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The Discovery of Macitentan – A Standard Medicinal Chemistry Approach?

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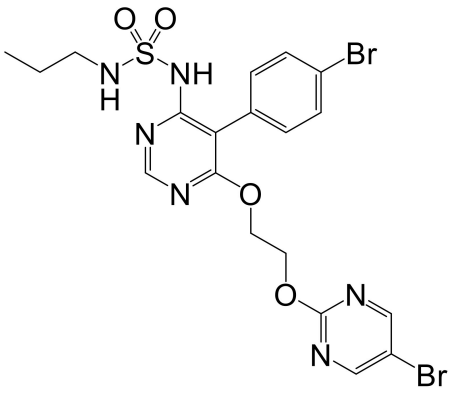

The dual endothelin receptor antagonist (ERA) macitentan (Opsumit®) is an approved drug for the treatment of pulmonary arterial hypertension.[1-4] The discovery efforts that led to its identification were motivated by the observation that bosentan, the first approved dual ERA, had several shortcomings. Based on this knowledge, our discovery efforts focused on three key compound properties: its in vitro potency on the endothelin receptors, its in vivo efficacy in reducing the mean arterial pressure (MAP) in hypertensive Dahl salt sensitive rats and its lack of interference with the hepatic bile salt transport in a rat model. Hence, in vitro assays for the two endothelin receptors served as a first filter to prioritize compounds for further profiling. More importantly, however, results obtained from in vivo experiments assessing compound efficacy and safety were clearly driving the discovery process. This 'in vivo'-driven approach was possible because both the pharmacological model as well as the liver safety assessment were easily accessable and allowed rapid and relevant testing of a relatively large number of compounds. Macitentan is about 100-fold more potent on ET_A and approximately ten times more efficacious in reducing MAP in Dahl S rats than bosentan and shows no sign of interfering with the hepatic bile salt transport. It was selected for clinical development after testing about 2500 compounds in vitro and almost 400 compounds in vivo. This talk shall not only describe the process that led to the discovery of macitentan and highlight some of its key properties but also illustrate how this successful approach can be applied to other drug discovery programs.

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Deciphering the catalytic mechanism of the sulfoxide synthase EgtB

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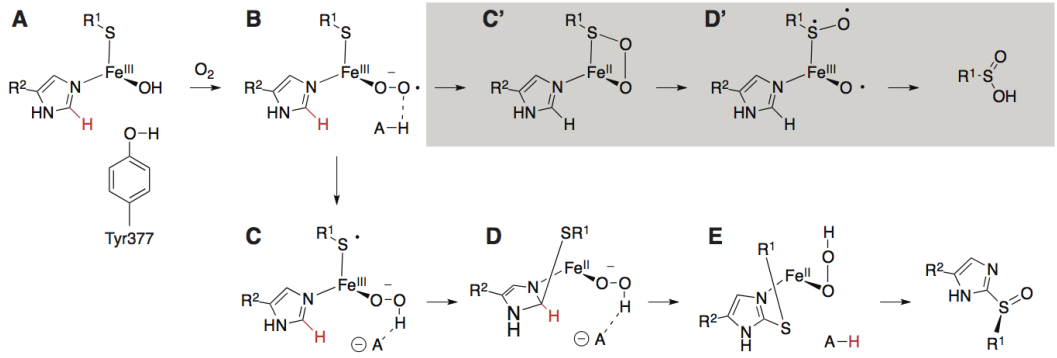
EgtB from *Mycobacterium thermoresistibile* is an unusual non-heme iron enzyme that catalyzes the formation of a sulfur-carbon bond between cysteine and N-alpha-trimethylhistidine. Based on the crystal structure of this enzyme, compounded with kinetic characterization of the wild type enzyme and active site mutants we devised a model for the catalytic mechanism of this enzyme (Figure). This model predicts that the rate-limiting step includes oxidation of the substrate thiolate to a thiyl radical. To test this proposition we engineered a hydrogen bond interaction to this thiolate. This intervention does not change substrate binding but significantly reduces k_{cat}. In this presentation we discuss these observation in view of our general understanding of biocatalytic sulfur-carbon bond formation.



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Discovery and Development of the Highly Potent, Highly Selective Cathepsin S Inhibitor RG7625 for the Treatment of Autoimmune Diseases

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The lysosomal cysteine protease cathepsin S plays an important role in antigen presentation by degrading the invariant chain fragment p10 to CLIP. This CLIP fragment is associated to the major histocompatibility complex MHCII. After exchange of CLIP by antigens the MHCII/antigen complex is transported to the surface on antigen presenting cells such as microglia, dendritic and B-cells. This complex may be recognised by e.g. T-cells which subsequently become activated. If this process is disturbed, occasional loading of MHCII by self antigens may occur followed by an autoimmune response. Therefore, inhibition of cathepsin S may be an effective treatment of autoimmune diseases.

This presentation will cover the medicinal chemistry optimization of a series of cathepsin S inhibitors culminating in the identification of RG7625 as a highly potent and highly selective cathepsin S inhibitor. Aspects of structure based design, enzyme kinetics and multi dimensional optimisation will be highlighted. The preclinical profiling of RG7625 and clinical Phase I data will be outlined as well.

A sticky interaction: Optimizing the hydrophobic stacking between the tyrosine gate of the bacterial lectin FimH with antagonists

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FimH is the main virulence factor of uropathogenic *E.coli* (UPEC) causing urinary tract infections (UTI) in humans. FimH attaches to mannosylated glycoproteins on urothelial cells preventing the clearance from the bladder and enabling infection of the host cells. Mannose-based antagonists have been developed to block this initial attachment step and thereby prevent UTI as a highly desired alternative to antibiotics treatment. Here we describe the rational development of FimH-antagonists with optimized interaction profiles for oral treatment of UTI.[1,2]
Special care was paid to the optimization of pharmacokinetic parameters and at the same time maintaining a high binding affinity.[2,3] The interaction between the tyrosine gate of FimH with hydrophobic aglycones of the antagonists was found to be crucial and could be analyzed by NMR spectroscopy and X-ray crystallography. NMR signals of key residues in the binding loop containing tyrosine 48 were identified as sensitive reporter for the conformation of the tyrosine gate. Finally, ITC profiles delivered full thermodynamic descriptions of the interactions guiding the further improvement of the antagonists.

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The discovery of a potent and orally available Dot1L inhibitor

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Dot1L is responsible for the methylations of lysine 79 of histone 3 (H3K79), with the H3K79me2 mark being associated with active transcription. Under physiological conditions, Dot1L is critical for normal hematopoiesis, however, misdirected catalytic activity (methyltransferase) is believed to be causative for certain acute leukemias. Several oncogenic fusion proteins including MLL-ENL, MLL-AF4 and MLL-AF9 aberrantly recruit Dot1L to ectopic loci, leading to local hypermethylation of H3K79 and misexpression of genes (including HoxA9, Meis1) which drive the leukemic phenotype[1]. Inhibition of the methyltransferase activity of Dot1L in MLL-rearranged leukemias (mixed lineage leukemia, MLL) is predicted to reverse ectopic H3K79 methylation, leading to repression of leukemogenic genes and tumor growth inhibition. The recent quest for Dot1L inhibitors is spearheaded by Epizyme and culminated in the discovery of EPZ-5676, a SAM-competitive, nucleoside-containing Dot1L inhibitor, which is currently being evaluated in MLL patients in Phase 1b clinical trials. The agent is administered by continuous intravenous (i.v.) infusion due to its physicochemical properties largely inherited from SAM cofactor from which it is derived. Herein, we will describe a structurally completely novel (non-SAM like) series of Dot1L inhibitor that is not interacting in the SAM cofactor binding pocket. This series was optimized to match the exquisite potency of the current clinical candidate and to achieve oral bioavailability with a suitable exposure profile in mouse.

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Optimization of 1,4-Disubstituted Benzodiazepines as Selective and Brain Penetrant Triple Calcium T-Channel Blockers

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Epilepsy affects more than fifty million people worldwide. Despite the availability of around thirty antiepileptic drugs (AED), used alone or in combination, 30% of the epileptic patients are not seizure free. More importantly, the current treatments affect only the symptoms and no available AED can prevent epileptogenesis. In this context, inhibition of the calcium T-channels offers a promising new way to treat this disorder [1,2].

At Actelion, our search for potent and selective brain penetrant triple blockers started with an HTS campaign and 1,4-disubstituted benzodiazepine hits were identified. Despite showing low nanomolar activity, these hits suffered notably from poor physico-chemical properties and were metabolically unstable. Herein we report the SAR studies and optimization of this class of compounds, leading to the discovery of a potent lead derivative with improved solubility and DMPK properties. The optimized compound showed interesting in-vivo efficacy in a model of absence epilepsy [3].

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A FUC/LecB system to crystallize versatile nucleic acid structures

P. Röthlisberger[1], C. Leumann[2], M. Hollenstein[1]*

[1]Institut Pasteur, [2]University of Bern

Over the last few decades, oligonucleotides consisting of base-modified nucleic acids found interest in applied material science.[1] In order to fully understand the properties of such oligonucleotides it is advantageous to know the exact three-dimensional structure. Functional nucleic acids, consisting of aptamers, DNAzymes and ribozymes have found applications in biosensing,[2] diganostics[3] and gene therapeutics.[4] A shortcoming of functional nucleic acids in *in vivo* applications is degradation by exonucleases, the requirement of high M^{2+} concentration to attenuate catalytic activity and a challenging cellular delivery. The chemical alteration of the functional nucleic acids to circumvent such problems without impeding their activity is a commonly used method. The knowledge of the exact structural arrangement allows a more specific chemical intervention and could therefore lead to more potent therapeutics of diagnostics. Herein, we report on the X-ray structural analysis of modified DNA duplexes and functional nucleic acids. In this context, we have developed a method for the elucidation of the three-dimensional structures that involves the derivatization of the oligonucleotide with an L-fucose (FUC) residue to enable a non-covalent conjugation to the protein lectin B (LecB) that serves as robust co-crystallization agent.[5]

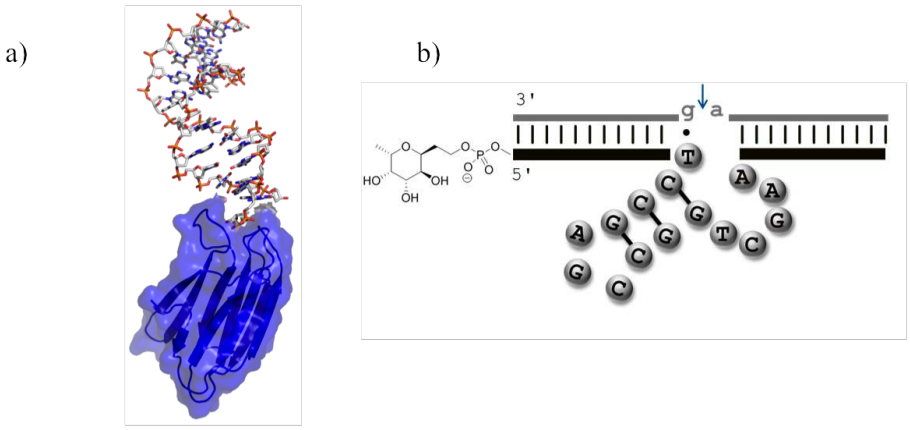

Figure 1. a) Representation of a DNA duplex crystallized with the FUC/LecB system; b) Schematic representation of a fucosylated DNAzyme binding to an RNA substrate.

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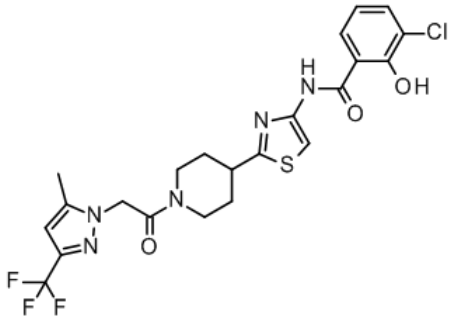
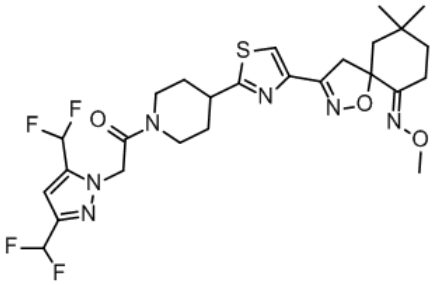
Synthesis and oomycete fungicidal activity of a new family of inhibitors targeting an oxysterol binding protein

M. Pouliot[1]

[1]Syngenta Crop Protection

Inhibitors targeting oxysterol binding protein have shown excellent fungicidal activity against late blight and downy mildew, plant diseases caused by oomycete pathogens. Oxathiapiprolin, discovered by DuPont researchers, have been the first compound of this class to reach the market and is commercialized by both DuPont and Syngenta under the trade names Zorvec™ and Orondis™ respectively. In this talk, we would like to present research done in Syngenta on oomycete fungicide inhibiting oxysterol binding protein. The synthesis and antifungal activity of new classes of bicyclic and spirocyclic isoxazolines will be presented, along with that of the *N*-thiazol-4-yl-salicylamide class which distinguished itself by its unique capacity at controlling damping-off disease caused by *Pythium ultimum*.



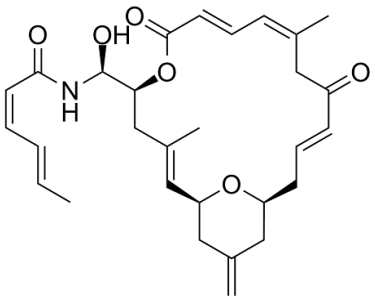


Stereoselective Synthesis and Biological Evaluation of Highly Potent New (-)-Zampanolide Derivatives

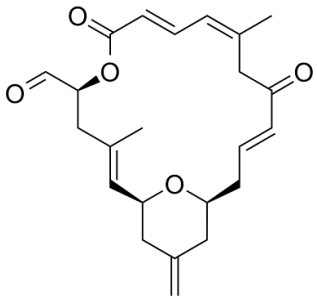
T. Brütsch[1], J. Miller[2], K.-H. Altmann[1]*

[1]ETH Zürich, [2]Victoria University of Wellington, School of Biological Sciences

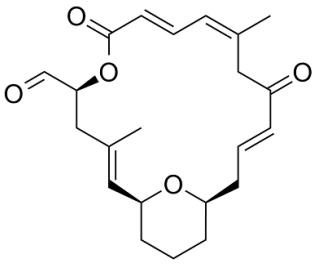
(-)-Zampanolide (**1**) is a polyketide natural product with a highly unsaturated macrolactone core structure and an uncommon N-acyl hemiaminal-linked side chain.[1] (-)-Zampanolide (**1**) is a microtubule-stabilizing agent that binds covalently to the luminal taxane site in b-tubulin through reaction of C9 with His^{229}.[2] We have previously shown that the removal of the the exo-methylene group at C13 in the related (-)-dactylolide **2** (leading to compound **3**) was well tolerated in terms of antiproliferative activity.[3]



(-)-Zampanolide (1)
IC_{50} (A549) = 3.2 nM

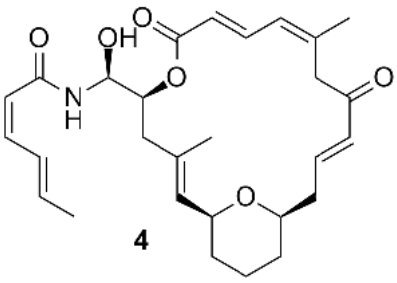


(-)-Dactylolide (2)
IC_{50} (A549) = 301 nM



3
IC_{50} (A549) = 149 nM

We have now also prepared 13-desmethylene-(-)-zampanolide (**4**) (from **3**) and we have found this analog to exhibit low nM antiproliferative activity. We have thus used **4** as the basis for extended SAR studies that have assessed the role of individual methyl groups and degree of unsaturation of the macrocyclic core structure. These studies were enabled by a newly developed stereoselective method for the installment of the N-acyl hemiaminal side chain.



4

This contribution will discuss the synthesis of a number of new (-)-zampanolide derivatives and will give new insights into the importance of specific structural features for antiproliferative and microtubules-stabilizing properties of **4**.

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High Kinetic Stability of T-Hg^{II}-T and DNA Polymerase Inhibition

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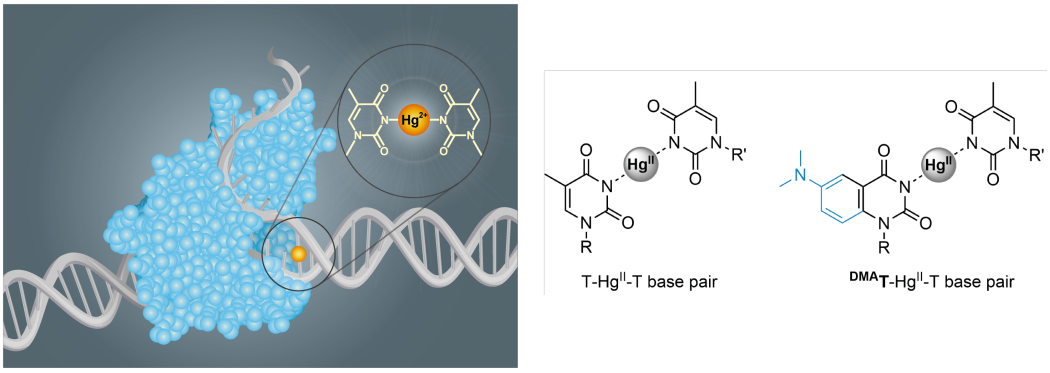
The mechanisms responsible for the infamous cytotoxic and mutagenic activities of Hg^{II} are only partly understood and are potentially the result of mercury-DNA interactions.[1] *In vitro*, T-T mismatches in duplex DNA stoichiometrically bind Hg^{II} ions to give T-Hg^{II}-T base pairs, which exhibit similar thermal stabilities and structural dimensions as T-A base pairs in duplex DNA.[2]



We have utilized the fluorescent thymidine analog ^{DMA}**T** to characterize the kinetic and thermodynamic parameters of Hg^{II} binding to discrete T-T sites in duplex DNA.[3,4] ^{DMA}**T** fluorescence quenching was used for the first reported kinetics study of T-Hg^{II}-T association and dissociation. The on- and off-rates of mercury were surprisingly slow, with association rate constants (k_{on}) = 0.8 – 9.0 x 10^4 $M^{-1}s^{-1}$, and dissociation rate constants (k_{off}) = 1.5 – 9.0 x 10^{-4} s^{-1}; giving equilibrium dissociation constants (K_d) in the range of 8 – 50 nM. With half-lives ranging from 0.3 to 1.3 h in duplex DNA, T-Hg^{II}-T base pairs exhibit high kinetic stabilities that can inhibit enzymatic DNA synthesis and strand-displacement reactions at biologically relevant concentrations. Our results demonstrate that T-Hg^{II}-T base pairs are kinetically distinct from T-A base pairs and therefore have the potential to disrupt DNA metabolism *in vivo*.[4]

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Discovery of a Potent and Selective Reversible BTK Inhibitor for the Treatment of Autoimmune Diseases

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Bruton's Tyrosine Kinase (BTK) is a cytoplasmic tyrosine kinase and a member of the TEC kinase family. It is expressed selectively in a subset of immune cells, including macrophages, mast cells, platelets and B cells. BTK is a key regulator of B cell antigen receptor signalling in B cells and of Fc receptor signalling in mast cells and macrophages. Based on a strong genetic and pharmacological validation, it is likely that a BTK inhibitor will have a positive impact on autoimmune diseases which are driven by autoreactive B cells and immune-complex driven inflammation, like e.g. Rheumathoid Arthritis (RA). In this presentation, we describe the discovery, optimization and preclinical characterization of highly selective reversible BTK inhibitors. Combination of an internal HTS hit with a tail fragment led to a potent and selective lead compound. A co-crystal structure of the lead with BTK proved binding to a specific inactive conformation of BTK underlying the excellent kinase selectivity of the scaffold. Unfortunately, the lead exhibited poor physicochemical properties resulting in low oral bioavailability in rat. We will describe SAR studies focussing on improving the physicochemical properties of the lead by reducing molecular weight, aromaticity and lipophilicity. In addition, a key *t*Bu-group was replaced in order to increase metabolic stability. Finally, the optimization yielded a development candidate with a good balance of potency, physicochemical and PK properties, combined with an excellent preclinical safety profile. The still low solubility of the compound could be mitigated by a tosylate salt, which provided adequate exposure of the compound in relevant preclinical safety species. The compound showed efficacy in two animal models depending on the B cell receptor pathway (sheep red blood cell model) and the Fc receptor pathway (therapeutic collagen induced arthritis).

Investigations for New Therapeutic Targets for Neurodegenerative Disease.

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Additional co-authors:

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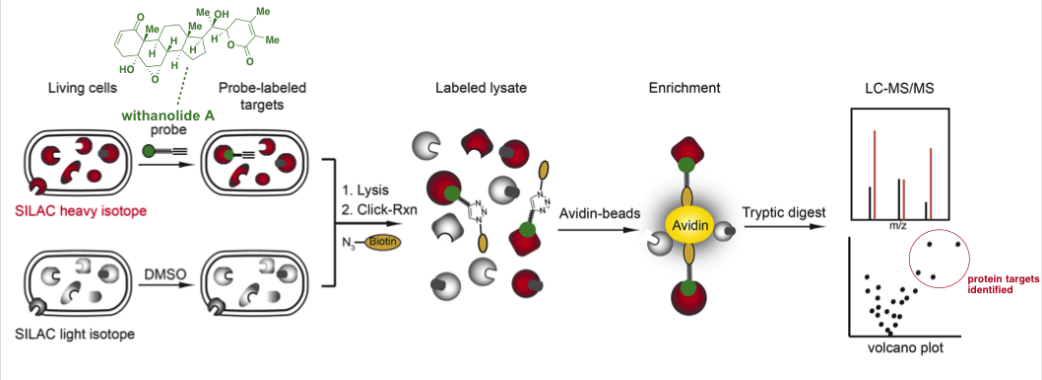
[1]University of Basel, [3]Technical University of Munich

In our quest to identify new potential therapeutic targets for neurodegenerative diseases, we initiated activity-based protein profiling (ABPP) studies with the neuritogenic natural product, withanolide A. Molecular probes were designed based on known reactivities of this scaffold from prior synthetic studies and six novel compounds were successfully prepared. Target profiling of these compounds revealed several relevant and novel hits in the context of neurodegeneration. Further investigations of these hits through molecular modeling with *VirtualToxLab* and several different types of *in vitro* assays were completed. As a result, new targets of relevance to the mechanism of action of withanolide A are suggested, providing more insight into these neuritogenic pathways.



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