Apotekarsocieteten
Swedish Pharmaceutical Society

4th Swedish Medicinal Chemistry Symposium

17-18 April 2024

Stockholm, Sweden

Sponsors

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17-18 April, 2024. Organizer: Section for Medicinal Chemistry, Swedish Pharmaceutical Society

April 17

09.15	Registration and coffee
09.45	**Opening remarks – House keeping** Chair: Lena Ripa, AstraZeneca
10.00	**An IL-17A crystallization system supporting small molecule structure-based drug design** *Maria Håkansson, SARomics Biostructures*
10.30	**Application of SYN321 in Osteoarthritis Management. The Science behind the Local Injection Technology HYDRO-LINK** *Antonio Bermejo Gómez, Synartro*
11.00	**Mimicking nature´s design – pseudoglucosinolates as prodrugs for bioresponsive isothiocyanate release** *Philipp Klahn, University of Gothenburg*
11.30	**Affibody-based hBCMA x CD16 dual engagers for NK cell-mediated killing of multiple myeloma cells** *Stefan Svensson Gelius, Oncopeptides*
12.00 – 13.30	*Lunch*
	Chair: Ulf Nilsson, Lund University
13.30	**The FragMAX facility for structure-based drug discovery at MAX IV Laboratory** *Tobias Krojer, MAX IV Laboratory*
14.00	**“Scratching the surface” with skin PKPD** *Stefan Eirefelt, LEO Pharma*
14.30	**Is functionalization of C-H bonds interesting in medicinal chemistry?** *Magnus Johansson, AstraZeneca*
15.00 – 15.45	*Coffee break – Poster session*
15.45	**Targeting metabolic reprogramming in leukemia** *Karin Lindkvist, Lund University*
16.15	**Discovery and pre-clinical characterization of AZD4604, a potent and selective inhaled JAK1 inhibitor in clinical development** *Magnus Nilsson, AstraZeneca*
16.45	**Accelerating drug design with AI & simulation** *Eva Nittinger, AstraZeneca*
17.15 – 19.00	**Poster session – Refreshments**

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April 18

08.45	**House keeping** Chair: Anders Karlén, Uppsala University
09.00	**Award Läkemedelskemiska priset** *Anders Karlén, Uppsala University*
09.15	**Award lecture Läkemedelskemiska Priset** *To be announced.*
10.00	*Coffee break – Poster session*
10.45	**Short presentations selected from abstracts** **Substrate-Derived Sortase A Inhibitors: Targeting Antimicrobial Resistance** Jordi Hintzen, *University of Gothenburg*
11.00	**Design, Synthesis and Evaluation of novel macrocyclic peptidomimetics targeting the insulin-regulated aminopeptidase (IRAP)** Esther Olaniran Håkansson, Uppsala University
11.15	**Optimization of the N Piperidinyl Benzimidazolone Derivatives as Potent and Selective Inhibitors of 8 Oxo Guanine DNA Glycosylase 1** *Zuzanna Szaruga*, Karolinska Institutet, SciLifeLab
11.30	**In vivo assembly of bioresorbable organic bioelectronics as a therapeutic modality** *Roger Olsson, Lund University*
12.00 – 13.30	*Lunch*
	Chair: Peter Sjö, Drugs for Neglected Diseases initiative (DNDi)
13.30	**Late Stage Chemical Process Development and Setting Efficient Control Strategies** *Hanna Cotton, Novo Nordisk*
14.00	**Development and Mechanism of the MTHFD1/2 Inhibitor TH9619** *Martin Henriksson, One-Carbon Therapeutics*
14.30	*Coffee break*
15.00	**Modulating Gene Expression with Small Molecules** *Erik Chorell, Umeå University*
15.30	**Poster award**
15.45 – 16.00	**Concluding remarks – End of Meeting**

Poster Abstracts

P1 Going beyond conventional virtual screening: charting molecular diversity in chemical space using 3D electrostatic field and shape descriptors

Oliver Hills*† & Nathan Kidley†

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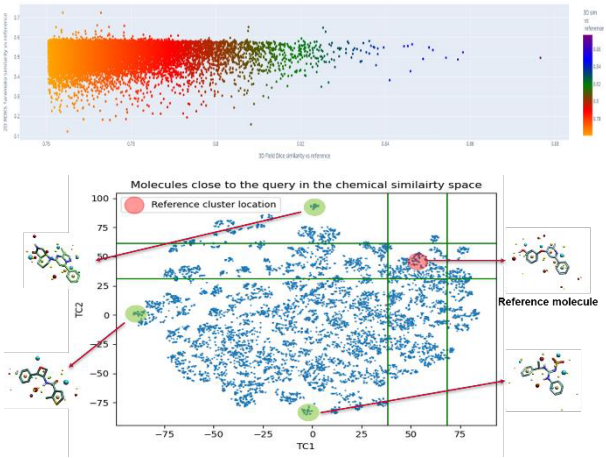
Aim: Using a prevalent Malaria target, this presentation illustrates the advantages of 3D field screening over, commonly used, 2D fingerprint similarity methods; as well as exemplifies the reduction and diversity enrichment of the vast chemical space explored.

Methods: Two known *P. falciparum* macrophage migration inhibitory factor (pfMIF) inhibitors were identified and prepared for virtual screening (poses taken from 4P7M.pdb and 4P7S.pdb). These inhibitors were then used as queries for Blaze™, Cresset's advanced ligand-based virtual screening platform (Cheeseright et al., 2007). Hits were identified as compounds most similar to the query in 3D electrostatics and shape, and the expanse of the chemical space explored was visualised using t-Distributed Stochastic Neighbour Embedded (t-SNE) projections of 2D RDK5 fingerprint similarities.

Results and Discussion: Analysis of the Blaze hits using 2D chemical similarity clearly demonstrates that Blaze identifies leads with a wide chemical diversity. Compounds at the 'fringes' of the explored chemical space belong to very different, and non-obvious, chemical series which do not represent intuitive design ideas. Yet, their 3D electrostatic and shape fields are conserved, representing molecules capable of forming similar interactions with the target protein than the query molecule.



Conclusions: The expanse of the chemical space charted, using the 3D virtual screening approach, is far larger than what's accessible using 2D. Screening on the basis of 2D fingerprint similarity fails to access the regions of chemical space that encompasses the novel, non-obvious, chemistry, required to progress molecule drug discovery, accessible when using the 3D electrostatic and shape field similarity.

References:

1. Cheeseright, T., Mackey PhD, M., Rose PhD, S., Vinter PhD, A., 2007. Molecular field technology applied to virtual screening and finding the bioactive conformation. Expert Opin. Drug Discov. 2, 131–144. https://doi.org/10.1517/17460441.2.1.131

P2 Structure-guided exploration of commercial chemical space: the Chemical Space Navigator

Flavio Ballante[1,2,*], Martin Scobie[3], Emma Rose Scaletti[4], Evert Homan[3], Ingrid Almlöf[3], Hao Liu[4], Mohit Narwal[4], Pål Stenmark[4], Thomas Helleday[3], Bengt Persson[2], and Jens Carlsson[2]

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Aim:

We have developed the "Chemical Space Navigator", a web tool to guide hit identification and lead development towards denser regions of tractable analogs from large chemical spaces.[1]

Methods:

The Chemical Space Navigator is designed to facilitate fast exploration of multi-billion commercial chemical space through structure-guided methods. This tool features substructure and similarity searches, enabling the identification of commercially available analogs to streamline drug discovery and development.

Results and discussion:

The tool was used to guide prospective virtual screening campaigns against the MutT homolog1 (MTH1) enzyme. Virtual screens led to the discovery of MTH1 inhibitors with IC_{50} values in the mid-nanomolar range (achieving a ~55 fold improvement from the initial hit) which were further studied by X-ray crystallography.

A web server that allows searching for analogs of the CBCS primary screening set through >5 billion commercially available compounds is currently accessible to the SciLifeLab Research Community. A second web server, tailored for fragments available at the MAX IV Laboratory, is under construction. Both web servers will soon be publicly accessible.

Conclusions:

The Chemical Space Navigator is beneficial in projects focusing on the discovery of chemical probes or drug candidates, as it allows to rationally uncover new compound hits from ultra-large chemical libraries. It represents a valuable tool in phenotypic and target-based drug discovery and can be used to facilitate fragment-based approaches, virtual or empirical screenings, and exploration of structure-activity relationships (SARs).

References:

[1]Ballante, et al. In preparation.

P3 Late Stage Defluorinative Functionalizations: Synthesis of Methyl-Dithioesters, Thioamides and Heterocycles from Trifluoromethylarenes

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Abstract

Aims: To develop a method for the synthesis of methyl-dithioesters from the readily available trifluoromethyl group via a defluorinative functionalization. The methyl-dithioester is a useful intermediate in various synthetic pathways, and can be used for a variety of further transformations, but known synthetic methods for the functional group suffer from toxic reagents and/or multistep protocols.

In addition, the trifluoromethyl group is relatively inert to many conditions, and with the prospect of its transformation into the reactive and versatile methyl-dithioester group, this method could serve as an excellent alternative for late-stage functionalization in medicinal chemistry synthesis.

Methods: The key reagent for the transformation of a trifluoromethyl group into a methyl-thioester was the commercially available BF_3SMe_2 complex. It functions as both a Lewis acid and a sulfur source, circumventing the handling of any additional odorous thiol sources. The transformation was achieved via microwave-assisted synthesis, an efficient and consistent method for high temperature reactions.

Results and Discussion: The method was successfully applied to a number of varying trifluoromethyl substrates affording the methyl-dithioester, and displayed good functional group tolerability, including halogens, N-acetyls, sulfonamides, amides and nitriles. The method could also be extended to a one-pot operation for the synthesis of thioamides and subsequent heterocycles from the trifluoromethyl group by taking advantage of the reactivity of the methyl-dithioester intermediate.

Finally, to illustrate the usefulness of the trifluoromethyl group as a late-stage handle in medicinal chemistry, the transformation of pharmaceuticals leflunomide, flufenamic acid and celecoxib into novel methyl-dithioester, thioamide and heterocyclic derivatives was demonstrated using these methods.

Conclusions: In conclusion, we developed a new strategy for the activation of the trifluoromethyl group and its transformation into methyl-dithioesters, and subsequently, the one-pot synthesis of biologically and synthetically important thioamides and heterocycles. These conversions from the trifluoromethylarenes opens up interesting new retrosynthetic disconnections and late-stage functionalizations, which has been exemplified with known pharmaceutical compounds.

P4 Nucleobase catalysts for the enzymatic activation of 8-oxoguanine DNA glycosylase 1 (OGG1)

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Current Address:Department of Pharmacy, Ludwig-Maximilians-Universität Munich, Germany

Aim:

The aim of this study is to explore the potential synthesis of a purine analogue inspired by OGG1's catalytic mechanism in DNA repair pathways. This analogue aims to enhance the organocatalytic function of 8-oxoG purine analogue activators, thereby increasing β-elimination efficiency.

Methods:

- Fluorescence based activator screening for OGG1 activators based on substrate similarities.
- ^{32}P-Radiolabelled substrate in investigating the effects of compounds on the AP-lyase function of hOGG1 on 8-oxoG:C containing DNA.
- Compound pKa investigation and protein stabilisation with the binding of activators by using Nano-DSF.
- X-ray co-crystal structure of mOGG1 with bound activator.

Results and discussion:

The initial screening of a 500-member in-house library identified primary hits predominantly composed of guanine-amine combinations at the 6-position. Subsequent investigation highlighted the 6-aniline modification as a favoured scaffold adjustment. These results suggest insights from previous OGG1 inhibitor synthesis can inform the optimization of nucleobase derived OGG1 activators. Moreover, the polar and nitrogen-rich nature of nucleobase scaffolds appears advantageous for facilitating proton abstraction during OGG1 biocatalysis. Our study also emphasizes the importance of considering pH conditions, with OGG1 exhibiting optimal function at pH close to 8, as reflected in our AC50 measurements at pH 7.5. Additionally, the assessment of AP-lyase activity across a pH range of 6.7 to 8.4 underscores the dynamic interplay between OGG1 activator and enzyme. These findings provide valuable groundwork for activator development, crucial for application in varied pH environments across distinct cellular compartments.

Conclusion:

We present the structure-activity relationship (SAR) analysis of two highly potent series of OGG1 nucleobase catalysts, or activators. Our findings illustrate the potential for reprogramming OGG1 biochemical function across various pH environments and substrate scopes. This discovery establishes a foundation for strategically manipulating base excision repair, offering potential applications in addressing neurodegenerative diseases, inflammation, obesity, and cancer.[1,2]

Reference:

1. C. Benitéz-Buelga, T. Helleday, M. Michel, Clinical and Translational Medicine2022, 12, e1035
2. M. Hussain, X. Chu, B. Duan Sahbaz, S. Gray, K. Pekhale, J.-H. Park, D. L. Croteau, V. A. Bohr, Free Radic Biol Med2023, 203, 34–44

P5

Phosphodiester Foldamers

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Aim

To construct a novel, conformationally stable backbone which can act as a scaffold for the molecular recognition of protein-protein interactions (PPIs).

Background

Phosphodiesters are ubiquitous throughout Nature and underpin many biological processes.[1,2] Despite their prevalence in biomacromolecules, they themselves do not act as a conformational determinant. Herein we report the investigation into novel abiotic aromatic phosphodiester architectures whereby conformation is precisely controlled via intramolecular hydrogen bonding, in accordance with the foldamer criteria outlined by Gellman.[3]

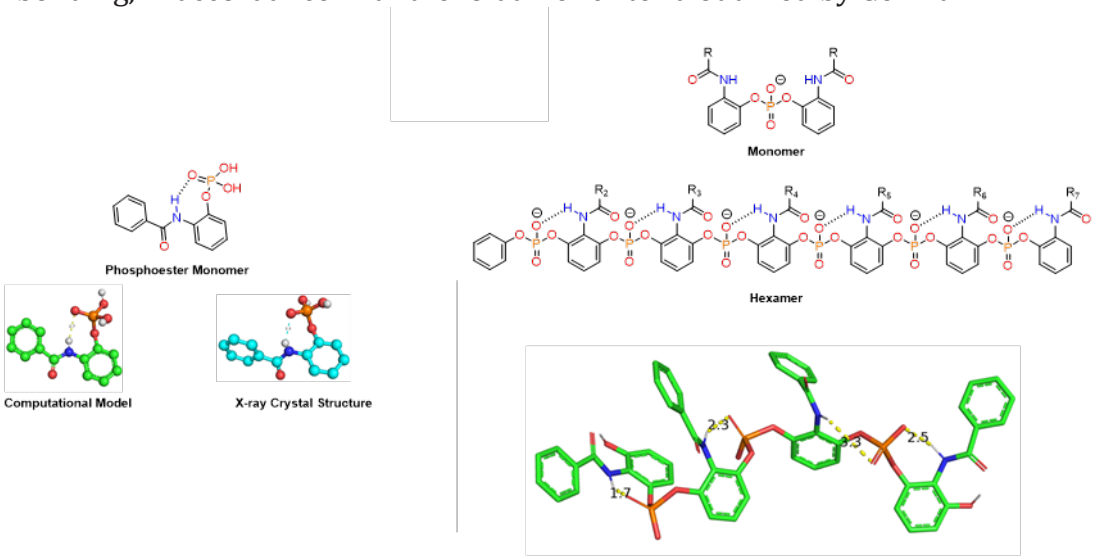

Figure 1. Left - Computational model (green) and X-ray crystal (blue) of a hydrogen bond stabilised phosphoester; Right - Extended structure of the phosphodiester foldamer backbone.

Methods, Results and Discussion:

X-ray crystallography, studies *in silico*, and NMR experimentation (^{1}H-^{31}P HSQC, HOESY, H-D exchange, and hydrogen bond acidity calculations) characterise novel intramolecular hydrogen bonds between the amide N-H and the phosphate P-O forming a stable 7-membered hydrogen bonded ring system.

Conclusions

These building blocks have been expanded into larger phosphodiester foldamer scaffolds. Biologically relevant "R" groups can be encoded into a specific sequence to recognise biomacromolecular surfaces and disrupt targeted PPIs.

References:

[1] Knowles, J. R, **1980**, *Annu Rev Biochem*, *49*, 877–919. [2] Rall, T. W., Sutherland, E. W., and Berthet, J., **1957**, *Journal of Biological Chemistry*, *224*, 463–475. [3] Gellman, S. H., **1998**, *Acc. Chem. Res.*, *31*, 173-180.

P6 AI/ML-Methodology Outperforms Animal Models and *In Vitro* Methods for the Prediction of Oral Bioavailability in Humans and Successfully Predicts for 300 Compounds Out of Reach for Laboratories

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Aim: Oral bioavailability (F) is a major determinant for systemic exposure of drug candidates. Traditionally, F measured in various animal species are used to predict F in humans. The correlation (R^2) between F in animals (mice, rats, dogs) and humans is 0.30 [1]. Predictions can also be done using *in vitro* data (Caco-2, hepatocytes, plasma protein binding, blood-plasma ratio). Published examples are, however, rare. Characteristic for *in vitro* methods is large portion of non-quantifiable compounds and major selection bias. Our PK prediction software ANDROMEDA (AI/ML) has been validated and benchmarked in many studies [1]. The aim was to use our AI/ML-methodology, integrated in ANDROMEDA, to predict F in humans and make head-to-head comparisons *vs* both animal and *in vitro* models.
Methods: Laboratory data from the literature were used to predict average F. A criterium for *in vitro* data was that complete and multiple measurements were available from various sources. Our AI/ML-methodology was used to predict F for these compounds. Predicted compounds were not included in model training sets. ANDROMEDA was also used to predict F for 300 compounds with non-predictable F due to non-quantifiable *in vitro* permeability, solubility, unbound fraction in plasma and/or intrinsic metabolic clearance.
Results and discussion: AI/ML outperformed both animal models (Q^2=0.50 *vs* R^2=0.30; n=156) and *in vitro* methodology (Q^2=0.58 *vs* R^2=0.20; n=29) and successfully predicted F for 300 compounds out of reach for *in vitro* methods (including many with very low fraction dissolved, absorbed, unbound and metabolized).
Conclusions: AI/ML was shown to clearly outperform (accuracy and range) animal models and *in vitro* assays for the prediction of F in humans, and succeeded in predicting F for all the compounds out of reach for *in vitro* methods. The encouraging results challenge the prevailing view of choice of methodology and enable improved cost-efficiency and 3R.

Reference: 1. Fagerholm, U., Hellberg, S., Spjuth, O., 2021. Advances in predictions of oral bioavailability of candidate drugs in man with new machine learning methodology. Molecules 26, 2572.

P7 A DNA-encoded compound library-based approach for the discovery of novel inhibitors

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Aim The aim of this work is to build DNA-encoded chemical libraries (DECLs) that can be used for rapid exploration of structure-activity relationships and identification of new chemical matter against antibiotic targets. This includes a general-purpose peptide-based library for a broader scope of drug targets.

Methods The DECL technology in drug discovery deviates from traditional methods by utilizing large compound collections synthesized on DNA without classical purification. DNA serves as both a reaction medium and information carrier, enabling rapid compound assembly through split-pool synthesis. Each compound in the collection is uniquely labeled with a DNA barcode, simplifying the screening process. The entire collection can be screened against a drug target in a single experiment, thanks to DNA barcodes facilitating easy binder identification through DNA sequencing.

Results and Discussion To enable the discovery of novel inhibitors, selections will be carried out against an "unbiased" peptide-based library, whose compounds have not been specifically designed using any known inhibitor. For the synthesis of this library, a split and pool, approach was used, involving three chemical steps. The library consists of 300.000 compounds, which were synthesized from a wide range of unnatural amino acid derivatives and carboxylic acids that exhibit variation in terms of structure, stereochemistry and functional groups.

Conclusion This research focuses on addressing the challenge of pan-drug resistant bacterial pathogens by employing a DNA-encoded compound library (DECL) approach for antibiotic discovery. DECL technology enables the rapid synthesis of a diverse set of compounds on DNA, while simplifying the screening process against drug targets. The study outlines the synthesis of a general-purpose peptide-based library comprising 300.000 compounds, emphasizing its potential for uncovering novel inhibitors.

References

Mannocci, L., Zhang, Y., Scheuermann, J., Leimbacher, M., Bellis, G. De, Rizzi, E., Dumelin, C., Melkko, S., Neri, D., 2008. High-throughput sequencing allows the identification of binding molecules isolated from DNA-encoded chemical libraries. Proc. Natl. Acad. Sci. 105, 17670–17675.

Salamon, H., Klika Škopić, M., Jung, K., Bugain, O., Brunschweiger, A., 2016. Chemical Biology Probes from Advanced DNA-encoded Libraries. ACS Chem. Biol. 11, 296–307.

P8 Fragment Docking for DNA Repair Inhibitors in Vast Chemical Space

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Aim: In this work we aimed to combine large-scale virtual screening and fragment-based drug discovery to identify inhibitors of 8-oxoguanine DNA glycosylase (OGG1), a DNA repair enzyme involved in diseases such as cancer and inflammation.

Methods: Chemical libraries of more than 14 million fragments were docked to the OGG1 active site, after which top-ranked molecules were selected for experimental evaluation by thermal shift and enzymatic assays. Binding modes of thermostabilizing fragments were elucidated by protein crystallography. The fragment hits were elaborated into potent leads guided by docking of readily synthesizable analogues into the active site. Inhibitors that displayed potent OGG1 inhibition were tested for target selectivity in the DNA repair enzyme family, cellular target engagement, anticancer and anti-inflammation activities in relevant cell models.

Results and discussion: Of the 29 top-ranked fragments that were experimentally evaluated, four binders were prioritized for protein crystallography. The experimentally derived binding mode of all four compounds agreed well with docking predictions. Fragment-to-lead optimization led to the discovery of potent and selective inhibitors with promising anti-inflammatory and anti-cancer activities.

Conclusions: We have developed efficient strategies to explore vast chemical spaces in pursuit of novel OGG1 inhibitors. Further optimization of these lead-like inhibitors to improve pharmacokinetic profiles can provide suitable candidates for *in vivo* studies.

P9 Design and validation of a GPCR-focused chemogenomics library to enable and unlock biology in the open

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AIM: The IMI2-funded EUbOPEN project (www.eubopen.org) aspires to compose a freely available chemogenomics library (CGL) of 3-5,000 small molecules targeting one third of the druggable genome. The library will cover all major target families including G Protein-Coupled Receptors (GPCRs). Each ligand will be annotated in-depth with selectivity data from the literature, complemented with data generated by the project, and then disseminated to the public through a web portal (gateway.eubopen.org). The final library will be made physically available to the research community.

Methods: KNIME workflows were built to query and filter cheminformatics databases such as ChEMBL, IUPHAR GtoPdb and Probes & Drugs for GPCR ligands and their associated activity data. A multi-objective scoring function was then used to rank ligands per target and modality. For selectivity profiling, PRESTO-Tango β-arrestin recruitment-based GPCR assays were established for a panel of 20 targets, selected to be aligned with the disease areas of focus for the project (CRC, IBD, MS & NASH). Additional in-depth ligand profiling is performed using the PDSP screening panels.

Results & Discussion: So far, 462 GPCR-targeted ligands have been acquired as CGL candidate members, collectively covering 170 different GPCRs. Screening of this ligand set confirmed all positive controls and uncovered additional activities for several ligands and targets.

Conclusions: Our results so far demonstrate that annotated chemogenomics libraries with subsets focusing on target families have the power to uncover novel activities of bespoke ligands as well as to identify novel ligands for understudied targets.

P10 T3P®-Promoted, One-Pot Synthesis of *N*-Alkenylated Heterocycles Using Ketones

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Aim: *N*-Alkenyl heterocycles are an interesting class of compounds and Galeterone, used for the treatment of prostate cancer, is the most notable member of this family (Njar and Brodie, 2015). Despite their utility, the available synthetic procedures suffer from several drawbacks including the use of strong acidic/basic conditions, advanced synthetic intermediates or expensive transition-metal catalysts, so new synthetic methods are desired. Our group previously described the Fischer indolization of phenylhydrazines with ketones/aldehydes using the cheap and green coupling reagent T3P® as an acid and water scavenger (Desroses et al., 2011). In our subsequent studies, we observed a recurrent side reaction leading to the formation of an *N*-alkenylated indole derivative. This observation prompted us to explore the development of a straightforward, metal-free synthesis of alkenylated *N*-heterocycles using T3P® as a water scavenger.

Methods: All reactions were carried out under MW at 120^{0}C for 20 min with a solution of the heterocycle, T3P® and the appropriate ketone in EtOAc. In case of poor conversion, the reaction was heated at 140 ^{0}C for 20 min or 160^{0}C for 1h. After reaction completion, triethylamine was added and the mixture was filtered through a silica plug to obtain the desired products.

Results and discussion: The scope of this methodology showed broad applicability and substrate tolerance. Several *N*H-heterocycles including benzo-fused and five-membered *N*-heteroarenes were produced at good to excellent yield (60-94%). The only exception was *N*H-heterocycles with electron-withdrawing groups at position 3 due to reduced nucleophilicity. The ketone scope also showed impressive compatibility and a small library of *N*H-alkenlyated indole derivatives was synthesized. Finally, the scope of this methodology was pleasingly extended to pharmaceutical-relevant scaffolds with a new two-step synthesis of Galaterone and an alternate synthesis of a key intermediate of the anti-tumoral drug Crizotinib.

Conclusions: A convenient synthesis of *N*-alkenylated heterocycles using the cheap and green coupling reagent T3P® as a water scavenger and ketone electrophiles under microwave irradiation was described. The protocol that we developed is metal and base-free, has easy product purification, and good functional group tolerance. The scope of this reaction was well demonstrated with more than 30 different examples of *NH* heterocycles and ketones, as well as being able to extend this procedure to pharmaceutically relevant scaffolds.

References:

Njar, V.C.O., Brodie, A.M.H., 2015. Discovery and development of galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer. J Med Chem 58, 2077–2087.

Desroses, M., Wieckowski, K., Stevens, M., Odell, L.R., 2011. A microwave-assisted, propylphosphonic anhydride (T3P®) mediated one-pot Fischer indole synthesis. Tetrahedron Lett 52, 4417–4420.

P11 Chemistry for potent small molecules as a modulator of Metacaspase targeting pathogenesis by *Trypanosoma*

Rajeshwari [*,1], Emelie Boström[1,2], Cecilia Lindgren[1], Mikael Lindberg[1], Thilde Andersson[1], Valentin Duvauchelle[1], Stina Berglund Fick,[1,2] Anna Linusson.[1]
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Aim-In tropical countries like Sub-Saharan Africa and in Latin America, protozoan parasite *Trypanosoma brucei* (*Tb*) is responsible for a disease called trypanosomiasis. WHO estimated the total number of people infected in 2023 at eight million with 30 000 new infection cases by *Tb* worldwide. Metacaspases (MCAs) are proteins found in lower eukaryotes instead of caspases with which they share a His-Cys catalytic dyad. They are cysteine proteases identified in a first place in plants, fungi, and protozoa and discovered to maintain homeostasis through programmed cell death (PCD) mechanism, but they are absent in mammals.[1,2] Herein, our work focuses on a *Tb* enzyme named *Tb*MCA-Ib, which cleaves substrates after arginine and lysine residues. *Tb*MCA-Ib can be detected in the bloodstream of mammals' host, and is thus considered as a potential therapeutic target.[2] *Tb*MCA-Ib zymogen (inactive form), require activation with Ca^{2+} prior to biochemical analysis. We aim to develop molecules able to modulate *Tb*MCA-Ib, from a high throughput screening (HTS), followed by the design, the synthesis and the evaluation of molecules as a modulator of *Tb*MCA-Ib to eradicate Trypanosomes-oriented diseases.

Method-We investigated the enzyme activation conditions based on SDS-PAGE and fluorescent-based assay to determine the optimal Ca^{2+} concentration and temperature to activate the system. The binding tendency of *Tb*MCAs to substrate (GGR-AMC) was determined with Michaelis-Menten constants (K_M) by measuring their initial rates (V_0) at different substrate concentrations. K_M values were obtained from a non-linear regression curve fit using the Michaelis-Menten equation. HTS was performed on Beckman Coulter NX pipetting robot for *Tb*K55E Mutant, and still ongoing for *Tb*K55 WT.

Results-The activity-based assay has been optimized with a 10 mM $CaCl_2$ concentration at 37˚C for the enzyme activation, through SDS-PAGE gel, and activity-based fluorescence assay. K_M values for *Tb*MCAs (K55 WT, and K55E Mutant) were, (149 µM and 171 µM) respectively. HTS of 25000 compounds against *Tb*K55E allowed for a conclusive examination (full dose-response assay) of a few hit compounds followed by a hit validation of three thiourea compounds through the determination of IC_{50}. Furthermore, HTS and investigation of activation mechanism of *Tb*K55 WT is ongoing.

Conclusion- Preliminary results provide information about the mode of activation and biochemical requirement of *Tb*MCA-Ib K55E (Mutant), the information can be implemented further to probe *Tb*MCA-Ib WT for structural characterization and functional kinetics.

Reference

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P12 Substrate-Derived Sortase A Inhibitors: Targeting Antimicrobial Resistance

Jordi C. J. Hintzen

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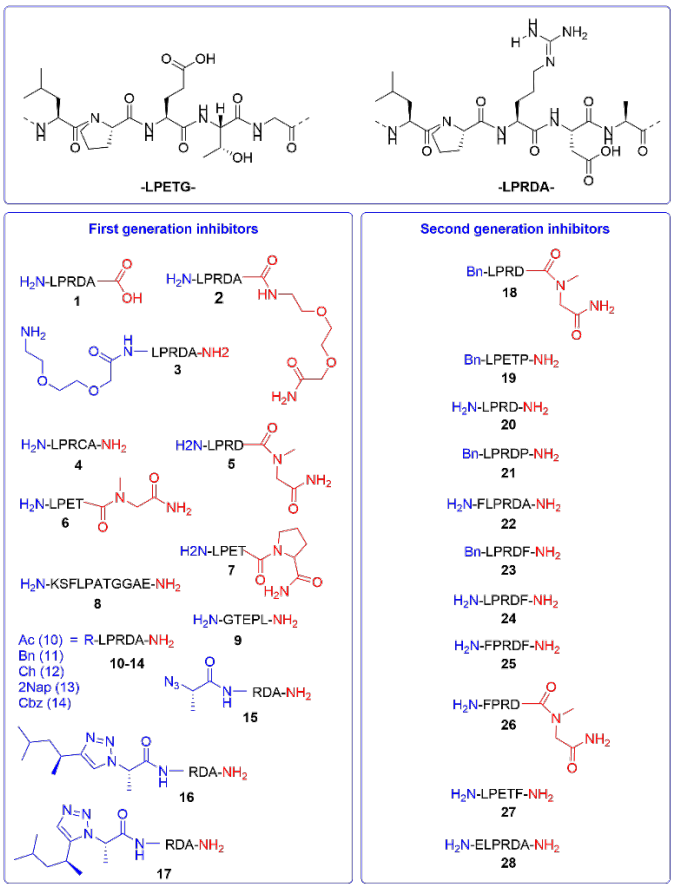
The rapid development of drug-resistant bacteria is a serious healthcare threat that prompted the exploration of new antibacterial targets. Targeting bacterial virulence, instead of developing bactericidal compounds, is a promising strategy that can block pathogenicity by disarming pathogens without affecting bacterial viability. Therefore, this approach exerts less selective pressure to induce the emergence of antibiotic resistance. In our work, we have targeted the bacterial enzyme Sortase A, known as a promising target for antivirulence treatment. Sortase A is a housekeeping transpeptidase of Gram-positive bacteria that anchors cell wall surface proteins into the peptidoglycan layer of the bacterial coat, a process which is crucial for bacterial adherence and host cell invasion. Sortase A relies on a five amino acid sorting signal, by which it recognizes its natural target. Here, we represent a series of peptidomimetic inhibitors of Sortase A, supported by computational investigations. *In vitro* inhibition was determined by employing a FRET-compatible substrate. Among our panel, we identified several promising inhibitors. Biofilm inhibition analysis revealed activity against pathogenic *S. aureus* in a low µM range which so far represents the most potent peptidomimetic inhibitors. Our results show great promise for further development as a novel antivirulence treatment.



P13 Novel Macrocycle-based Compounds for Oral Treatment of Visceral Leishmaniasis

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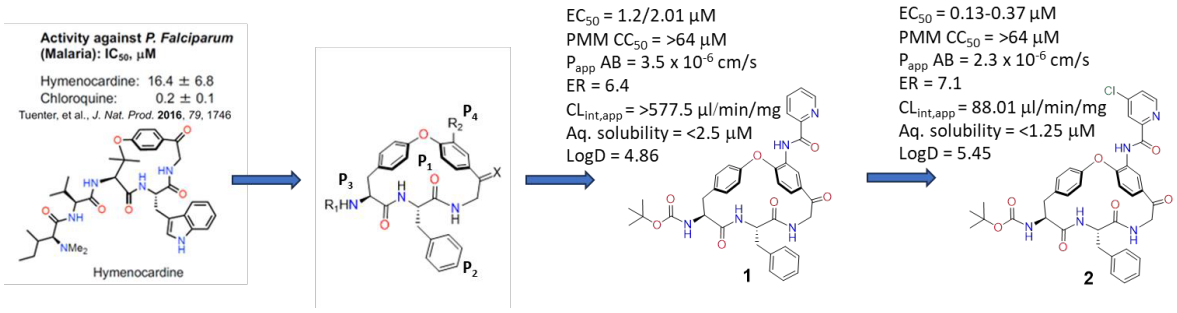
Aim: Visceral leishmaniasis (VL) is the most severe form of leishmaniasis, a neglected tropical disease,[1] caused mainly by *L. infantum and L. donovani*.[2] Miltefosine, approved in 2002, is the only peroral drug for VL, but it is associated with teratogenicity.[3] Our aim is to develop a novel series of macrocycles as an orally bioavailable treatment with improved efficacy and side-effect profile. This will have a significant impact for patients in the developing world, the majority of which are children.

Methods: Our research is focused on *in silico* mining of natural products to discover new leads for difficult-to-drug targets. As part of this project, we designed and synthesized a series of macrocycles, taking inspiration from antiparasitic natural products.[4]

Results and discussion: The series of macrocycles showed promising inhibition of *L. infantum* (EC_{50}: 1.2 µM for **1**) in infected primary mouse macrophages (PMM). Importantly, this series did not show host cell toxicity (PMM CC_{50} >64 µM). Enlarging the macrocyclic ring (**P1**) led to loss of potency against *L. infantum.* Substitution of Phe in the **P2** position by other amino acids, such as Ala and Leu, revealed that hydrophobic side chains are preferred. Replacement of the Boc group in **P3** by an acetyl group led to a reduction in potency, just as removal of the aniline moiety or acetylation of it in **P4**. The compounds had low-moderate solubility and moderate permeability across Caco-2 cells. While microsomal clearance is still too high for **1**, introduction of a chlorine atom in **1** to give **2** improved microsomal stability as well as the antiparasitic activity 5-10 fold.

Conclusions: Compounds **1**-**2** are the starting point for a Lead Optimization process towards an oral Drug Candidate for the treatment of visceral Leishmaniasis.



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P14 Selective Inhibition of a pH-Dependent Metacaspase in *Arabidopsis thaliana*

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Aim: Metacaspases were discovered in 2000 as a type of cysteine protease found in plants, fungi, and protozoa, named originally for their structural homologies with the catalytic domain of caspases.[1,2] These works are focused on the type II family and particularly *At*MCA-IIf which is a common model from *Arabidopsis thaliana* that requires acidic conditions to be active.[3] Metacaspases are synthesized as inactive pro-enzymes called zymogens that require autocatalytic processing to enable the substrates to access the active site. They are also subject to autocleavage over a short period of time, making their monitoring and study very challenging.[4] Our main interest is to develop small, specific chemical probes in order to modulate and investigate the system *in vitro*.

Methods: Two hit series were identified in a high-throughput screening. The hits have been synthesized, and once the results have been confirmed in our hands, two chemical libraries were synthesized (10-15 compounds per series). Inhibition kinetics were performed in addition to circular dichroism and SDS-PAGE to further assess the potency of the best compound in each series and determine the mode of inhibition towards *At*MCA-IIf.

Results and discussions: Using a fluorescent-based assay with Ac-VRPR-AMC as a substrate, we were able to determine that the two series showed interesting results with an IC_{50} in the low micromolar range, a selectivity toward *At*MCA-IIf and no activity on the two Ca^{2+} dependent enzymes *At*MCA-IIa,b. We also observed that the compounds bind to the active protein form following a competitive inhibition mechanism.

Conclusions: These preliminary results are promising and support the purpose we have, which is to develop the first selective probe able to bind to *At*MCA-IIf as an alternative tool to investigate the overall functioning of *At*MCA-IIf.

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P15 AG5 is a novel non-steroidal anti-inflammatory and immune regulator that minimizes Cytokine Release Syndrome and preserves innate immunity

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Aim. An archetypal anti-inflammatory compound against cytokine storm would inhibit it without suppressing the innate immune response.

Methods. AG5, an anti-inflammatory compound, has been developed as synthetic derivative of andrographolide [1], which is highly absorbable and presents low toxicity.

Results and discussion. We found that the mechanism of action of AG5 is through the inhibition of caspase-1. Interestingly, we show with in vitro generated human monocyte derived dendritic cells that AG5 preserves innate immune response. AG5 minimizes inflammatory response in a mouse model of lipopolysaccharide (LPS)-induced lung injury and exhibits in vivo anti-inflammatory efficacy in the SARS-CoV-2-infected mouse model [2]. Furthermore, AG5 showed interesting antiviral activity against SARS-CoV-2 in humanized mice.

Conclusion. AG5 opens up a new class of anti-inflammatories, since contrary to NSAIDs, AG5 is able to inhibit the cytokine storm, like dexamethasone, but, unlike corticosteroids, preserves adequately the innate immunity. This is critical at the early stages of any naïve infection, but particularly in SARS-CoV-2 infections.

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P16 NBP is an equally good solvent as DMF for Microwave Assisted Solid Phase Peptide Synthesis

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Aim

Solid-Phase Peptide Synthesis (SPPS) is the prevailing method for synthesizing therapeutic peptides today. However, SPPS is associated with a significant environmental concern due to the utilization of hazardous solvents such as N,N-dimethylformamide (DMF) or N-methylpyrrolidone (Ferrazzano et al., 2022). In light of this, our research endeavors to identify more environmentally friendly solvents for SPPS.

Methods

We assessed the suitability of five green solvents as alternatives to DMF in automated SPPS. The solvents evaluated include Cyrene, ethyl acetate, 1,3-dioxolane, tetrahydro-2-methylfuran, and N-butylpyrrolidone (NBP). Our investigation encompassed all stages of the synthesis process, from resin swelling, dissolution of reagents, and synthesis of five diverse peptides by microwave-assisted SPPS.

Results and discussion

We systematically evaluated 37 different solvents and solvent mixtures through a comparative analysis with DMF. From the initial resin swelling, roughly half of the solvents proved ineffective. Subsequent dissolution of reagents and starting materials further narrowed down the selection. This resulted in the identification of eight solvents and solvent mixtures for testing in the microwave-assisted SPPS of our model peptide [Asp^5]-Vasopressin. Our findings showed that NBP demonstrated performance on par with DMF. Additionally, we explored the convenience of synthesis by further investigating NBP, establishing its comparable performance with DMF even in the synthesis of the intricate peptides ACP 65-74, Peptide 18A, Thymosin α1, and Jung-Redemann.

Conclusions

Our findings indicate that NBP emerged as a strong contender, performing on par with DMF in all tested syntheses. This research contributes to the pursuit of more sustainable and environmentally conscious practices in peptide synthesis.

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P17 Phthalimide as Protecting Group of Amines: Expanding the Toolbox of the DNA Encoded Chemical Library (DECL) Technology

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Aim:

To investigate the feasibility of phthalimide as an amine-protecting group on DNA. This will include the identification of reaction conditions for deprotection of the phthalimide group. Further aims are to demonstrate the utility of the developed method in a DECL synthetic sequence.

Background and Methods:

In our research group, we focus on discovering new antibacterial molecules. Antibiotic resistance is a severe global problem, where the medicines we use to fight infections become less effective against bacteria as they develop resistance against the drugs. Chemists have dedicated significant efforts to finding new, improved drugs and drug combinations to combat this emerging problem. DNA-encoded chemical library (DECLs) technology provides an efficient and cost-effective drug discovery tool for new starting points. In this approach, large libraries containing millions to billions of compounds attached to unique DNA barcodes are created and used to screen against various isolated drug targets for novel binders. As DNA is fragile, mild synthetic strategies are needed which can ensure selective reactions on building blocks attached to the DNA molecule. Here, DNA-compatible protecting groups of amines are for example limited. Protecting groups need to be stable in conditions suitably of choice, easily attached and removed.

Results and Discussion:

We have investigated the DNA-compatibility method of deprotection of phthalimide. For this, we developed methods for attaching phthalimide to an amine on double-stranded DNA and for removal of the phthalimide, freeing the amine. This allows us to more easily work with DNA and gain better control over the use of amines on DNA molecules.

Conclusion:

Successfully our objectives of this project, *to develop reaction conditions for DNA-compatible phthalimide protection and deprotection* were discovered. Future work within our project is to investigate the usage of phthalimide as a protecting group of amines on DNA for application in DNA-encoded library synthesis tested bacterial activity assays.

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P18 Development of artificial 5-OP-RU-derived MAIT cell modulators for AICs against microbial infection

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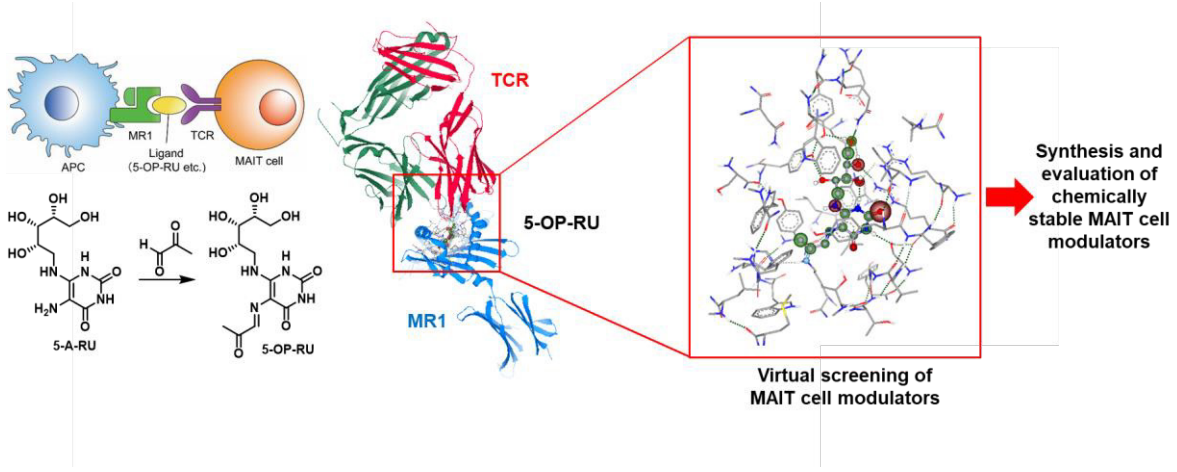
Mucosal-associated invariant T (MAIT) cells play a key role in antibacterial immunity as their membrane-bound T cell receptors (TCRs) recognize microbial antigens presented by surface proteins on antigen-presenting cells (APCs). The ligand-dependent activation of MAIT cells is mediated by the monomorphic MHC (major histocompatibility complex) class I-related (MR1) protein. MR1 binds the bacterial metabolite 5-OP-RU originating from the riboflavin biosynthesis and the resulting complex is presented on the surface of the APC, which leads to MAIT cell activation through formation of a ternary complex with their TCR receptor.[1] MAIT cells are considered to provide efficient protection against acute bacterial infections and seem to play crucial roles in other diseases, such as viral infections and cancer.[2]



However, medical application of natural MAIT modulators, such as 5-OP-RU and 5-A-RU, is limited by a short half-life time due to chemical instability. We aim to synthesize stabilized MAIT ligands as immunomodulators to utilize them in APC-targeted antibody-immunomodulator conjugates (AICs). Here, we report on the virtual in silico screening and chemical synthesis of the MAIT activators as well as preliminary immunological biological evaluation.

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P19 From Anti-inflammatory to Antibiotic? Progress Towards Novel Antibiotics Against *C. difficile*

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Aim

Fentiazac, originally developed as an anti-inflammatory drug, was recently identified as a selective inhibitor of *Clostridioides difficile* growth.[1] The current project aims to synthesize a library of analogues to assess the structure activity relationship of Fentiazac, and then apply this towards novel antibacterial compounds with improved inhibition.

Methods

A reliable and efficient synthetic route has been established for analogue generation, and a small library of analogues has been synthesized to explore changes at specific positions of the structural scaffold. All analogues were tested against *C. difficile* in a growth-inhibition assay.

Results and discussion

Only analogues with changes at one position were still able to inhibit growth, suggesting the other groups are essential for activity. Four analogues, all sharing similar structural motifs, had improved growth inhibition compared to Fentiazac.

Conclusion

Current progress has identified a key area for assessing the structure activity relationship of Fentiazac, and utilized this to synthesize compounds with improved activity. These will inform the next round of synthesis and will lead to additional screening against commensal gut bacteria to assess if selectivity for *C. difficile* has been retained.

References

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P20 Optimization of the N-Piperidinyl-Benzimidazolone Derivatives as Potent and Selective Inhibitors of 8-Oxo-Guanine DNA Glycosylase 1

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Aim: The aim of this study was to develop a series of potent and selective inhibitors of OGG1 in the DNA damage repair process as well as study their binding to the active site.

Methods: In an excision-incision screening of an in-house compound library, two N-piperidinylbenzimidazolone compounds were revealed as weak inhibitors of OGG1. Analogues were synthesised via a multistep route utilising Boc protection of the piperidinyl substituent. The potency of the analogues was tested in fluorescence-based biochemical assays, target stabilisation assessed using differential scanning fluorimetry and selectivity determined against other base excision repair enzymes. CETSA was used to evaluate cellular target engagement of lead compound TH8535. Cell viability in transformed and non-transformed cell lines with TH8535 was tested in a 5-day single-drug exposure fluorescence-based assay.

Results and discussion: Two compounds containing the N-piperidinylbenzoimidazolone structure were identified as weak inhibitors of OGG1 (IC_{50} 10 and 33 µM). A series of analogues of the stronger compound with varying substituents on benzoimidazolone were synthesised, with 4-bromobenzoimidazolone giving the best results (IC_{50} 300 nM). We were unable to optimize the piperidinyl motif and the urea linker further, however, the N atom followed by a non-heteroaromatic sp^2 hybridised system was proven to be essential to the binding of the inhibitor to OGG1. TH8535, combining the 4-bromobenzoimidazolone and 3'-methoxy-4'-methylbenzene substitution patterns, showed an IC_{50} of 200 nM against OGG1. Out of several other base excision repair and DNA glycosylases, TH8535 and 4-halogen substituted analogues were found to weakly inhibit SMUG1 as well as partly Fbg (all IC_{50} > 100 µM). The series was classified as selective inhibitors within the base excision repair process. A co-crystal structure of mouse OGG1 and TH8535 was obtained, showing three hydrogen bonds and a range of hydrophobic interactions within the active site. Through a series of computational experiments, it was discovered that the more potent OGG1 inhibitors adopt a chair conformation within the N-piperidinyl linker as opposed to a less potent analogue, present in a twisted boat conformation. This change causes a shift in hydrogen bonding and a loss of stability. TH8535 was found to lower the cell viability in a number of cancer cell lines in the µM range while the non-transformed cell lines tolerate the compound better.

Conclusions: The N-piperidinylbenzoimidazolone series was optimized to give TH8535 with an IC_{50} of 200 nM against OGG1. The lead compound is a selective OGG1 inhibitor within the base excision repair pathway and is active in transformed cell lines. The SAR of TH8535 was explored and a preference for a chair conformation of the N-piperidinyl linker was revealed. TH8535 has therapeutic potential in chronic inflammation and targeted cancer therapy.

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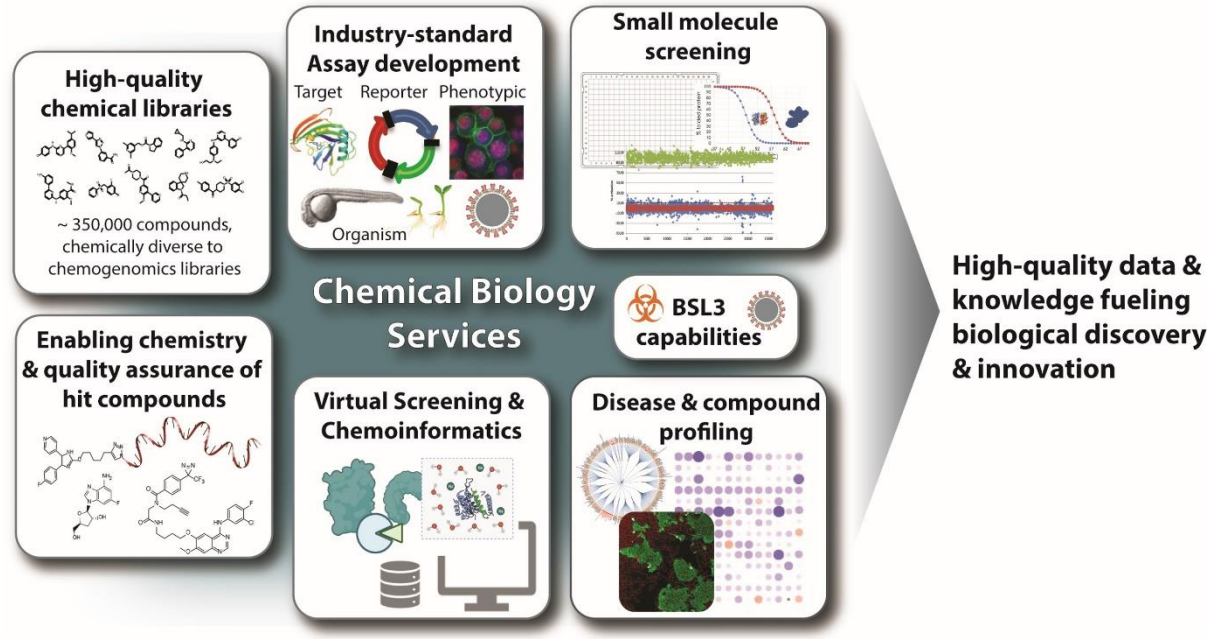
P21

The Chemical Biology Consortium Sweden

Flavio Ballante and Tobias Koolmeister, Karolinska institutet

The Chemical Biology Consortium Sweden (CBCS) (www.cbcs.se) is a national research infrastructure in Sweden funded by the Swedish Research Council, Science for Life Laboratory, and the host universities. The aim of CBCS is to provide world-leading expertise in the field of chemical biology, as well as strengthen research in chemical biology at a national level. Along this line, CBCS is a core part of the Chemical Biology and Genome Engineering (CBGE) facility at SciLifeLab, which also includes the CRISPR Functional Genomics and Chemical Proteomics platforms.

CBCS provides support to academic researchers in assay development and screening, medicinal and computational chemistry, along with disease and compound profiling. Additionally, CBCS provides access to a high-quality compound collection (~350 000 compounds) that originates from pharmaceutical companies and has been developed and expanded with compounds from various commercial vendors. The available screening collections include chemically diverse sets, approved drugs and pharmacological tool compounds. CBCS has well-functioning logistics and routines for distribution of assay ready plates and library compounds nationally.



Collectively, CBCS has been involved in more than 300 collaborative projects. The screening projects cover a wide range of model systems and readouts, including isolated protein targets, targeted cell-based assays, phenotypic cell-based assays, and whole organisms, such as parasites and plants. All research is conducted in close collaboration with users and project outputs range from scientific publications to commercialization of projects and technology developments.

P22 Small-molecule activation of OGG1 increases oxidative DNA damage repair by gaining a new function

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Aim: The aim of this study is to investigate the mode of action of a small molecule, TH10785, in the DNA damage repair process of OGG1.
Methods: TH10785 was chosen from various compounds on stabilization in differential scanning fluorimetry. In vitro OGG1 activity reaction rate was assessed by a fluorescence-based biochemical assay in presence of TH10785 or apurinic endonuclease 1 (APE1). Further, the same assay was performed with generated AP sites by UNG2 which were resolved as well. In cells, a thermal shift assay and confocal microscopy to measure OGG1-green fluorescent protein recruitment were employed to confirm target engagement. DNA binding capacity to OGG1 was tested by measuring amounts of OGG1 substrates with qPCR after U2OS cell oxidation by $KBrO_3$ for 1h. This was also determined by modified comet assay. LC.MS/MS and immunofluorescence were employed to discriminate between glycosylase and AP-lyse activity. Cell viability was measured by IF in combination with PNKP1i.
Results and discussion: TH10785 stabilised OGG1 by 3.8 ± 0.7°C (100 µM) and increased reaction rate at maximum enhancement of 6.25 µM. Unexpectedly, when testing OGG1 β-lyse activity in presence of TH10785 two products were observed. In both molecular dynamic simulations and K_D measurements it was shown that affinity of TH10785 to OGG1 increased with inclusion of an AP analogue with double-stranded DNA. In determination of enzymatic turnover v_{max} we showed a 20-fold increase. It was also shown that in binding to OGG1 TH10785 does not compete with 8-oxodG. Regarding the role of TH10785 it was discovered that it binds to OGG1 as an essential cofactor, by enhancing β-elimination as well as inducing a newly described δ-elimination by OGG1. This is possible due to providing a correctly positioned nitrogen base which mediates protein abstraction, which leads to a cascade of β,δ-elimination. Overall, it was shown that TH1078-activated OGG1 favours AP sites over 8-oxoG. Even more, the induction of β,δ-elimination was determined by decrease in cell viability for TH10785 in combination with PNKP1i due to the change in pathway from monofunctional OGG1 to β,δ-bifunctional complexed with TH10785.
Conclusions: We present findings of a new enzyme-directed small molecule catalysis of β,δ-lyse activity of OGG1 at its active site. Small molecule TH10785 increases OGG1 enzymatic activity 10-fold. We also show that this function of TH10785 shows a preference for AP sites over 8-oxoG and shows a dependency of PNKP1 in vitro and in cells as well. These results have implications for various conditions such as inflammation, cancer, Alzheimer's disease, and aging[1].

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P23

Chemical Space Docking

Mining Billion-Sized Compound Collections for ROCK1 and PKA Inhibitors

Alexander Neumann

Aim: The ongoing explosion of accessible compound collections represents a challenge for common screening methods. To efficiently uncover relevant chemistry in a structure-based approach for identifying the best drug candidates, novel methods are required that can process these vast numbers of molecules without compromising the quality of predictions.

Methods: We developed Chemical Space Docking: a synthon-based combinatorial method that delivers synthetically accessible results while requiring only a fraction of the computational resources typically associated with exhaustive docking procedures.

Results and discussion: Chemical Space Docking was employed on two kinase drug discovery campaigns. In the first scenario, a combinatorial chemical space comprising approximately one billion compounds was screened using preliminary docking of fragment-like synthons at the ROCK1 kinase domain. Follow-up on the most promising candidates resulted in an overall hit rate of 39% for the generated compounds, with the most potent inhibitor displaying a K_i value of 38 nM. The second scenario focused on the discovery of protein kinase A (PKA) inhibitors using fragment-bound complexes as starting points. The screening of a Chemical Space containing 2.7 billion compounds achieved a 43% hit rate. The best compound (K_i = 740 nM) exhibited a 13,500-fold gain in affinity, supported by six crystal structures confirming the predicted binding modes of the most promising binders.

Conclusions: In both studies, Chemical Space Docking successfully identified novel molecular scaffolds within a single iteration cycle.

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P24 Organocatalytic switches of OGG1 control *de novo* AP-lyase function *via* compound pK_a

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Aim

To investigate the impact of compound pK_a on potency as organocatalytic switches of OGG1 to enhance DNA damage repair through promotion of a *de novo* AP-lyase function.

Method

Variably substituted pyridine and pyrimidine-containing organocatalytic molecules were synthesized. The pK_a values were predicted computationally. All compounds were tested *in vitro* in serial dilution at pH 6.7, 7.1, 7.5, and 8.0. Controls were APE1 coupled and DMSO conditions. A duplex oligonucleotide substrate containing U:C was digested by UNG2 prior to assay to determine OGG1/compound complex activity against AP-sites. Initial rates of reaction were measured by ΔFU/sec as DNA cleavage separated a fluorophore and a quencher producing a fluorescence signal.

Results and Discussion

The synthesized organocatalytic switches reliably caused OGG1 to exhibit *de novo* AP-lyase activity. Remarkably, some compounds activated OGG1 to such an extent that the OGG1/compound complex outperformed the APE1 coupled control condition. Compounds with pH-sensitive substituents (i.e., -OH, -COOH, $-NH_2$) were weaker, less predictable activators of OGG1 despite high pK_a values. Meanwhile, compounds with high pK_a values and a pH-stable substituent (i.e., -Me, -OMe) proved highly potent activators of a *de novo* OGG1 AP-lyase function. Native enzyme pH 7.5 gave rise to maximum rates of reaction for all compounds.

Conclusions

We present an SAR foundation for rational design of organocatalytic switches of OGG1. This work reinforces the possibility of enhancing DNA damage repair through acceleration of the OGG1 repair mechanism.[1] Elucidation of organocatalytic switch SAR and modalities will inform future efforts to unlock and enhance *de novo* functions in other enzymes critical to human health. Given the role of OGG1 in BER and inflammation pathways, acceleration of its function may have applications as varied as cancer, chronic inflammation, Alzheimer's disease, and aging.[2,3,4]

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