S.L.; Nagai, T.; Akiyama, S.K.; Reeves, E.J.; Grzegorzewski, K.; Olden, K. *Cancer Commun*. **1991**.
[2] Gerber-Lemaire, S.; Popowycz, F.; Rodriguez-Garcia, E.; Carmona Asenjo, A.T.; Robina, I.; Vogel, P, *ChemBioChem* **2002**, *5*, 466.
[3] Fiaux, H. ; Popowycz, F. ; Favre, S. ; Schütz, C. ; Vogel, P. ; Gerber-Lemaire, S. ; Juillerat-Jeanneret, L. *J. Med. Chem*., in press.

Medicinal Chemistry **55**

Novel Highly Stereoselective Synthesis of (±)-(2R*,4S*,5S*)-Hydroxyethylene Transition State Mimetics

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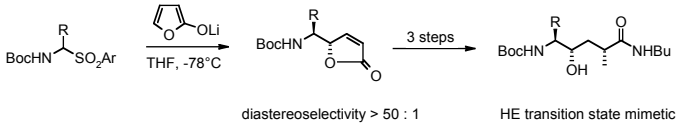
For the structure-based design of inhibitors of aspartic proteases the hydroxyethylene (HE) transition state mimetic (TSM) plays an important role.



diastereoselectivity > 50 : 1 HE transition state mimetic

We developed a highly selective 5-step procedure for the preparation of racemic HE TSM's starting from aldehydes. The scope of the method and the mechanism of the key-step are discussed. The usefulness of the method is illustrated with the synthesis of inhibitors for BACE (Beta-site APP Cleaving Enzyme).

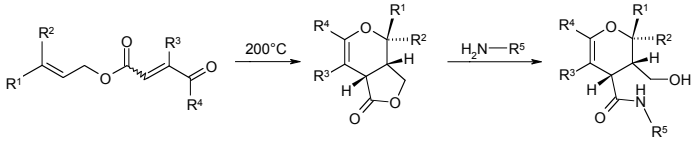
Medicinal Chemistry **56**

Synthesis and Further Derivatisation of Furo[3,4-*c*]pyranones with Antiproliferative and Apoptotic Activity

Cyril Fuhrer[a], Stephan Ruetz[b], Eric Grüter[a], and Robert Häner[a]

[a]Department of Chemistry, University of Bern, CH-3012 Bern, Switzerland
[b]Novartis Institutes of Biomedical Research, CH-4002 Basel, Switzerland

Different furo[3,4-*c*]pyranones **2** have been synthesized through an intramolecular hetero-Diels-Alder (HDA) reaction which was highly stereoselective. In all cases, formation of a single product was observed. Structural elucidation revealed a *cis*-configuration of the two rings. The α,β-unsaturated γ-ketoesters **1** were obtained as isomeric mixtures (*E:Z*-ratio approximately 1:2) through a *Horner-Wadsworth-Emmons* reaction of diethyl phosphonate esters with α-diketones [1]. Subsequent derivatisations included the aminolysis of the lactone moiety with different amines.



(*E*)/(*Z*) **1** **2** **3**

Compounds **2** were tested in cell proliferation assays of two different human cancer cell lines (A549 and KB31). Both cell lines showed a significant accumulation of cells in the G2/M phase when treated with compounds **2**. Furthermore, the cell viability tests showed that compounds **2** also induced apoptosis in KB31 cells while no programmed cell death was observed in A549 cells.

[1] C. Fuhrer, R. Messer, R. Häner; *Tetrahedron Lett*. **2004**, *45*, 4297-4300.

Medicinal Chemistry **57**

Discovery of novel, potent and fully selective MAO-B inhibitors using an elegant Hit to Lead Paradigm

Rosa María Rodríguez Sarmiento, Alexander Alanine, Andrea Cesura, Andrew W. Thomas & René Wyler

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

Mononamine oxidases (MAO) are flavin adenine dinucleotide (FAD) containing enzymes located in the outer membrane of mitochondria. They catalyze oxidative de-aminations involving the reduction of FAD to $FADH_2$ and the conversion of substrate amine to an aldehyde in a process accompanied by the formation of hydrogen peroxide from oxygen.
MAO-A and B catalyze the oxidative deamination of brain neurotransmitters such as dopamine and serotonin as well as a variety of biogenic and xenobiotic amines. The first generation of MAO inhibitors were irreversible and unspecific for the two MAO isoforms and had to be curtailed because of the occurrence of side effects such as hypertensive crises (cheese effect) when given tyramine rich food. A fully selective and reversible MAO-B inhibitor would have a better safety profile and therapeutic scope in a range of neurodegenerative diseases such as Parkinson´s disease (PD) and Alzheimer´s disease (AD) (eg: mechanism based inhibitor deprenyl for PD and AD, Lazabamide for PD) and in anti-addiction therapies such as smoke cessation.
A high throughput screening (HTS) campaign was initiated with the goal to discover a novel molecular class that would fulfil our internal quality control milestone, lead series identified (LSI), with potential to rapidly be transformed into a development candidate.
We will disclose our successful efforts in the discovery of an attractive Lead Series and describe a lead generation process that facilitated the rapid identification of high quality, low molecular weight, selective, potent and *in vivo* active compounds demonstrating a broad SAR with a range of novel molecular sub-classes emerging from exploration of the parent hit series.

Medicinal Chemistry **58**

Identification of the Precursor of (*S*)-3-Methyl-3-Sulfanylhexan-1-ol, the Sulfury Malodour of Human Axilla-Sweat.

Christian Starkenmann*, Yvan Niclass, Myriam Troccaz and Anthony J. Clark

Firmenich SA, Division Recherche & Développement, B.P. 239, CH-1211 Genève 8

Human axillary sweat malodour is produced by the action of skin microflora on proteinaceous material excreted from apocrin glands. A careful study of human axillary microflora led us to the identification of a new strain of *Staphylococcus haemolyticus*. The role in axillary malodour formation of this micro-organism was compared to those of *Corynebacterium xerosis* and *Staphylococcus epidermidis,* upon incubation on sterile human ecrine and aprocrine axilla sweat. *St. haemolyticus* was responsible for the strongest sulphury malodour and the volatile sulfur compound (VSC), (*S*)-3-methyl-3-sulfanylhexan-1-ol was detected for the first time. We investigated then the non-volatile precursors of VSCs. Human axillary sweat was collected, fractionated and analysed by HPLC-APCI-MS (High Pressure Liquid Chromatography coupled to Atmospheric Pressure Chemical Ionisation Mass Spectrometry). The precursor of (*S*)-3-methyl-3-sulfanylhexan-1-ol was identified as [1-(2-hydroxyethyl)-1-methylbutyl]-(*L*)-cysteinylglycine (Cys-Gly-(*S*)-conjugate). Because Cys-Gly-(*S*)-conjugates are key intermediates in the glutathione biodetoxification pathway, glutathione-(*S*)-conjugate and Cys-(*S*)-conjugate were prepared. Synthetic (*S*)-conjugated homologues were incubated with *C. xerosis*, *St. heamolyticus* and *St. epidermidis*. We observed efficient conversion of precursors Cys-Gly-(*S*)-conjugate and Cys-(*S*)-conjugate to form VSCs when incubated with *St. haemolyticus*, with a clear preference for the natural occurring Cys-Gly-(*S*)-conjugate.

M. Troccaz, C. Starkenmann, Y. Niclass, M. van de Waal, A. J. Clark, *Chem. Biodiversity* **2004**, *1*, 1022.

Medicinal Chemistry **59**

Cloning and Characterization of Cysteine Mutants of the Asialoglycoprotein Receptor H1-CRD

Karin Johansson, Rita Born, Daniela Stokmaier, Daniel Ricklin, Said Rabbani, Beat Ernst

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The asialoglycoprotein receptor (ASGP-R) is a C-type lectin, abundantly expressed on hepatocytes, and responsible for the clearance of desialylated glycoproteins from the circulation. The receptor consists of two subunits, H1 and H2. Each subunit contains a carbohydrate recognition domain (CRD), which binds to ligands containing terminal galactose and *N*-acetylgalactosamine residues with high specificity [1]. Therefore, the ASGP-R can be considered as a potential candidate for targeted drug delivery to hepatocytes.

The CRD of H1 contains seven conserved cysteines, shown to form three disulfide bonds [2]. However, the structural importance of these disulfide bridges for the functionality of the subunit and of the odd cysteine, which is giving rise to dimerization, has not previously been studied. The functional role of one of the bridges is of particular interest as it neither takes part in the actual binding site, nor does it act as a link between the *N*- and *C*-terminus.

Site-directed mutagenesis was used to create mutants of H1-CRD in order to investigate the functional role of the three cysteines closest to the *N*-terminus. Changes in activity and dimer formation, arising as a result of the mutation(s), were analyzed and compared to wild-type H1-CRD.

[1] Weigel, P. H., Yik, J. H. N. *Biochim Biophys Acta*. **2002**, *1572*, 341-363
[2] Meier, M., Bider, M. D., Malashkevich, V. N., Spiess, M., Burkhard. P. *J. Mol. Biol*. **2000**, *300*, 857-865

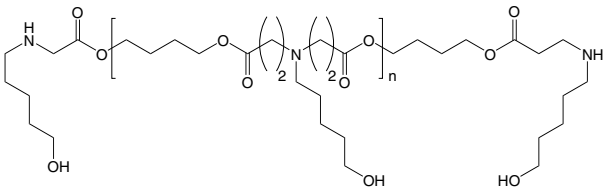
Medicinal Chemistry **60**

Gene Therapy Using Degradable Poly(β-amino esters)

Andreas Zumbuehl, Daniel G. Anderson, Robert Langer*

MIT, 77 Massachusetts Avenue, Cambridge, MA, USA-02139

Applying straightforward *Michael*-type conditions, a large library of several thousand different cationic poly(β-amino esters) was synthesized [1]. Plasmid DNA-delivery to COS-7 cells *in vitro* established lead-structures that showed enhanced transfection efficiency over the benchmark polyethylene imine (PEI). *In vivo* experiments further identified a polymer (C-32, see figure) that delivered DNA intratumorally 4-fold better than one of the best commercially available reagents, jetPEI [2].



In this communication we present synthetic efforts towards the modification of the first-generation polymers. In particular the introduction of groups allowing the targeting of specific cell lines, and side-chain modification to fine-tune the charge-distribution of the polymers.

[1] D. G. Anderson, D. M. Lynn, R. Langer, *Angew. Chem. Int. Ed.*, **2003**, *42*, 3153–3158.
[2] D. G. Anderson, W. Peng, A. Akinc, N. Hossain, A. Kohn, R. Padera, R. Langer, J. A. Sawicki *PNAS*, **2004**, *101*, 16028–16033.

Medicinal Chemistry **61**

A Model for Cell Surface Exposed Carbohydrate Units

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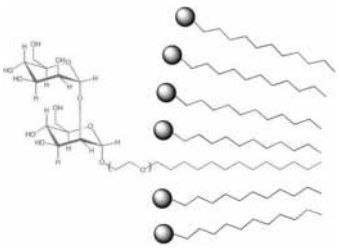
*Institute of Organic Chemistry, University of Zurich, CH-8057 Zürich
‡Fachbereich Chemie, Universität Konstanz

Cell-cell recognition is an event of prime biological importance in a variety of biological phenomena and interactions with carbohydrate units on the cell surfaces are of prime importance[1-2]. We have developed a model system for oligosaccharide units presented on cell surfaces. Therein, carbohydrates are linked via a flexible methyleneglycol linker to a lipid chain:



The model system was verified by testing its binding capability to cyanovirin N. Cyanovirin (CNV) is a cyanobacterial protein that interacts through high-affinity carbohydrate-mediated interactions with the surface-envelope glycoprotein gp120 from various HIV and SIV strains and thereby blocks HIV entry[3]. CNV is currently under preclinical investigation and has structurally been characterized by NMR[4]. We describe methods to characterize the glycoconjugates when integrated into the micelles and present data on the protein-carbohydrate interaction.

[1] Brandley, B.K., *Semin Cell Biol,* **1991**. 2, 281-7.
[2] Sharon, N. and H. Lis, *Sci Am*., **1993**. 268, 82-9.
[3] Boyd, M.R., *Antimicrob. Agents Chemoth*. **1997**, 41, 1521-30.
[4] Bewley, C. et al., *Nat. Struct. Biol.*, **1998**, 5, 571-8.

Medicinal Chemistry **62**

CB-1 Antagonists: From Knowledge Based Design to Lead Optimization

L. Alig, A. Alanine, M. Andjelkovic, A. Benardeau, K. Bleicher, A. Bourson-Sleight, P. David-Pierson, W. Guba, J. Higelin, D. Kube, T. Lübbers, S. Mohr, W. Neidhart, J.-M. Plancher, C. Rocha, M. Rogers-Evans, S. Röver, G. Schneider, J. Smit & S. Taylor

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

Here we report on the results of a knowledge based approach via evolutionary de novo design and rapid parallel synthesis to finding CB-1 receptor antagonists, culminating in the identification of 2,2-diphenylbenzo[1,3]dioxole-5-carboxylic acid amides. This formed part of a fast follower project aimed at the identification of novel small molecules with a profile competitive to Rimonabant, a Sanofi CB-1 antagonist currently in late phase clinical trials for smoking cessation and obesity treatment. The presentation will focus primarily upon the tools used within the Roche Hit and Lead Generation department which enabled the rapid identification of the lead series within just 7 months.

Medicinal Chemistry **63**

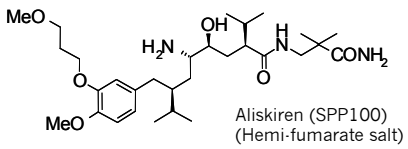
Aliskiren, A Structurally Unique Oral Renin Inhibitor

Jürgen Maibaum

Novartis Institutes of BioMedical Research, Novartis Pharma AG, CH-4002 Basel, Switzerland

The recognition of active site non-substrate binding sites has opened new avenues for the structure-based design of more drug-like, small molecule transition-state peptidomimetics of the aspartyl protease renin. Blockade of the renin-angiotensin-aldosterone system at source by selective inhibition of the rate-limiting renin has been well recognized as highly attractive target.

The iterative target X-ray structure-based topographical design concept leading to the discovery of aliskiren [1], a first-in-class orally efficacious renin inhibitor currently in clinical Phase III for the treatment of hypertension, will be discussed.



Aliskiren (SPP100)
(Hemi-fumarate salt)

[1] J. M. Wood, J. Maibaum, J. Rahuel, M. G. Gruetter, N.C. Cohen, V. Rasetti, H. Rueeger, R. Goeschke, S. Stutz, W. Fuhrer, W. Schilling, P. Rigollier, Y. Yamaguchi, F. Cumin, H.-P. Baum, Ch. R. Schnell, P. Herold, R. Mah, Ch. Jensen, E. O'Brien, A. Stanton, M. P. Bedigian. Structure-based design of aliskiren, a novel orally effective renin inhibitor. *Biochem. Biophys. Res. Comm.* **2003**, *308(4)*, 698-705.

Medicinal Chemistry **64**

Fluorescent trivalent Gal/GalNAc-terminated ligands for the asialoglycoprotein receptor (ASGP-R)

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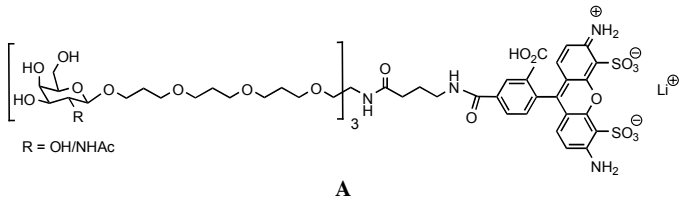
Institute of Molecular Pharmacy, University of Basel, Klingelbergstrasse 50, Basel CH-4056, Switzerland

A series of fluorescent ligands for the ASGP-R [1], each bearing three β-linked galactose or *N*-acetylgalactosamine moieties linked to flexible spacers for efficient interaction with the receptor [2], has been synthesized and tested on cells. The spacers are connected to a central TRIS-derived core, which is itself conjugated to a linker bearing a terminal amino group. The latter has been linked to a fluorescent label via amide coupling to the succinimidyl-activated ester of Alexa Flour® 488 dye.
The final constructs **A** are efficiently endocytosed by HepG2 cells (ASGP-R-positive human hepatocellular carcinoma) in a process that can be monitored by laser scanning confocal microscopy (LSCM).
These results indicate that compounds of type **A** can be used for targeted drug delivery to the liver.



R = OH/NHAc

A

[1] Ashwell, G.; Harford, J. *Annu. Rev. Biochem.* **1982**, *51*, 531.
[2] Biessen, E. A. L.; Beuting, D. M.; Roelen, H. C. P. F.; van de Marel, G. A.; van Boom, J. H.; van Berkel, T. J. C. *J. Med. Chem.* **1995**, *38*, 1538.

Medicinal Chemistry **65**

Discovery of a New Class of Angiogenesis Inhibitors by Molecular Modeling

Pascal Furet, Karl-Heinz Altmann, Guido Bold, Paul Manley, Thomas Meyer, Juergen Mestan, Wilhelm Stark.

Novartis Institutes for Biomedical Research, CH- 4002 Basel , Switzerland

Inhibition of tumor induced angiogenesis is a promising strategy in anticancer drug research. In this area, our main target is the tyrosine kinase activity of the vascular endothelial growth factor receptor (VEGF-R). Early medicinal chemistry efforts in this direction have resulted in Valantanib, a first generation VEGF-R kinase inhibitor, currently undergoing phase 3 clinical trials.
In this presentation, the molecular modeling and structural biology aspects of an effort that has led to the discovery of a novel class of VEGF-R kinase inhibitors will be discussed.

Medicinal Chemistry **66**

Peptide- and Protein-Capped Inorganic Semiconductor Nanocrystals for Photodynamic Therapy

V. V. Nikandrov,[1] A. P. Savitsky,[2] E. K. Ruuge,[3] V. A. Nadtochenko,[4] S. P. Martsev,[5] S. Mann,[6] and J.-E. Moser[7]

[1] Inst. of Biochemistry, RAS, Moscow; [2] Moscow State University; [3] Cardiology Research Center, Moscow; [4] Inst. of Chemical Physics, RAS, Chernogolovka, Russian Federation; [5] Inst. of Bio-organic Chemistry, Minsk, Belarus; [6] University of Bristol, UK; [7] Inst. of Chemical Sciences & Engineering, Ecole Polytechnique Fédérale de Lausanne, Switzerland.

Photodynamic therapy (PDT) has been extensively developed for the treatment of some types of cancer and skin diseases. PDT is based on the use of photosensitizers, which are able of accumulating in cancerous tissues. This inter-disciplinary project focuses on inorganic semiconductor nanocrystals (SNs) as photosensitizers for PDT. These have been shown to be very reactive towards redox species in solution and to induce strong phototoxicity. They are more active and stable than organic sensitizers.

Nanosized cadmium sulfide SNs capped with peptides (glutathione, cysteine) and proteins (bovine serum albumin, hexokinase, trypsine, and R-phycoerythrin red fluorescent protein) were obtained by crystallization during reaction between $CdCl_2$ and Na_2S aqueous protein solutions. TEM micrographs showed that CdS nanocrystals capped with bovine serum albumin (BSA) typically display a mean diameter of 37 Å, with BSA units forming a tetrahedral structure around the mineral core.

Specific delivery of SNs to tumors can be achieved by fusing capping proteins with anti-ferritin antibodies or their recombinant fragments targeted to ferritin-exposing malignant cells. Human ferritins, modified to incorporate a recombinant VL fragment of the anti-ferritin antibody F11, were expressed and isolated. Conjugates of semiconductor nanocrystals with ferritins were then prepared by including various amounts of hydrous ferric oxide, CdS, or CdSe in the cavity of the modified apo-protein.

Medicinal Chemistry **67**

Cloning, Expression and Preparative Use of a Mutated, Bifunctional α(2-3/2-8)-Sialyltransferase from *Campylobacter jejuni*

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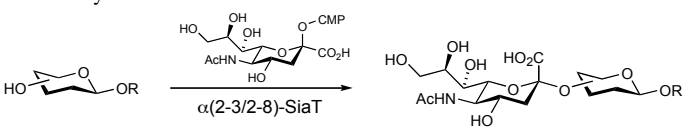
Carbohydrates from glycolipids and glycoproteins with terminal sialic acids are involved in a broad variety of biological recognition and adhesion phenomena.[1] Although numerous glycosylation methods are known, the chemical synthesis of complex oligosaccharides is still a cumbersome and time-consuming procedure. In particular, the formation of α(2-8)-linked sialic acids is one of the most difficult reactions in carbohydrate chemistry. Additionally, chemical sialidations suffer from poor stereoselectivity due to the lack of neighboring group participation. A convenient alternative is the use of enzymatic sialidations.[2]



For the enzymatic synthesis of mono- and bis-sialylated oligosaccharides, a bifunctional, recombinant α(2-3/2-8)-sialyltransferase (Δ32Cst-II) from *Campylobacter jejuni*, as well as a mutated variant, were cloned and overexpressed as His-tagged proteins in *E. coli*.[3],[4] These enzymes were kinetically analyzed and their potential for sialidations of various substrates on a preparative scale was explored.

[1] R.A. Dwek *Chem. Rev.* **1996**, *96*, 683.
[2] R. Öhrlein *Topics Curr. Chem.* **1999**, *200*, 227.
[3] M. Gilbert, M.F. Karwaski, S. Bernatchez, N.M. Young, E. Taboada, J. Michniewicz, A.M. Cunningham, W.W. Wakarchuk, *J. Biol. Chem.* **2002**, *277*, 327.
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Medicinal Chemistry **68**

Exploring The Binding Site on MAG

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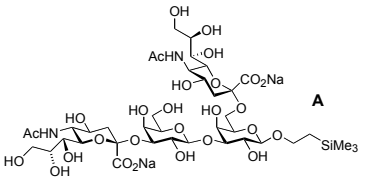
The myelin-associated glycoprotein (MAG) has been identified as one of the neurite outgrowth inhibitory proteins.[1] Several gangliosides like GD1a, GT1b, GQ1bα have been shown to be potent inhibitors of MAG.[2] In previous SAR studies with partial structures of GQ1bα (e.g. tetrasaccharide **A**), the α(2-3)- and α(2-6)-linked sialic acids were found to be important pharmacophores.[3] In addition, the disaccharide core acts as a linker between the neuraminic acids. To improve binding properties, various sialic acid derivatives were synthesized yielding the lead compound **B**. For its further optimization the Topliss operational scheme[4] was applied.



To obtain more potent MAG antagonists, second binding sites in the close vicinity of the lead compound were identified using second binding site screening by NMR[5] and click chemistry.[6]

[1] C.J. Woolf, S. Bloechlinger, *Science* **2002**, *297*, 1132-1134.
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Medicinal Chemistry **69**

N-Benzyl Derivatives of (−)-Conduramine B-1 are β-Glucosidase Inhibitors: Potential Drugs against Gaucher`s Disease

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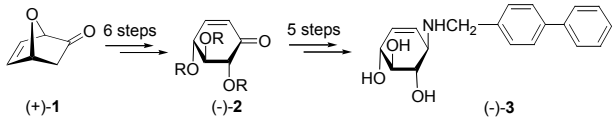
Gaucher´s disease is the most prevalent of the lysosomal storage disorders [1]. Patients with Gaucher´s disease exhibit hepatosplenomegaly, anemia, bone lesions and respiratory failure, with or without progressive neurological disorders. Current therapeutic strategies include expensive enzyme replacement and substrate depletion [2]. Unfortunately, the efficacy to neurological symptoms of this therapy is low [3].



N-Benzyl derivatives of (−)-conduramine B-1, such as (−)-**3**, are good, competitive and selective β-glucosidase inhibitors [4]. Because of their relative important hydrophobicity, they should be tested for their ability to act as chemical chaperones and for their therapeutic potential for the Gaucher´s disease, in analogy with other β-glucosidase inhibitors [5].

[1] A. Beutler, G. A. Grabowski, In *The Metabolic and Molecular Bases of Inherited Disease;* 8th ed., C. Servier, A. L. Beaudet, D. Valle, W. S. Sly, B. Childs, K. W. Kinzler, A. Vogelstein, Eds., McGraw-Hill, New York, **2001**, p. 3635.
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Medicinal Chemistry **70**

Toward an Anti-cancer Vaccine: Clusters Synthesis of C-disaccharide Mimetics of the Thomsen-Friedenreich Antigen

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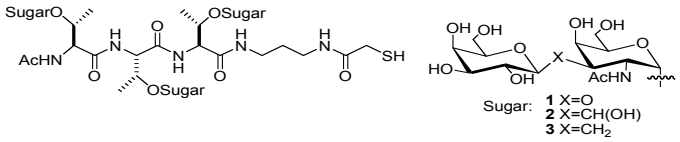
The Thomsen-Friedenreich antigen (T antigen) is a cancer-associated disaccharide which plays an important role in tumor cell-cell recognition. The immunodominant part of the T antigen consists of the disaccharide Galβ1→3GalNAcα→O linked to serine or threonine. The great potential of clustered antigen motifs such as **1** for antitumor vaccines has been demonstrated. [1]



C-linked disaccharide analogues offer stability towards hydrolysis which is catalysed by ubiquitous glycosidases. We wish to present here the extension of our previous efforts [2] towards the synthesis of C-disaccharide analogues of the T antigen based on a Baylis-Hillman type of condensation between a D-galactose-derived aldehyde and isolevoglucosenone. [3-4] Cluster of type **2** have been prepared and conjugated to KLH.

[1] S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupathi, P. O. Livingston, S. J. Danishefsky, *J. Am. Chem. Soc.* **1998**, *120*, 12474.
[2] Y.-H. Zhu, P. Vogel, *Synlett* **2001**, 79.
[3] Y.-H. Zhu, P. Vogel, *Tetrahedron Lett.* **1998**, *39*, 31; Y.-H. Zhu, R. Demange, P. Vogel, *Tetrahedron: Asymmetry* **2000**, *11*, 263; [4] L. Awad, J. Riedner, P. Vogel, *Chem. Eur. J.* **2005**, 11, in press

Medicinal Chemistry **71**

Correlation between drug uptake and efficacy of Platinum (IV) anti-cancer drugs with functionalized aromatic carboxylate ligands

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Despite the success of platinum-based drugs in treating a broad variety of tumors, drug resistance remains a severe limitation to their chemotherapeutic effectiveness **[1]**. One strategy to overcome resistance is to design and build specific functionalities onto Pt compounds, to enhance uptake or inhibit mechanisms of resistance **[2, 3]**. Aryl groups have been known to improve uptake of drugs by conferring greater lipophilicity and facilitating transport across cell membranes **[4, 5]**. We have therefore synthesized a series of functionalized *trans*-Pt(IV) aryl carboxylate complexes, based on the *cisplatin* moiety, with the objective of studying their efficacy as a relationship to drug uptake. The compounds were found to be 5-20 fold more cytotoxic than cisplatin across tested breast, lung and colon cell lines. There was also a strong correlation between their drug efficacy and intracellular accumulation which could account for their rapid activity compared to cisplatin.

[1] M. Fuertes, C. Alonso, J. Perez, *Chem. Rev.* **2003,** 103, (3), 645-662.
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[3] W. Ang, I. Khalaila, C. Allardyce, L. Juillerat-Jeanneret, P. Dyson, *J. Am. Chem. Soc.* **2005,** 127, (5), 1382-1383.
[4] Y. Berger, A. Greppi, O. Siri, R. Neier, L. Juillerat-Jeanneret, *J. Med. Chem.* **2000,** 43, (25), 4738-4746.
[5] S. Hanessian, L. Zhan, R. Bovey, O. Saavedra, L. Juillerat-Jeanneret, *J. Med. Chem.* **2003,** 46, (17), 3600-3611.

Medicinal Chemistry **72**

In Vitro and in Vivo Evaluation of Ruthenium(II)-Arene PTA Complexes

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The antitumour activity of the organometallic ruthenium(II)-arene complexes, $RuCl_2(\eta^6\text{-arene})(PTA)$, (arene = *p*-cymene, toluene, benzene, benzo-15-crown-5, 1-ethylbenzene-2,3-dimethylimidazolium tetrafluoroborate, ethyl benzoate, hexamethylbenzene; PTA = 1,3,5-triaza-7-phosphaadamantane), abbreviated RAPTA, has been evaluated. *In vitro* biological experiments demonstrate that these compounds are active toward the TS/A mouse adenocarcinoma cancer cell line whereas cytotoxicity on the HBL-100 human mammary (nontumour) cell line was not observed, which indicates selectivity of these ruthenium(II)-arene complexes to cancer cells.[1] Analogues of the RAPTA compounds, in which the PTA ligand is methylated, have also been prepared, and these prove to be cytotoxic toward both cell lines. RAPTA-C, $RuCl_2(\eta^6\text{-}C_{10}H_{14})(PTA)$,[2] and the benzene analogue RAPTA-B were selected for *in vivo* experiments to evaluate their anticancer and antimetastatic activity. The results show that these complexes can reduce the growth of lung metastases in CBA mice bearing the MCa mammary carcinoma in the absence of a corresponding action at the site of primary tumor growth. Pharmacokinetic studies of RAPTA-C indicate that ruthenium is rapidly lost from the organs and the bloodstream.

[1] C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava, P. J. Dyson, *J. Med. Chem.* **2005**, *in press.*
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Medicinal Chemistry **73**

2,3-Diaminopropionic Acid as Tridentate Ligand for Potential Radiopharmaceutical Application

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As a part of ongoing efforts to find new radiopharmaceutical cores in our group[1][2], a series of ligand with different coordination patterns (O-N-O, N-N-O and N-N-N) have been examined, among which, 2,3-diaminopropionic acid was found to be an efficient tridentate (N-N-O) chelator for Re(I) or ^{99m}Tc(I) (Figure 1).

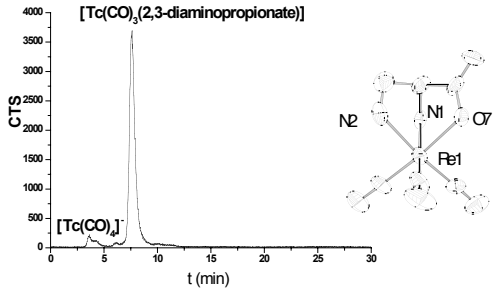

Fig. 1 Ortep drawing of [Re(2,3-diaminoproionate)$(CO)_3$] and HPLC trace of a labeling experiment with [^{99m}Tc$(CO)_3(H_2O)_3]^+$-kit.

In addition, the labeling data further indicated that the species formed by 2,3-diaminopropionic acid and ^{99m}Tc$(CO)_3(H_2O)_3^+$ was stable in the presence of histidine, cysteine and O_2.

[1] R. Alberto, *et al*, *J. Am. Chem. Soc.* **1998**, *120,* 7987..
[2] R. Alberto, *et al*, *J. Am. Chem. Soc.* **1999**, *121,* 6076.

Medicinal Chemistry **74**

Modified Pyrenes for Two Points Attachment/Incorporation

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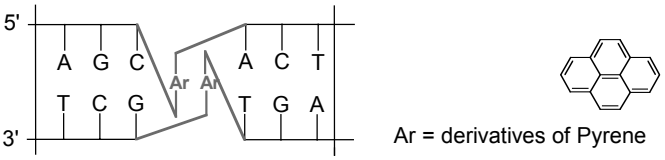
Pyrene compounds are expanding their place in research of functional materials and medicinal chemistry because of advanced photophysical properties they hold. Rich fluorescence properties of pyrene systems allowed using them as microenvironment sensors (for pH, anions/cations, viscosity, polarity etc monitoring), as well to serve as dendrimers/polymers fluorescent modificators and DNA probes.



We involved in a program of non-nucleosidic aromatic building blocks incorporation into DNA, their synthesis and study.[1] [2]
Usually modified pyrenes bear only one functional group that allows incorporating the pyrene unit into more complex system via only single covalent bond and there are very few compounds that have two or multiple attachment.
Herein we will present our development on pyrene modification that include of introduction of (1) two functional groups (ester or amide) for *Two Points Incorporation* into oligonucleotides; and (2) one/two additional substituents in pyrene core that allow to tune its stacking properties.

[1] A. Stutz, S.M. Langenegger, R. Häner, *Helv. Chim. Acta* **2003**, 3156.
[2] S. M. Langenegger, R. Häner, *Chem. Commun*. **2004**, 2792.

Medicinal Chemistry **75**

Interactions of Chlorin e6 and Monoaspartyl-Chlorin e6 with model membranes

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Chlorin e6 (CE) and its mono-L-aspartyl ester (MACE) are promising candidates for Photodynamic Therapy (PDT) against various types of carcinoma [1] [2]. PDT is based on the combination of a photosensitizer and its activation by light leading to a selective photodamage of the tumor tissue. The amphiphilic structure of CE and MACE may enhance their membrane solubility which is believed to be important for PDT efficiency [2]. The aim of this study was to examine the interactions of CE and MACE with model membranes with respect to their membrane solubility, location at the membrane, and the factors modulating these interactions. Solution state NMR spectroscopy was used as main tool to probe CE/MACE model membrane systems. Small unilamellar vesicles (SUVs) served as model membranes.
Analysis of the ^{1}H NMR spectra obtained for the chlorin-SUV systems led to the following conclusions: i) a split phospholipid (PL) choline signal suggests interaction of CE and MACE with the outer PL head groups, ii) time-dependent changes of the split choline chemical shifts of CE-SUVs indicate slow distribution of CE across the PL bilayer, iii) interactions are pH-dependent, iv) decrease in T_1 and T_2 relaxation times of PL protons suggest restricted mobility of PL molecules in the presence of CE. NMR data indicate that CE and MACE are self-associated in neutral aqueous solutions. For the applied concentration range disaggregation could not be detected in the presence of SUVs. Further studies are aimed at analyzing the aggregation behavior of CE/MACE in the absence and presence of model membranes.

[1] Nyman,Emma S.; Hynninen, Paavo H., R. *J. Photochem. Photobio. B.* **2004**, *73,* 1-28.
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Medicinal Chemistry **76**

DNA targeting radiotherapy with ^{99m}Tc based on the [2+1] approach

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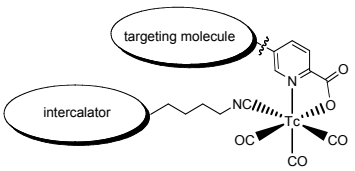
The most effective electrons for inducing DNA single and double strand breaks by direct and indirect pathways are those with initial energy of 50 to 250 eV because these electrons are able to produce clusters of inelastic interactions within a radius of a few nm[1]. In the case of ^{99m}Tc, the emitted electrons with the highest cytotoxic potential would thus be the MXY Auger as well as MMX and NNX Coster-Kronig ones.
Although the theoretical aspect of the in vivo toxicity of ^{99m}Tc is well known, the in vivo cytotoxicity of ^{99m}Tc has only been experimentally tested by three research groups[2].



In this work we design molecules using the [2+1] method. This offers the possibility to coordinate an intercalator via one and a targeting molecule via another ligand. This will allow us to determine the cytotoxic effect of such systems on living cell.

[1] S. Ftacnikova et. al., *Radiat. Prot. Dosim.*, **2000**, 92, 269.

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Medicinal Chemistry **77**

Species-dependent differences in MAO B inhibitor specificity

Laura Novaroli[a)], Marianne Reist[a)], Elisabeth Favre[a)], Antoine Daina[a)], Marco Catto[b)], Angelo Carotti[b)], Pierre-Alain Carrupt[a)]

[a)]Ecole de Pharmacie Genève-Lausanne, LCT - Pharmacochimie, Section des sciences pharmaceutiques, Quai Ernest-Ansermet 30, CH-1211 Genève 4, Suisse, [b)]Dipartimento Farmaco-Chimico, Università di Bari, Bari, Italy

Interest in inhibitors of monoamine oxidase type-B (MAO B, EC 1.4.3.4) has grown in the last years, due to their therapeutic potential in aging related neurodegenerative diseases, as Parkinson disease (PD) and Alzheimer's disease (AD) [1].
In the literature, brain, liver and, especially, blood platelets have been extensively described as human MAO B sources to screen inhibitors. However, for practical and ethical reason, numerous authors have preferred to employ animal models as rat brain or rat liver. Indeed, rat tissues are more easily accessible sources for *in vitro* screening of MAO B inhibitors than human tissues. Nevertheless, species-dependent differences of critical importance for comparative studies of drugs involved in MAO B metabolic pathway have been described by several authors [2], [3]. In an attempt to provide a better understanding of the limitations of the rat model two different classes of compounds, coumarin (n=30) and 5*H*-Indeno[1,2-*c*]pyridazin-5-one derivatives (n=33), known as rat MAO B inhibitors, have been tested on human cloned MAO B obtained from a *Baculovirus* expression system (Supersomes™ MAO B, BD Gentest). Recently, the reliability in using this enzyme source has been reported [4].

[1] N. Castagnoli Jr., J. P. Petzer, S. Steyn, K. Castagnoli, J. Chen, M. A. Schwarzschild, C. J. Van der Schyf, *Neurology* **2003,** *61,* S62-S68.
[2] R. M. Geha, K. Chen, J. C. Shih, *J. Neurochem.* **2000,** *75,* 1304.
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Medicinal Chemistry **78**

Antiapoptotic properties of combined acetylcholinesterase, monoamino oxidase-B inhibitors for the treatment of Alzheimer's Disease

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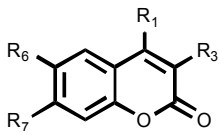
[b] Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Italy

Alzheimer's disease (AD) is a progressive and fatal disorder of the central nervous system characterized by deficits in cholinergic function and progressive cell death of selective neurons in the brain. Apoptosis is considered to be a common type of neuronal cell death in neurodegenerative diseases. The potential to prevent apoptosis in rat pheochromocytoma differentiated PC-12 cells of coumarin derivatives, already identified as potent and selective inhibitors of MAO B and/or AChE [1, 2], was investigated.



General structure of investigated coumarin derivatives

7-[3-(chloro)benzyloxy]-3,4-dimethylcoumarin, showing both MAO-B and AchE inhibitory activity [1, 2], was found to reduce cell death induced by serum-NGF withdrawal in differentiated-PC12 cells. Hence, this compound was identified as an interesting multi-functional hit for the treatment of AD.

[1] C. Gnerre, M. Catto, F. Leonetti, P. Weber, P. A. Carrupt, C. Altomare, A. Carotti, B. Testa, *J. Med. Chem* **2000,** *43,* 4747.
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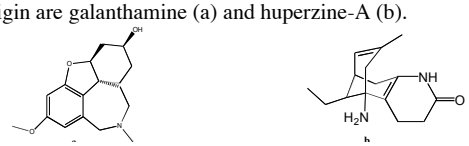
Medicinal Chemistry **79**

Natural Products as Source of Novel Bioactive Agents in Alzheimer's Disease Therapy: Comparison of Two Screening Tests for Inhibitors of Acetylcholinesterase.

Saviana Di Giovanni[a], Marianne Reist[a] , Aurélie Urbain[b], Andrew Marston[b] , Kurt Hostettman[b], Pierre-Alain Carrupt[a]

[a]LCT - Pharmacochimie, [b]Laboratoire de Pharmacognosie et Phytochimie, Ecole de Pharmacie Genève-Lausanne, Section des sciences pharmaceutiques, Quai Ernest-Ansermet 30, CH-1211, Genève 4, Suisse

Alzheimer's disease (AD) is characterized by selective neuronal loss in cholinergic population and by massive deposits of aggregated proteins [1]. Direct analysis of neurotransmitter content in the cerebral cortex shows a striking and disproportionate deficiency of acetylcholine. Classical examples of acetylcholinesterase inhibitors (AChEIs) used for the treatement of AD from plant origin are galanthamine (a) and huperzine-A (b).



In view of the potential of plants for the discovery of new AChEIs a microplate assay which allows rapid and complete kinetic analysis of molecules and in which the activities of compounds were determined by Ellman's method [2] was used and compared to a simple and rapid enzyme assay on TLC plates [3].

[1] M. Bolognesi, V. Andrisano, M. Bartolini, R. Banzi, C. Melchiorre, *J .Med. Chem.* **2005**, *48*, 24.
[2] G. L. Ellman, D. Courtney, V. Andres Jr., R. M. Featherstone, *Biochem. Pharmacol.* **1961**, 7, 88.
[3] A. Marston, J. Kissingl, K. Hostettman, *Phytochem. Anal.* **2002**, 13, 51.

Medicinal Chemistry **80**

Computational Aspects of ATP Analogue Binding in Engineered Protein Kinases

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Protein kinases play an important role in controlling diverse signal transduction pathways in cells, many of which that are disease related. The elucidation of these pathways is therefore essential to understand the molecular mechanics of the disease and to identify viable drug targets, but the identification of the cellular substrates of individual protein kinases remains one of the central challenges in the field.

The group of Shokat et al has developed a method to directly tag the substrates of protein kinases. The ATP binding site of a given protein kinase is mutated to accept a radiolabelled ATP analogue (A*TP) as the phosphodonor, allowing the identification of the substrates of the protein kinase [1]. The challenge of this method consists of finding a suitable combination of ATP analogue and mutation site of the enzyme so it retains its activity, but does not alter the substrate specificity. Usually this problem is addressed empirically, and therefore computational approaches offer an attractive alternative to investigate the binding properties of prospective ligands.

In this work, six ATP analogues and two different protein kinases, for which experimental data is available, are used to develop a method for investigating ligand binding properties of the ATP analogues to the given engineered protein kinase

[1] Shah,K.L.Y., Deirmengian, C. and Shokat, K.M. *Proc. Natl. Acad. Sci USA*, **1997**, 94, 3565

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Cyclopropanation of 3,4-Dihydro-1*H*-benzo[e] [1,4]diazepine-2,5-diones

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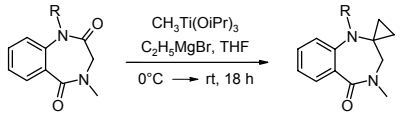
A fast and efficient two step parallel synthesis protocol for the preparation of 1*N*-substituted spirobenzodiazepineones is described. Treatment of 4-methyl-3,4-dihydro-1*H*-benzo[e][1,4]diazepine-2,5-dione with a series of alkyl halides using a microwave-assisted heating protocol provided *N*-derivatized compounds which were transformed to the corresponding cyclopropylamines employing optimized Kulinkovich-type reaction conditions. X-ray structural analysis provided conclusive evidence of the newly created spiro center and revealed a significant flattening of the 7-membered ring



system compared with the benzodiazepinedione system which allows to access different exit vectors. The physicochemical parameters log *D*, pKa, solubility and membrane permeability of both starting materials and cyclopropanated compounds were assessed showing interesting trends depending on the substitution pattern. The spirocycle benzodiazepinone backbone represents an interesting novel template that offers several possibilities for further modification which might lead to new biologically active compounds.

[1] Kulinkovich, O. G.; Sviridov, S. V. *Zh. Org. Khim.* **1989**, *25*, 2244-2245.
[2] Chaplinski, V.; de Meijere, A. *Angew. Chem. Int. Ed.* **1996**, *35*, 413-414.

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