Synthesis and biological evaluation of imidazole and pyrazole derivatives of Deferasirox targeting KDM4A demethylase

Barthes, N. P. F.,[1,*] Wenzler, S.,[1] Spiske, M.,[1] Schmidtkunz, K.,[1] Robaa, D.,[2] Ngo, V.,[3] Sippl, W.,[2] Hein, L.,[3] Schüle, R.,[4] and Jung, M.[1]

[1] Institut für Pharmazeutische Wissenschaften, Pharmazeutische und Medizinische Chemie, Albert-Ludwigs-Universität Freiburg, Albertstraße 25, 79104 Freiburg, Germany
[2] Martin-Luther Universität Halle-Wittenberg, Department of Pharmaceutical Chemistry, Langenbeckstraße 4, 06120 Halle/Saale, Germany
[3] Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Albertstraße 25, 79104 Freiburg, Germany
[4] Urologische Klinik und Zentrale Klinische Forschung, Klinikum der Universität Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany
[*] nicolas.barthes@pharmazie.uni-freiburg.de

KDM4A, a lysine specific demethylase belonging to the family of the JumonjiC domain-containing (JmjC) demethylases, specifically recognizes the trimethylated lysine residues H3K9 and H3K36 and demethylates them thanks to its Fe(II) center and α-ketoglutarate (αKG) and molecular oxygen (O_2) used as co-substrates.[1] Poorly expressed in normal cells, its overexpression has been observed in different cancer tissues (e.g. lung, breast, colon), making it an highly promising epigenetic target.[2]

Since KDM4A depends on an iron in its active site for its enzymatic activity, our group tested clinically used iron-chelator drugs in order to identify new motives enable to target this demethylase. More particularly, deferoxamine mesylate (1968), deferasirox (2005), and deferiprone (1994 – Europe & Asia and 2011 – USA) were tested and appeared to be highly potent in vitro, with IC_{50} in the low micromolar range. However, our results suggested that deferoxamine and deferiprone, in contrast to deferasirox, seem to inhibit the enzyme solely by sequestration of the iron ions in solution and not by competing with αKG. Because of its high affinity for KDM4A, deferasirox was selected and analogues were synthesized, but, unfortunately, without increase in the potency.[3] Later on, thiazole derivatives of deferasirox, exhibiting activity in the low micromolar range, were also synthesized.

In this context, a library of pyrazole derivatives was prepared using the well-known Knorr-pyrazole synthesis. On the same model, imidazoles, isomers of pyrazoles, were also prepared using the Debus-Radziszewski multicomponent approach and, contrary to the former families synthesized, imidazoles have the advantage of bearing a side chain, allowing them to generate more interaction within the active site, and bringing high potential for the future of this family. Herein, the synthesis of such derivatives and their biological evaluation will be presented. More particularly, active compounds (until 2 µM IC_{50}) have been obtained and hypermethylation of H3K36 has been observed in Human oesophageal cancer KYSE-150 cell line.

We thank for funding by the DFG (CRC992 Medical Epigenetics) – Project A04

[1] L. Morera, et al.: *ChemMedChem*, **2016**, *11*, 2063–2083.
[2] N. Rüger, et al.: *ChemMedChem*, **2015**, *10*, 1875–1883.
[3] M. Roatsch, et al. *ACS Chem. Biol.* **2019**, *14*, 1737–1750.

Pre-clinical Development of PQR530, a Highly Potent Dual PI3K/mTOR Kinase Inhibitor

Lucinda Batchelor[1], Denise Rageot[1], Thomas Bohnacker[1], Erhan Keles[1], Jacob A. McPhail[2], Reece M. Hoffmann[2], Anna Melone[1], Chiara Borsari[1], Rohitha Sriramaratnam[1], Alexander M. Sele[1], Florent Beaufils[1], Paul Hebeisen[3], Petra Hillmann[3], John E. Burke[2], Matthias P. Wymann[1]

[1]Department of Biomedicine, University of Basel, Switzerland;
[2]Department of Biochemistry and Microbiology, University of Victoria, Canada,
[3]PIQUR Therapeutics AG
email: lucindakate.batchelor@unibas.ch

Constitutive activation of the PI3K/mTOR signaling pathway promotes progression of malignant tumors. Dual class I pan-PI3K/mTOR inhibitors – targeting this pathway at multiple nodes – appear to have a broad activity profile in cancer therapy, and are currently explored in numerous clinical studies.

The development of the potent PI3K/mTOR inhibitor PQR530 aimed to improve both the potency and specificity for class IA PI3K isoforms and mTOR kinase. Here, we present a detailed structure-activity relationship study which led to the identification of PQR530, a drug-like adenosine 5'-triphosphate site P3IK/mTOR kinase inhibitor, as a brain penetrable clinical candidate.

PQR530 qualifies as a clinical candidate due to its potency and specificity for both PI3K and mTOR kinases and its pharmacokinetic properties including brain penetration. PIQ530 shows excellent selectivity over a wide panel of kinases and an excellent selectivity against unrelated receptor enzymes and ion channels. Moreover, PQR530 prevented cell growth in a cancer cell line panel. The pre-clinical in vivo characterization of PQR530 in an OVCAR-3 xenograft model demonstrated good oral bioavailability, excellent brain penetration and efficacy. Initial toxicity studies in rats and dogs qualify PQR for further development as a therapeutic agent in oncology. [1]

[1] D. Rageot, T. Bohnacker, E. Keles *et al*., *J. Med. Chem.* **2019**, *62*, 6241-6261

Structure-Activity Relationship of a Factor H-binding peptide to ward off undesired host complement attack on transplants and biomaterials

Clément Bechtler[1], Amal Hamid[1], Lukas Kaufann[1], Said Rabani[1], Christoph Q. Schmidt[2], John D. Lambris[3], Daniel Ricklin[1]

[1] Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland, [2] Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Helmholtzstraße 20, 89081 Ulm, Germany, [3] Department of Pathology and Laboratory Medicine, University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104-4238, USA

The complement system is a first in line defence system against pathogens, leading to inflammation and pathogen removal. However, it can also be a driving force in pathologies where the undesired recognition of non-self surfaces, such as in ischemic reperfusion injury following transplantation, leads to clinical complications.
One possibility to prevent such complications is to recruit plasma-borne complement regulators, such as Factor H (FH), by synthetic tethers to biomaterial surfaces and consequently prevent complement attack *in situ*.
Pursuing this idea, a disulphide-bridged cyclic peptide (5C6) was previously discovered by our group through phage display screening. 5C6 showed nanomolar binding affinity to FH and could act as a bridge between FH and model surfaces when combined with appropriate tethering motifs, resulting in reduced complement activation.
To further develop 5C6 towards a preclinical candidate, we identified and addressed three key aspects for improving the affinity and stability. First, we replaced the disulphide with a variety of other functional groups, affecting activity to different degrees. Second, we altered the macrocycle size, enabling us to define the ideal ring size. Third, we replaced individual amino acids with natural and unnatural amino acids to successfully improve the affinity of 5C6.

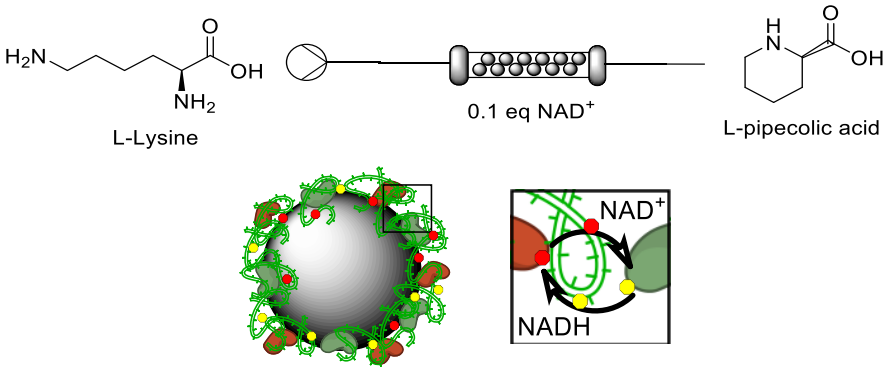
[1] YQ. Wu, H. Qu, G. Sfyroera, A. Tzekou, BK. Kay, B. Nilsson, KN. Ekdahl, D. Ricklin, JD. Lambris, *Journal of Immunology,* **2011**, *186(7),* 4269-77.
[2] PH. Nilsson, KN. Ekdahl, PU. Magnusson, H. Qu, H. Iwata, D. Ricklin, J. Hong, JD. Lambris, B. Nilsson, Y. Teramura*, Biomaterials,* **2013***, 34(4),* 985-94.

Ex vivo and sustainable production of *L*-pipecolic acid: a key block in pharmaceutical synthesis

David Roura Padrosa[1,2], Ana I. Benítez Mateos[1,2], Liam Calvey[2] and Francesca Paradisi[1]

[1]Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012, Bern Switzerland.
[2]School of Chemistry, University of Nottingha, University Park NG7 2RD, Nottingham, UK.
david.roura@dcb.unibe.ch and ana.benitez@dcb.unibe,ch

Pipecolic acid is a non-proteinogenic cyclic α-amino acid with great importance as a building block for a plethora of APIs [1,2]. As an alternative to the traditional chemical synthesis, which normally involve harsh conditions and/or hazardous chemicals, biosynthetic routes inspired by the natural pathways have been mined in whole cell biocatalysts [3–5] but only few examples of the *ex vivo* synthesis have been reported. In this work, two *ex-vivo* strategies using free and immobilized enzymes have been designed for the production of L-pipecolic acid. In the first strategy, the combination of a transaminase from *Halomonas elongata*[6] capable of lysine deamination has been coupled with a pyrroline-5-dehydrogenase. This strategy yielded 60% conversion of lysine at the 50 mM scale with free enzymes, but the reaction required a basic pH which affected enzyme stability and reusability even after immobilization. As an alternative to overcome these limitations, a redox neutral system has been constructed by coupling a lysine-6-dehydrogenase from a thermophilic organism in combination with the pyrroline-5-decarboxylase. Co-immobilization of both enzymes, as well as the cofactor in the same biocatalytic unit has been successfully applied for the batch synthesis of pipecolic acid. Moreover, the bienzymatic biocatalyst with catalytic amount of free cofactor has been applied in a flow reactor for the synthesis of L-pipecolic acid with a molar conversion of 100% and a volumetric productivity up to 2.5 g/L/h, 10 times higher productivity than the highest reported in literature for whole cell fermentation.



1) V. Vranova, L. Lojkova, K. Rejsek, P. Formanek, *Chirality* **2013**, *25*, 823.
2) H. Návarová, F. Bernsdorff, A.-C. Döring, J. Zeier, *Plant Cell* **2012**, *24*, 5123.
3) H. Ying et al., *Microb. Cell Fact.* **2017**, *16*, 52.
4) F. Pérez-García, J. Max Risse, K. Friehs, V. F. Wendisch, *Biotechnol. J.* **2017**, *12*, 1.
5) S. Byun, H. J. Park, J. C. Joo, Y. H. Kim, *Biotechnol. Bioprocess Eng.* **2019**, *24*, 215.
6) L. Cerioli, M. Planchestainer, J. Cassidy, D. Tessaro, F. Paradisi, *J. Mol. Catal. B Enzym.* **2015**, *120*, 141.

Targeted Therapy for Neurological Disorders: A Novel, Orally Available, and Brain-Penetrant mTOR Inhibitor (PQR626)

Chiara Borsari[1], Erhan Keles[1], Denise Rageot[1], Thomas Bohnacker[1], Anna Melone[1], Lucinda Kate Batchelor[1], Martina De Pascale[1], Paul Hebeisen[2], Petra Hillmann[2], Doriano Fabbro[2], Matthias Wymann[1]

[1]Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland
[2]PIQUR Therapeutics AG, Hochbergerstrasse 60, 4057 Basel, Switzerland

chiara.borsari@unibas.ch

Mechanistic target of rapamycin (mTOR) is a key regulator of cell growth and survival. The mTOR pathway is dysregulated in many diseases including cancer and neurological disorders. Among Central Nervous Systems disorders, mTOR is implicated in Parkinson's, Alzheimer's and Huntington's disease and epilepsy. Rapalogs have recently been explored to alleviate epileptic seizures in Tuberous Sclerosis Complex (TSC).

Herein, we combined pharmacophore features of PQR620[1], the first-in-class brain penetrant ATP-competitive mTOR kinase inhibitor showing efficacy in a TSC mouse model, and PQR617[2], a potent, highly selective mTOR inhibitor. An extensive chemical exploration on the morpholine ring led to the discovery of PQR626, a highly potent, selective, brain penetrant inhibitor of mTORC1/2 kinase.

PQR626 displayed an excellent brain penetration compared to everolimus and AZD2014, that possess a limited ability to cross the blood-brain-barrier. PQR626 did not trigger an increase in insulin and glucose plasma levels, suggesting its potential application in the treatment of chronic diseases. It showed very good tolerability in mice and efficacy studies, using mice with conditional inactivation of the *Tsc1* gene primarily in glia (*Tsc1*^{GFAP}CKO mice), were performed. PQR626 (50 mg/kg, BID – twice a day) showed a significant effect on survival and significantly prevented/decreased mortality.

On the basis of its favorable pharmacological parameters, excellent brain penetration, safety profile and efficacy in *Tsc1*^{GFAP}CKO mice, PQR626 qualifies as a novel mTOR inhibitor with potential application in the treatment of epilepsy and neurological disorders.

[1] D. Rageot, T. Bohnacker, A. Melone, J.B. Langlois, C. Borsari, P. Hillmann, A.M. Sele, F. Beaufils, M. Zvelebil, P. Hebeisen, W. Löscher, J. Burke, D. Fabbro, M.P. Wymann. *Journal of Medicinal Chemistry,* **2018**, *61*, 10084-10105.

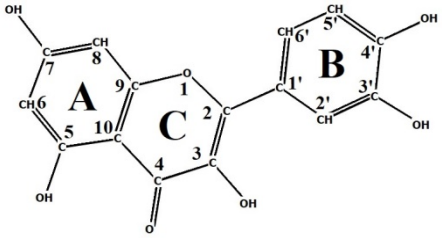
[2] C. Borsari, D. Rageot, A. Dall'Asen, T. Bohnacker, A. Melone, A.M. Sele, E. Jackson, J.B. Langlois, F. Beaufils, P. Hebeisen, D. Fabbro, P. Hillmann, M.P. Wymann, *Journal of Medicinal Chemistry,* **2019**, *62*, 8609-8630.

Unique properties of the biologically active quercetin molecule: A detailed investigation

Brovarets' Ol'ha, Hovorun Dmytro

Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Akademika Zabolotnoho Street, 03680, Kyiv, Ukraine
o.o.brovarets@gmail.com

Quercetin molecule (3, 3', 4', 5, 7 - pentahydroxyflvanone, C15H10O7) is known to be an effective pharmaceutical compound of a plant origin [1]. It is known to act as a natural drug molecule with a wide range of treatment properties, like an antioxidant, antitoxic etc. and also is involved in the process of drug delivery from the site of the administration to the therapeutic target [2].



In this study by using the quantum-mechanical modelling at the MP2/6-311++G(2df,pd) // B3LYP/6-311++G(d,p) level of theory together with Bader's "Quantum Theory of Atoms in Molecules" it was established for the first time unique properties of the quercetin molecule:

- it was established 48 stable conformers, corresponding to local minima on the potential energy hypersurface of the isolated quercetin molecule, and characterized in details [3];
- it was revealed 123 different prototropic tautomers of the most stable conformer **1** of the quercetin molecule with Gibbs free energies in the range 0.0-172.8 kcal·mol^{-1} [4];
- it was investigated the pathways of the tautomeric transformations for the most stable conformers of the isolated quercetin molecule *via* the intramolecular proton transfer [5];
- it was explored the conformational interconversions in the 24 pairs of the conformers of the quercetin molecule through the rotation of its non-deformable (A+C) and B rings around the C2-C1' bond [6];
- it was explored conformational variety of the isolated quercetin molecule due to the mirror-symmetrical hindered turnings of the O3H, O5H and O7H hydroxyl groups, belonging to the A and C rings, around the exocyclic C-O bonds [7];
- it was explored novel conformational mobility of the quercetin molecule due to the turnings of the O3'H and O4'H hydroxyl groups, belonging to the B ring, around the exocyclic C-O bonds [8].

Altogether, investigated physico-chemical properties of the quercetin molecule enable to shed light on its unique properties, which could be further used for the pharmaceutical applications.

[1] S. Nathiya, M. Durga, T. Devasena, *Int. J. Pharm. & Pharm. Sciences*, **2014**, *10*, 20-26.
[2] A.V.A. David, R. Arulmoli, S. Parasuraman, *Pharmacogn. Rev.*, **2016**, *10*, 84–89.
[3] O. Brovarets', D. Hovorun, *JBSD*, **2019**, doi:10.1080/07391102.2019.1656671.
[4] O. Brovarets', D. Hovorun, *JBSD*, **2019**, doi: 10.1080/07391102.2019.1691660.
[5] O. Brovarets', D. Hovorun, *PLoS ONE*, **2019**, *14*, e0224762.
[6] O. Brovarets', D. Hovorun, *JBSD*, **2019**, doi: 10.1080/07391102.2019.1645734.
[7] O. Brovarets', D. Hovorun, *Symmetry*, **2020**, submitted.
[8] O. Brovarets', D. Hovorun, *Appl. Sci.*, **2020**, submitted.

2nd Anglo-Swiss Symposium 2020

Using Chemical Biology to Identify New Targets for Medicinal Chemistry

Michaela, Buerdsell
Durham University, 14b Frederick Street North, Meadowfield, DH78NB Durham, United Kingdom
michaela.l.buerdsell@durham.ac.uk

Leishmaniasis is a neglected tropical disease with 0.7-1 million new cases and an estimated 26-65 million deaths per year. [1] The current chemotherapeutic options are toxic with long treatment times as well as increasing resistance. Therefore, it is important for new drug targets to be identified and characterised. Inositol phosphorylceramide synthase (IPCS) is a membrane bound enzyme that has no direct human homologue and converts ceramide to inositol phosphorylceramide (IPC) through the action of a highly conserved HHD catalytic triad. [2] Inhibiting this enzyme has shown to decrease parasitaemia. [3] To further explore this enzyme, a probe was designed and synthesised based on the structure of diethyl pyrocarbonate (DEPC), a compound known to bind covalently to nucleophilic histidine residues. [4] This probe has been shown to bind histidine, inhibit IPCS in an *in vitro* assay and produce a fluorescent band in an acid phosphatase model. Current ongoing work seeks to verify the selective binding of the probe to the active site histidine and produce a protein profile in *Leishmania mexicana*.

References:

1: WHO. (2019). *Leishmaniasis*. [online] Available at: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis [Accessed 14 Jan. 2020].
2: Mina, J., Mosely, J., Ali, H., Denny, P. and Steel, P. (2011). Exploring Leishmania major Inositol Phosphorylceramide Synthase (LmjIPCS): Insights into the ceramide binding domain. *Organic & Biomolecular Chemistry*, 9(6), p.1823.
3: Norcliffe, J.L., Mina, J.G., Alvarez, E. *et al.* Identifying inhibitors of the *Leishmania* inositol phosphorylceramide synthase with antiprotozoal activity using a yeast-based assay and ultra-high throughput screening platform. *Sci Rep* **8,** 3938 (2018) doi:10.1038/s41598-018-22063-9
4: Miles, E. (1977). Modification of histidyl residues in proteins by diethylpyrocarbonate. New York, etc.: Academic Press, pp.431-442.

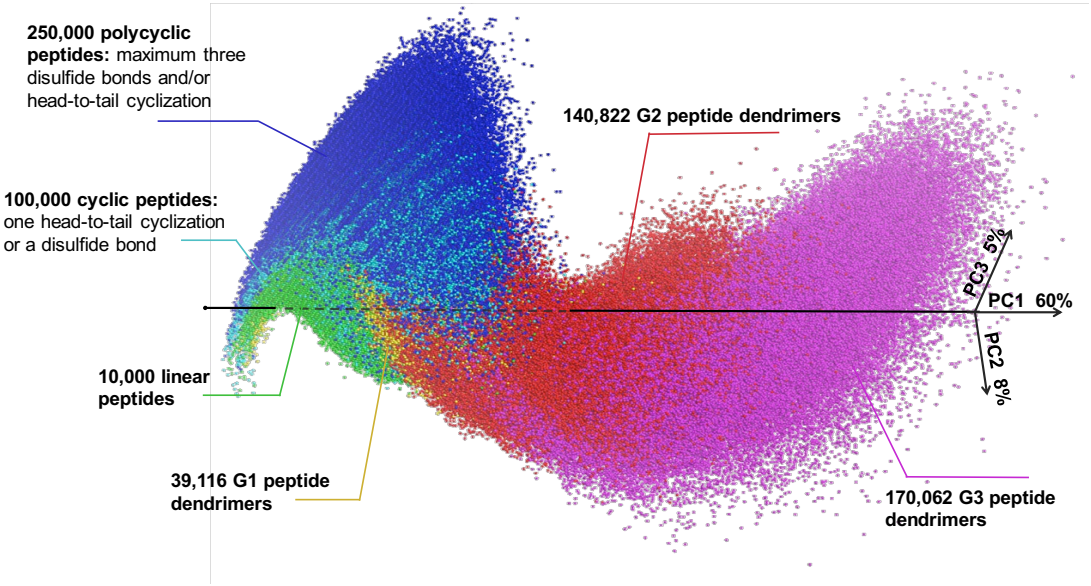
Peptide Chemical Space

Alice Capecchi
University of Bern, Department of Chemistry and Biochemistry, 3012 Bern, Switzerland
alice.capecchi@dcb.unibe.ch

To visualize a subset of the extremely large (>10^{33}) peptide chemical space, we sampled one million sequences among linear, (poly)cyclic and dendritic sequences.

To encode the peptides properties and topology, we used the Macromolecule extended atom pair fingerprint (MXFP) that has been shown to be able to describe different classes of macromolecules[1]. The obtained chemical space was visualized in the 3D visualization tool Faerun[2].

Furthermore, we developed a MXFP driven Peptide design genetic algorithm (PDGA) which returns peptide analogs of any given target and therefore allows the otherwise impractical exploration of such large chemical space.



[1] Capecchi, A.; Awale, M.; Probst, D.; Reymond, J.-L. PubChem and ChEMBL beyond Lipinski. *Molecular Informatics* **2019**.

[2] Probst, D.; Reymond, J.-L.; Wren, J. FUn: A Framework for Interactive Visualizations of Large, High-Dimensional Datasets on the Web. *Bioinformatics* **2018**, 34 (8), 1433–1435.

Development of Peptides as Potential Drug Leads for the Treatment of Inflammatory Bowel Diseases

Claudia Cobos, Paramjit S. Bansal, Severine Navarro, David Wilson, Paul Giacomin, Laurianne Don, Alex Loukas, Norelle L. Daly*

Centre for Biodiscovery and Molecular Development of Therapeutics, AITHM, James Cook University, Cairns, QLD, Australia

Email :claudia.cobos@univie.ac.at

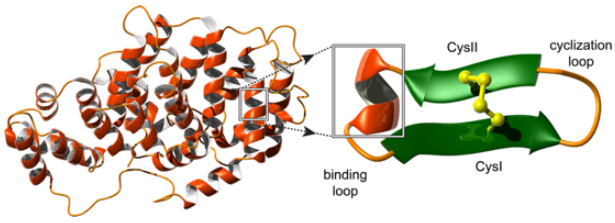
Inflammatory bowel diseases (IBDs) are a set of complex and debilitating diseases, for which there is no satisfactory treatment[1]. The two major conditions are Ulcerative Colitis and Crohn's disease. IBDs carry a significant burden of disease for Australia with an estimated 75,000 Australians affected by the disease, costing the country approximately 2.7 billion dollars annually[1]. IBDs can cause severe abdominal pain, weight loss, constipation, diarrhoea, fatigue, malnutrition, nausea and impaired growth[2].

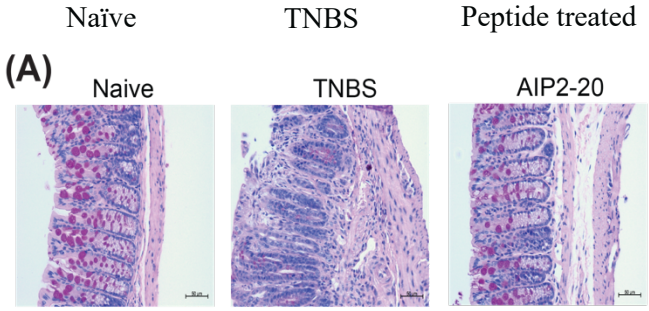
Peptides are excellent molecules for the design of novel therapeutics due to the intrinsic properties and specificity that they have. New techniques have been used for the development of biologically-active peptides that are safe and cheap to manufacture compared with protein-based drugs[3, 4].

The first part of the project involved improving the stability a range of small peptides that may have potential in the design of novel drug leads for the treatment of IBD by grafting them into stable disulfide-rich peptide scaffolds to enhance their therapeutic potential[5,6]. Using this approach, we designed novel disulfide-rich peptides, which comprise a small bioactive peptide from the annexin A1 protein grafted into two known stable disulphide-rich peptides. This engineered cyclic peptide maintained the overall fold of the naturally occurring cyclic peptide, was more effective at reducing inflammation in a mouse model of acute colitis than the bioactive peptide alone, and showed enhanced stability in human serum[7].

In the second part of the project, we have studied the correlation between a decline in the prevalence of parasites such as hookworms and the rise in autoimmune conditions in developed countries[8,9]. This correlation has led to studies that have identified hookworm proteins with therapeutic activity in inflammatory bowel disease (IBD) and asthma[10]. Analysis of three-dimensional protein structures provide clues to regions important in activity, which are a promising starting point for the design of peptide-based lead molecules for the treatment of inflammatory diseases such as IBD.

References





1. Health Direct Australian Government. (2013) Crohn's disease and ulcerative colitis.
2. Ament, M. E. Inflammatory disease of the colon: ulcerative colitis and Crohn's colitis. J. Pediatr. (1975), 86, 322-33
3. Lennardjones, J. E. Classification Of Inflammatory Bowel-Disease. Scand. J. Gastroenterol, (1989), 24, 2-6
4. Wong, C.T.T., et al., Orally Active Peptidic Bradykinin B-1 Receptor Antagonists Engineered from a Cyclotide Scaffold for Inflammatory Pain Treatment. Angewandte Chemie-International Edition, (2012). 51(23): p. 5620-5624.
5. Wang, C.K., et al., Molecular Grafting onto a Stable Framework Yields Novel Cyclic Peptides for the Treatment of Multiple Sclerosis. Acs Chemical Biology, (2014). 9(1): p. 156-163.
6. Luckett, S., et al., High-resolution structure of a potent, cyclic proteinase inhibitor from sunflower seeds. Journal of Molecular Biology, (1999). 290(2): p. 525-533.
7. Cobos Caceres, C., et al., An engineered cyclic peptide alleviates symptoms of inflammation in a murine model of inflammatory bowel disease. J Biol Chem, (2017). 292 (24). pp. 10288-10294.
8. Molodecky, N. A., Soon, I. S., Rabi, D. M., Ghali, W. A., Ferris, M., Chernoff, G., Benchimol, E. I., Panaccione, R., Ghosh, S., Barkema, H. W., and Kaplan, G. G. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review. Gastroenterology, (2012), 142, 46-54
9. Prescott, S., and Allen, K. J. Food allergy: Riding the second wave of the allergy epidemic. Pediatr. Allergy Immunol, (2011), 22, 155-160
10. Weinstock, J. V., Summers, R. W., Elliott, D. E., Qadir, K., Urban, J. F., and Thompson, R. The possible link between de-worming and the emergence of immunological disease. J. Lab. Clin. Med., (2002), 139, 334-338

Preclinical Development of PQR514, a Highly Potent PI3K Inhibitor Bearing a Difluoromethyl-Pyrimidine Moiety

Martina De Pascale[1], Chiara Borsari[1], Denise Rageot[1], Florent Beaufils[2], Thomas Bohnacker[1], Erhan Keles[1], Ivan Buslov[1], Anna Melone[1], Alexander M. Sele[1], Paul Hebeisen[2], Doriano Fabbro[2], Petra Hillmann[2], Matthias P. Wymann[1]

[1]Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland
[2]PIQUR Therapeutics AG, Hochbergerstrasse 60, 4057 Basel, Switzerland
martina.depascale@unibas.ch

The phosphoinositide 3-kinase (PI3K) – mechanistic target of rapamycin (mTOR) signaling pathway plays a key role in many cellular processes, including cell growth, proliferation and survival. Hyperactivation of the PI3K/mTOR pathway can occur at multiple levels of this signaling cascade, finally promoting cancer growth and progression. Therefore, PI3K inhibitors represent a valuable asset in cancer therapy. Recently, we have reported on PQR309 (bimiralisib), a brain-penetrant pan-PI3K inhibitor, which also moderately targets mTOR kinase. PQR309 is currently in phase II clinical trials for the treatment of lymphoma and solid tumors and first phase I clinical results have been disclosed.[1-2]

Herein, we have developed a novel anti-cancer agent, the potent pan-PI3K inhibitor PQR514, which is a follow-up compound for the phase II clinical compound PQR309. PQR514 has an improved potency both *in vitro* and in cellular assays with respect to its predecessor compounds. Pharmacokinetic studies of PQR514 showed good oral bioavailability and a minimal brain permeability, which suggests the possible application in the treatment of systemic tumors, with the advantage of avoiding putative neurological side effects. PQR514 showed superiority in the suppression of cancer cell proliferation, and demonstrated significant anti-tumor activity in an OVCAR-3 xenograft model, at concentrations ~8-fold lower than the parental Phase II inhibitor PQR309. On the basis of its remarkable PI3K affinity, favorable pharmacological parameters, safety profile, and *in vivo* antitumor efficacy, PQR514 qualifies as an optimized candidate for the treatment of systemic tumors.[3]

1. Beaufils, F.; Cmiljanovic, N.; Cmiljanovic, V.; Bohnacker, T.; Melone, A.; Marone, R.; Jackson, E.; Zhang, X.; Sele, A.; Borsari, C.; Mestan, J.; Hebeisen, P.; Hillmann, P.; Giese, B.; Zvelebil, M.; Fabbro, D.; Williams, R. L.; Rageot, D.; Wymann, M. P., 5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-amine (PQR309), a Potent, Brain-Penetrant, Orally Bioavailable, Pan-Class I PI3K/mTOR Inhibitor as Clinical Candidate in Oncology. *J Med Chem* **2017,** *60* (17), 7524-7538.
2. Wicki, A.; Brown, N.; Xyrafas, A.; Bize, V.; Hawle, H.; Berardi, S.; Cmiljanovic, N.; Cmiljanovic, V.; Stumm, M.; Dimitrijevic, S.; Herrmann, R.; Pretre, V.; Ritschard, R.; Tzankov, A.; Hess, V.; Childs, A.; Hierro, C.; Rodon, J.; Hess, D.; Joerger, M.; von Moos, R.; Sessa, C.; Kristeleit, R. First-in human, phase 1, dose-escalation pharmacokinetic and pharmacodynamic study of the oral dual PI3K and mTORC1/2 inhibitor PQR309 in patients with advanced solid tumors (SAKK 67/13). *Eur J Cancer* **2018,** *96*, 6-16.
3. Borsari, C.; Rageot, D.; Beaufils, F.; Bohnacker, T.; Keles, E.; Buslov, I.; Melone, A.; Sele, A. M.; Hebeisen, P.; Fabbro, D.; Hillmann, P.; Wymann, M. P. Preclinical Development of PQR514, a Highly Potent PI3K Inhibitor Bearing a Difluoromethyl-Pyrimidine Moiety. *ACS Medicinal Chemistry Letters* **2019** [Epub ahead of print].

BODIPY-Tagged Dinuclear Trithiolato-Bridged Ruthenium(II)-Arene Complexes – Photophysical Properties and Bioactivity

Oksana Desiatkina[1], Emilia Păunescu[1], Nicoleta Anghel[2], Ghalia Boubaker[2], Yosra Amdouni[2], Andrew Hemphill[2], Julien Furrer[1]

[1]Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland
[2]Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggass-Strasse 122, 3012 Bern, Switzerland
oksana.desiatkina@dcb.unibe.ch

Cationic trithiolato-bridged dinuclear ruthenium(II)-arene complexes presenting symmetric or 'mixed' structures (general formula $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR})_3]^+$ and, respectively, $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR}^1)_2(\mu_2\text{-SR}^2)]^+$) were acknowledged as prospective antiparasitic agents against *Toxoplasma gondii*[1] and *Neospora caninum*.[2] Interestingly, the mitochondrion was identified as the major target in both parasites.[1,2] The identification of their possible mechanism of action and the monitorization of their fate in cells are aimed.

Fluorophore-labeled conjugates were identified as a versatile approach for the intracellular visualization of organometallic compounds by means of confocal fluorescence microscopy. BODIPY-tethered ruthenium complexes were shown to be a useful tools for cellular bioimaging.[3,4] As such, a series of eleven trithiolato-bridged dinuclear ruthenium(II)-*p*-cymene conjugates tagged with BODIPY fluorophores were synthesized. The impact of various structural features (i.e. nature and length of the linker between the fluorophore and the organometallic moiety) upon the photophysical and antiparasitic properties of the conjugates was evaluated. Fluorescence measurements revealed that anchoring dinuclear ruthenium(II)-arene moieties to the BODIPY dye induced an important, yet acceptable, fluorescence quenching for all conjugates.

In a first biological activity screening, human foreskin fibroblast (HFF) host cells and *T. gondii* apicomplexan parasite grown in HFF cells were exposed to each compound of interest (BODIPY-tethered conjugates, non-modified dinuclear ruthenium(II)-arene complexes and free BODIPY dyes) at two concentrations (1 and 0.1 µM). From this early assessment, five conjugates emerged as interesting for further biological studies. Interestingly, none of the compounds affected HFF host cells growth at dosage of up to 2.5 µM, but they inhibited *T. gondii* proliferation with IC_{50}s (50% inhibitory concentrations) in the 0.34-0.54 µM range, the four ester conjugates being slightly more active compared to the amide derivative. Transmission electron microscopy of *T. gondii* tachyzoites exposed to two ester conjugates detected ultrastructural alterations in the matrix of the parasite mitochondria already 24 h after treatment initiation.

[1] A. P. Basto, J. Müller, R. Rubbiani, D. Stibal, F. Giannini, G. Süss-Fink, V. Balmer, A. Hemphill, G. Gasser, J. Furrer, *Antimicrob. Agents Chemother.* **2017**, *61*, e01031-17.
[2] A. P. Basto, N. Anghel, R. Rubbiani, J. Müller, D. Stibal, F. Giannini, G. Süss-Fink, V. Balmer, G. Gasser, J. Furrer, A. Hemphill, *Metallomics* **2019**, *11*, 462-474.
[3] T. Wang, Y. Hou, Y. Chen, K. Li, X. Cheng, Q. Zhou, X. Wang, *Dalton Trans*. **2015**, *44*(*28*), 12726-12734.
[4] S. Tasan, O. Zava, B. Bertrand, C. Bernhard, C. Goze, M. Picquet, P. Le Gendre, P. Harvey, F. Denat, A. Casini, E. Bodio, *Dalton Trans*. **2013**, *42*(*17*), 6102-6109.

Development of Positron-Emission Tomography and Fluorescent Tracers for the Imaging of Calcium Sensing Receptors (CaSR) in Parathyroid Glands

Luca Dick, Martin Lochner

Institute of Biochemistry and Molecular Medicine, University of Berne

Abstract

Primary hyperparathyroidism (HPT) is caused by the pathological growth of parathyroid glands and the resulting increased secretion of parathyroid hormone (PTH) [1]. Elevated PTH levels raise the Ca^{2+}-concentration in the blood which affects kidneys, digestive system, bones, muscles and brain and significantly impairs the well-being of patients [2]. Surgical removal of the affected glands is the treatment of choice. However, for the optimization of the surgery, a pre-surgical localization of the affected gland is necessary [3]. The development of a selective tracer for the parathyroid gland would enhance the spatial and temporal resolution and reduce the likelyhood of false negative results compared to existing localization-tools [4].

^{18}F-positron emission tomography (^{18}F-PET) can detect ^{18}F-labeled tracer-molecules in a living organism and hence image biological protein targets [5]. The calcium sensing receptor (CaSR) is highly expressed on the surface of the parathyroid glands. Previously, highly potent CaSR inhibitors have been developed for the treatment of osteoporosis [6]. We have used these CaSR inhibitors as design templates to develop tracers for the imaging of CaSR in parathyroid glands.

In general, the challange is to preserve the high CaSR activity of these compounds when synthetically altering their structures. For the PET tracers, the fluorine-18 (half-life of 110 minutes) has to be introduced into the molecule efficiently in the last step which requires an appropriate leaving group [5]. We synthesized several non-radioactive ^{19}F-fluorinated CaSR inhibitor analogues and have tested their activity in CaSR-expressing fibroblast cells using a FLIPR-assay. In addition, we have also synthesized a few CaSR inhibitors with appended small fruorescent dyes. Such fluorescent CaSR tracers are currently validated in fluorescent binding and fluorescent-activated cell sorting (FACS) assays. The most promising compounds will be ^{18}F-labeled and evaluated as PET tracers in live animals.

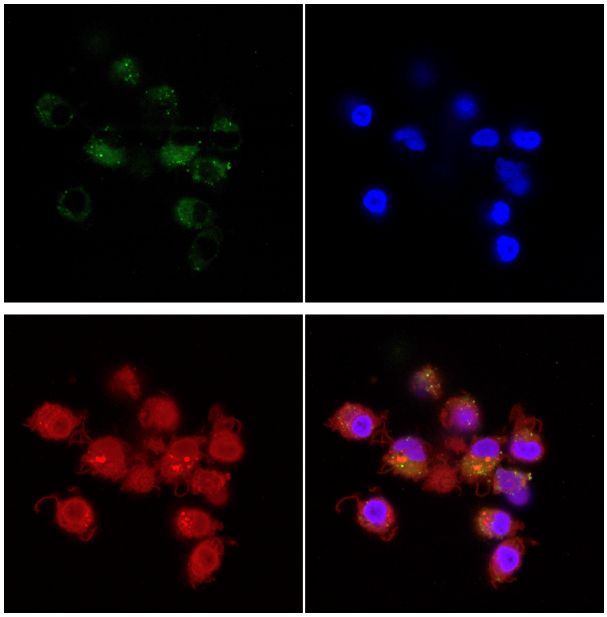
Literature

1. Taniegra, E. D. Hyperparathyroidism. *AFP* **69**, 333–339 (2004).
2. Madkhali, T., Alhefdhi, A., Chen, H. & Elfenbein, D. Primary hyperparathyroidism. *Ulus Cerrahi Derg* **32**, 58–66 (2016).
3. Iacobone, M., Carnaille, B., Palazzo, F. F. & Vriens, M. Hereditary hyperparathyroidism--a consensus report of the European Society of Endocrine Surgeons (ESES). *Langenbecks Arch Surg* **400**, 867–886 (2015).
4. Beheshti, M. *et al.* 18F-Fluorocholine PET/CT in the assessment of primary hyperparathyroidism compared with 99mTc-MIBI or 99mTc-tetrofosmin SPECT/CT: a prospective dual-centre study in 100 patients. *Eur. J. Nucl. Med. Mol. Imaging* **45**, 1762–1771 (2018).
5. Miller, P. W., Long, N. J., Vilar, R. & Gee, A. D. Synthesis of 11C, 18F, 15O, and 13N radiolabels for positron emission tomography. *Angew. Chem. Int. Ed. Engl.* **47**, 8998–9033 (2008).
6. Widler, L. Calcilytics: antagonists of the calcium-sensing receptor for the treatment of osteoporosis. *Future Med Chem* **3**, 535–547 (2011).

Peptide Dendrimer Analogues of the Multiple Sclerosis Drug Glatiramer Acetate

Dina Erzina, Alice Capecchi, Sacha Javor and Jean-Louis Reymond*

Department of Chemistry and Biochemistry, University of Bern
dina.erzina@dcb.unibe.ch



Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system. One of the most used first-line treatment for MS is glatiramer acetate (GA), a random copolymer of glutamic acid, lysine, alanine, tyrosine in 1.4/3.4/4.2/1 ratio with molecular weight 4.7-11 kDa consisting of a mixture of over 10^{30} potentially active peptides.[1] To identify a peptide dendrimer mimicking GA, we synthesized selected compounds from a virtual library of dendrimers designed to match GA in terms of size and composition using our chemical space approach for peptide design.[2,3] By testing the synthesized peptide dendrimers for modulation of IL1Ra production by monocytes,[4] we identified two peptide dendrimers with GA-like immunomodulatory properties. Herein we discuss the library design and screening approach and the detailed characterization of the effect of our peptide dendrimers on human primary monocytes by cytokine profiling and confocal microscopy of fluorescence labeled analogs.

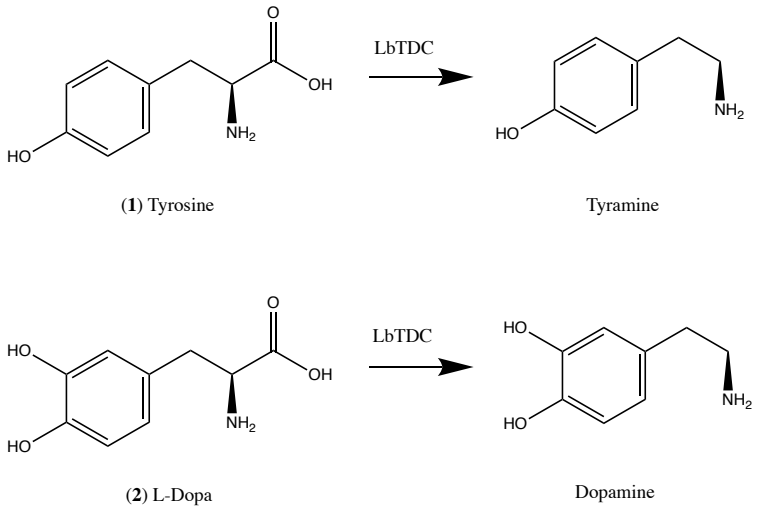
[1] R. Arnon, R. Aharoni, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101 Suppl 2*, 14593–14598.
[2] I. Di Bonaventura, X. Jin, R. Visini, D. Probst, S. Javor, B. H. Gan, G. Michaud, A. Natalello, S. M. Doglia, T. Kohler, et al., *Chem. Sci.* **2017**, *8*, 6784–6798.
[3] T. N. Siriwardena, A. Capecchi, B. H. Gan, X. Jin, R. He, D. Wei, L. Ma, T. Kohler, C. van Delden, S. Javor, et al., *Angew. Chem., Int. Ed. Engl.* **2018**, *57*, 8483–8487.
[4] R. Carpintero, K. J. Brandt, L. Gruaz, N. Molnarfi, P. H. Lalive, D. Burger, *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 17692–17697.

Decarboxylation in flow: covalent immobilization of *lb*tdc on methacrylic polymer resins

Stefania Gianolio, David Roura Pedrosa, Francesca Paradisi

Departement für Chemie und Biochemie, *Universität Bern,*
Bern, Switzerland
email: Stefania.Gianolio@dcb.unibe.ch

Bacterial tyrosine decarboxylases have been previously identified [1, 2, 3] however the soluble expression of these enzymes in heterologous hosts is challenging. On the contrary, the soluble expression of tyrosine decarboxylase from *Lactobacillus brevis* (*Lb*TDC) was reported on 2011 [4]. Here, we have successfully expressed, purified and immobilized *Lb*TDC on different supports. With the best conditions, up to 20% of activity was maintained and, as the soluble enzyme, immobilized *Lb*TDC is active not only towards its natural substrate, L-Tyrosine (**1**), but also L-Dopa (**2**) [4]. This new biocatalyst was applied in a flow bioreactor reaching complete conversion of 5mM of tyrosine and L-Dopa. Furthermore, by improving the solubility of the aromatic substrates with the addition of surfactant, we reached a production of 16,45 g L^{-1} h^{-1} of tyramine, 7 times higher than previously reported [5].



(**1**) Tyrosine Tyramine

(**2**) L-Dopa Dopamine

[1] Liu, Fang, et al. "Heterologous expression and characterization of tyrosine decarboxylase from Enterococcus faecalis R612Z1 and Enterococcus faecium R615Z1." *Journal of food protection* 77.4 (2014): 592-598.

[2] Lucas, Patrick, et al. "The tyrosine decarboxylase operon of Lactobacillus brevis IOEB 9809: characterization and conservation in tyramine-producing bacteria." *FEMS microbiology letters* 229.1 (2003): 65-71.

[3] Bargossi, Eleonora, et al. "Tyrosine decarboxylase activity of enterococci grown in media with different nutritional potential: tyramine and 2-phenylethylamine accumulation and tyrDC gene expression." *Frontiers in microbiology* 6 (2015): 259.

[4] Zhang, Kai, and Ye Ni. "Tyrosine decarboxylase from Lactobacillus brevis: soluble expression and characterization." *Protein expression and purification* 94 (2014): 33-39.

[5] Jiang, Mingyang, et al. "Improving Soluble Expression of Tyrosine Decarboxylase from Lactobacillus brevis for Tyramine Synthesis with High Total Turnover Number." Applied biochemistry and biotechnology 188.2 (2019

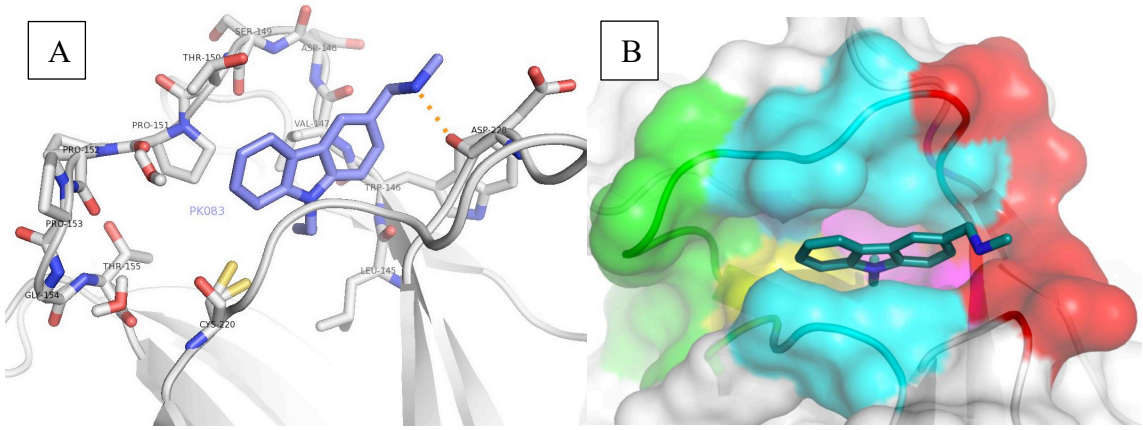
Towards the Design and Synthesis of a p53 Mutant Y220C Rescue Drug

Raysa Khan Tareque, Rhiannon Jones, Matthias Bauer, Matthias Baud,
Andreas C Joerger, and John Spencer

University of Sussex, Falmer, BN1 9RH, Brighton, United Kingdom
rk302@sussex.ac.uk

The p53 mutant Y220C, tyrosine to cysteine mutation, affects approximately 100,000 new cancer cases annually worldwide. The mutation causes p53 to be de-stable under physiological conditions. This mutation creates the merging of two shallow pockets which leads to a druggable extended surface crevice.

The protein mutant generally has a short half-life of about 4 minutes. The half-life was raised to over 15 minutes through a selective binding of the small molecule PK083 to the mutant crevice, presenting an ideal starting point for drug design to target the p53 mutant.



A: Co-crystal structure of PK083 in Y220C showing hydrogen bond between benzylic amine and Asp228 backone C=O, shown in dashed orange line. (PDB: 2VUK). B: PK083 bound to p53-Y220C (PDB: 2VUK). Mutation induced cavity is subdivided into: Subsite I (red), Subsite II (green), central cavity (cyan), Y220C mutation (yellow) and subsite III (pink).

A library of carbazoles was designed and synthesized, guided by SAR studies, crystallographic information and computational chemistry, with the aim of optimizing the compound toward a more potent PK083 analogue.

Over 100-folds increase in affinity was achieved through SAR studies. A scan of heterocyclic molecules was carried out targeting the proline-rich subsite II and by exploitation of direct fluorine-protein interactions between ligand and the backbone carbonyls of Leu145 and Trp146 and the thiol of Cys220.

[1] M. Bauer, R. Jones, M. Baud, R. Wilcken, F. Boeckler, A. Fersht, A. Joerger, and J. Spencer, *ACS Chemical Biology*, **2016**, 11 (8), 2265-2274
[2] M. Bauer, R. Jones, M. Baud, R. K. Tareque, B. Springett, F.A. Dingler, L. Verduci, K. J. Patel, A. Fersht, A. Joerger, and J. Spencer, *Future Med Chem*, **2019**, 11 (19), 2491-2504

Aglo-Swiss Symposium abstract

Functionalized Proline-Rich Peptides Bind the Bacterial Second Messenger c-di-GMP

Simon Loosli, Carlotta Foletti and Helma Wennemers*
Laboratorium für Organische Chemie, D-CHAB, ETH Zürich, CH-8093 Zürich

C-di-GMP is an attractive target in the fight against bacterial infections since it is a near ubiquitous second messenger that regulates important cellular processes of pathogens, including biofilm formation and virulence.[1-3] Screening of a combinatorial peptide library enabled the identification of the proline-rich tetrapeptide Gup-Gup-Nap-Arg, which binds c-di-GMP selectively over other nucleotides in water. Computational and CD spectroscopic studies provided a possible binding mode of the complex and enabled the design of a pentapeptide with even higher binding strength towards c-di-GMP. Biological studies showed that the tetrapeptide inhibits biofilm growth by the opportunistic pathogen *P. aeruginosa*.

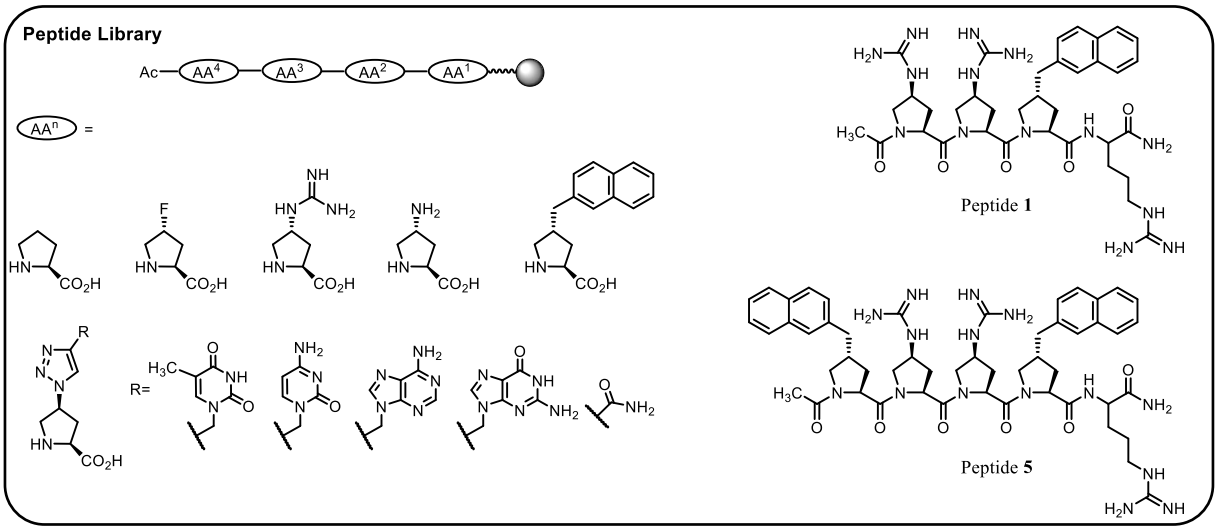


Figure 1: Left: general structure of the peptide Library. Right: the peptide **1** obtained from the peptide library and the optimised peptide **5**

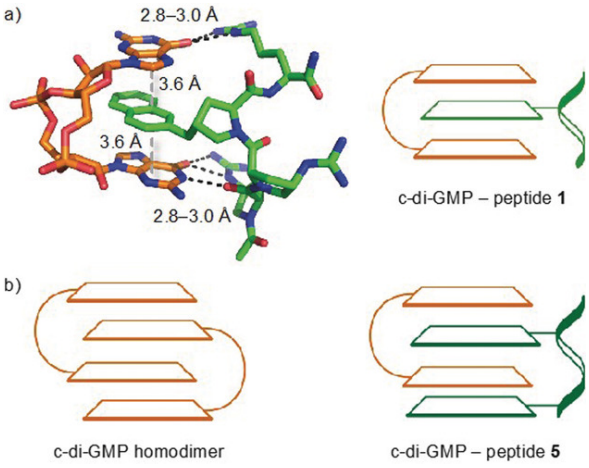


Figure 2: a) Lowest-energy structure of the complex between peptide 1 (green) and c-di-GMP (orange) and schematic representation. b) Illustration of the omodimer of c-di-GMP (left) and complex between c-di-GMP and peptide 5 (right)

Table 1: Binding affinities of peptides 1-4, 1a-1f and 5 with c-di-GMP, c-di-AMP and pGpG

Peptide	ΔG [kcal mol^{-1}] c-di-GMP	c-di-AMP	pGpG
1 Ac-GupGupNapArg-NH_2	−6.2	−4.7	−4.7
2 Ac-NapArgArgArg-NH_2	−6.3	−5.0	−5.1
3 Ac-GupNapArgArg-NH_2	−6.2	−5.1	−5.1
4 Ac-NapGupGupArg-NH_2	w.b.	w.b.	w.b.
1a Ac-ProGupNapArg-NH_2	w.b.	w.b.	w.b.
1b Ac-GupProNapArg-NH_2	w.b.	w.b.	w.b.
1c Ac-GupGupProArg-NH_2	−5.1	−5.9	w.b.
1d Ac-GupGupNapAla-NH_2	w.b.	−4.1	w.b.
1e Ac-GupGupNapDArg-NH_2	−5.4	−5.0	−5.6
1f Ac-GupGupNap-NH_2	w.b.	w.b.	w.b.
5 Ac-NapGupGupNapArg-NH_2	−7.9	−6.5	−6.5

[a] Binding affinities at 25 °C in deionized water, error ±0.1 kcal mol^{-1}. w.b. = weak binding, indicates that ΔG is higher than −4–−4.5 kcal mol^{-1}, the lowest ΔG values detectable under the experimental conditions.

[1] U. Jenal *et al. Nat Rev. Microbiol.*, **2017**, 15, 271-284.
[2] M. Gomelsky *et al. Microbiol. Mol. Biol. Rev.*, **2013**, 77, 1-52
[3] R. Hengge, *Nat. Rev. Microbiol.*, **2009**, 7, 263-273.

Advanced building blocks for medicinal chemistry from the chemical universe database GDB

Kris Meier, Jean-Louis Reymond
University of Bern, Freiestrasse 3, CH-3012 Bern

With more than 30 drug candidates in clinical trials, fragment-based drug discovery (FBDD) has become a very successful strategy to identify bioactive molecules during the past 20 years.[1] However, the success of FBDD is often dependent on the quality of the fragment library. It is therefore critical to access new and advanced fragments or building blocks. Reymond *et. al.* extensively enumerated the chemical space of small molecules with up to 17 heavy atoms in the chemical space project. This project lead to different chemical universe databases, GDB-11 (26.4 million molecules), GDB-13 (970 million molecules), and GDB-17 (166 billion molecules). The vast majority of the molecules in these databases have not been described in synthetic literature and are therefore a source of inspiration for novel fragments or building blocks.[2-4]

The use of GDB for drug discovery was exemplified with the successful identification of new Glutamate Transporter 1 (GLT-1) inhibitors (Fig. 1). First, Aspartate (**1**) and Glutamate were systematically diversified using GDB. High-throughput virtual screening followed by synthesis of high-ranking molecules then yielded a new Norborane-type inhibitor (**rac-25a**). These Aspartate analogues showed not only good micromolar activity but also high selectivity towards GLT-1.[5]

Extensive analysis of molecules described in literature compared to molecules in GDB revealed that a large part of novelty resides in polycyclic compounds.[6] In this work we focussed on the synthesis of conformationally restricted structures bearing one quaternary carbon. So here we present a new approach to the design of the synthesis of novel polycyclic molecules from GDB, displaying pharmacophoric features of interest for medicinal chemistry.

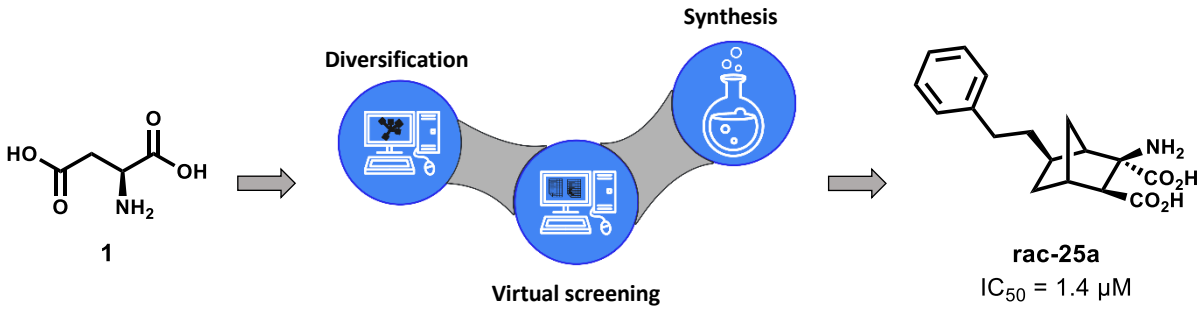


Figure 1: General workflow for GDB based drug discovery shown for Glutamate Transporter 1 (GLT-1) as a target. Asparagate (**1**) was used as a starting point to find novel Norborane-type analogue (**rac-25a**) with good activity and selectivity.[5]

[1] D. A. Erlanson, S. W. Fesik, R. E. Hubbard, W. Jahnke, H. Jhoti, *Nat. Rev. Drug Discov.*, **2016**, *15*, 605—619
[2] L. C. Blum, J.-L. Reymond, *J. Am. Chem. Soc.*, **2009**, *131*, 8732—8733
[3] L. Ruddigkeit, R. van Deursen, L. C. Blum, J.-L. Reymond, *J. Chem. Inf. Model.*, **2012**, *52*, 2864—2875
[4] http://gdb.unibe.ch/tools/ (25.04.2019)
[5] E. Luethi, K. T. Nguyen, M. Bürzle, L. C. Blum, Y. Suzuko, M. Hediger, J.-L. Reymond, *J. Med. Chem.*, **2010,** *53*, 7236—7250
[6] R. Visini, J. Arús-Pous, M. Awale, J.-L. Reymond, *J. Chem. Inf. Model.*, **2017**, *57*, 2707—2718

Glycosyl Donors for Chemoenzymatic Reconstitution of the Dolichol Pathway

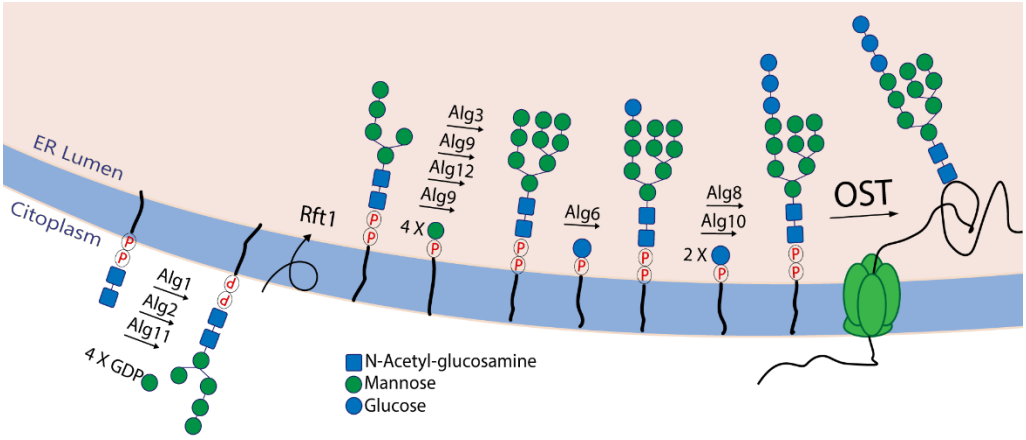
*Giorgio Pesciullesi[1], Joël S. Bloch[2], Tamis Darbre[1], Kaspar P. Locher[2] and Jean-Louis Reymond[1]**

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

[2] Institute of Molecular Biology and Biophysics, ETH Zürich, Otto-Stern-Weg 5, 8093 Zürich, Switzerland

Abstract

Eukaryotic protein N-glycosylation involves the gradual build-up of a lipid-linked oligosaccharide (LLO) starting from a chitobiose connected to a C_{70}-C_{105} dolichol via an α-diphosphate linkage ($GlcNAc_2$-α-PP-dol70-105) through the sequential action of 11 membrane bound glycosyl transferases (GTs) within the endoplasmic reticulum, followed by transfer of the resulting tetradecasaccharide to the asparagine side chain of an acceptor protein catalyzed by oligosaccharyl transferase (OST).[1] Synthetic LLOs and glycosyl donors are essential tools in structural and functional studies of these enzymes,[2,3] which together form the so-called dolichol pathway. We previously reported that eukaryotic OST accepts chitobiose linked to a relatively short C_{25} dolichol ($GlcNAc_2$-α-PP-dol25) as minimal substrate,[4] and developed a chemo-enzymatic procedure to elongate this substrate up to $Man_5GlcNAc_2$-α-PP-dol25 using purified GTs and commercially available GDP mannose.[5] Here we report the synthesis of two additional synthetic donors, namely man-β-P-dol25 and glc-β-P-dol25, which make it possible to access the remaining intermediates of the dolichol pathway by further elongating $Man_5GlcNAc_2$-α-PP-dol25 using purified GTs. We further prepared and tested two differently functionalized azide bearing substrates (6 and 4 position), as well as a non-hydrolysable Glc-β-phosphonate as enzyme inhibitor to test the specificity of GTs along the dolichol pathway.



References

1. M. Aebi. *Biochimica et Biophysica Acta* **2013**, 2430 –2437
2. J. M. Boilevin, Jean-Louis Reymond. *Synthesis* **2018**, 50, 2631–2654
3. Camilo Perez, Sabina Gerber, Jérémy Boilevin, Monika Bucher, Tamis Darbre, Markus Aebi, Jean-Louis Reymond & Kaspar P. Locher. *Nature* **2015, 524 (7566), 433**
4. Ana S. Ramírez, Jérémy Boilevin, Rasomoy Biswas, Bee Ha Gan, Daniel Janser, Markus Aebi, Tamis Darbre, Jean-Louis Reymond, and Kaspar P Locher. *Glycobiology* **2017**, vol. 27, 525–535
5. Ana S. Ramírez, Jérémy Boilevin, Chia-Wei Lin, Bee Ha Gan, Daniel Janser, Markus Aebi, Tamis Darbre, Jean-Louis Reymond, and Kaspar P Locher. *Glycobiology* **2017**, vol. 27, no. 8, 726–733

Activity-based protein profiling (ABPP) of serine hydrolases for target discovery against Leishmaniasis.

Exequiel O. J. Porta, Jaime Isern, Kalesh Karunakaran-Nair-Ana, Paul W. Denny, Patrick G. Steel.

Department of Chemistry, Durham University, DH1 3LE, Durham, UK.
exequiel.o.porta@durham.ac.uk

The leishmaniases are a group of diseases caused by parasitic protozoa of the genus *Leishmania*. The diseases range from self-healing cutaneous forms to debilitating mucocutaneous disease, and lethal visceral leishmaniasis. Approximately 12 million people in 98 countries in tropical and sub-tropical regions bear the burden of disease. The available treatments are inadequate for many reasons, including parenteral administration, varying efficacy, toxicity and increasing resistance. New agents, with new modes of action, are urgently needed to treat leishmaniasis. Whilst it is well-known that the superfamily of human serine hydrolases contains many validated and druggable targets, the biological role(s) of the serine hydrolases counterpart in *Leishmania* parasite remain unexplored. In order to fill this void, we have generated a series of chemical probes containing an electrophilic fluorophosphonate moiety to covalently label active site serine residues within the *Leishmania* serine hydrolase proteome. Robust workflows have been established for the selective profiling of the serine hydrolases in *Leishmania mexicana* parasites. Details of this, together with studies exploring protein deconvolution, identification and quantitation through competitive ABPP experiments and quantitative proteomics mass spectrometry (MS) analyses will be described.

Anti-Infective Target Enzyme IspE
- optimising the balance between cellular and enzymatic activity

H.-K. Ropponen[1,2], E. Diamanti[1], B. Illarionov[3],
J. Haupenthal[1], M. Fischer[3] and A. K. H. Hirsch[1,2]

[1] Department for Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research (HIPS) − Helmholtz Centre for Infection Research (HZI), Campus Building E8.1, 66123 Saarbrücken, Germany. [2] Department of Pharmacy, Saarland University, Campus Building E8.1, 66123 Saarbrücken, Germany. [3] Hamburg School of Food Science, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany.

E-mail: henni-karoliina.ropponen@helmholtz-hips.de

Biosynthesis of isoprenoids *via* the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway is vital for bacteria, parasites and plants. Since the universal isoprenoids precursors are biosynthesised *via* the distinct mevalonate pathway in humans, the MEP pathway possesses many attractive anti-infective drug targets.[1] In the search for novel inhibitors of the central enzyme IspE of the MEP pathway, we embarked on *in silico* virtual screening (VS) of 106,801 compounds using the crystal structure of *E. coli* IspE (1OJ4).

Most of the reported IspE inhibitors against Gram-negative *Escherichia coli* have low-micromolar enzyme activity but lack activity in cell-based assays.[2] To address this translational gap, filtering according to the Gram-negative eNTRy rules, namely presence of a primary amine, globularity and rotatable bonds [3,4], led to the selection of 24 compounds and identification of a fragment-like hit compound HIPS5242 with K_d of 600-800 µM for *Ec*IspE. HIPS5242 possesses a promising profile against Gram-negative pathogens including *Escherichia coli* (MIC = 97.0 ± 4.2 µM), *Acinetobacter baumannii* (MIC = 100 ± 0.7 µM) and *Pseudomonas aeruginosa* (%-Growth inhibition = 46 ± 6% @100 µM). Further medicinal-chemistry optimisation is ongoing to investigate the necessity of the ionisable amines and includes testing against *E. coli* mutant strains such as Δ*tolC*, Δ*acrB* and D22 whilst further improving the antibacterial spectrum against the clinically relevant Gram-negative pathogens.

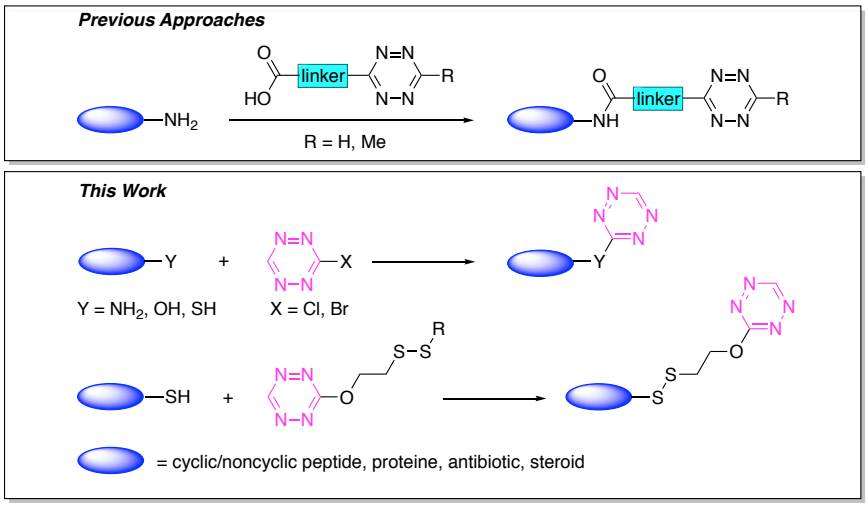
References: [1] Frank, A.; Groll, M. *Chem. Rev.* **2017**, *117*, 5675–5703. [2] Masini, T.; Hirsch, A. K. H. *J. Med. Chem.* **2014**, *57*, 9740–9763. [3] Richter, M. F.; Drown, B. S.; Riley, A. P.; Garcia, A.; Shirai, T.; Svec, R. L.; Hergenrother, P. J. *Nature* **2017**, *545*, 299–304. [4] Richter, M. F.; Hergenrother, P. J.; *Ann. N.Y. Acad. Sci.* **2019**, *1435*, 18–38.

3-Bromotetrazine: Labelling of Macromolecules via Monosubstituted Bifunctional *s*-Tetrazines

Simon D. Schnell, Lukas V. Hoff, Advaita Panchagnula, Simon Sieber, Anthony Linden, Karl Gademann

Department of Chemistry, University of Zurich,
Winterthurerstrasse 190, 8057 Zurich, Switzerland
simon.schnell@uzh.ch

In recent years, the inverse electron demand Diels-Alder (IEDDA) reaction gained interest in the field of chemical biology, due to its bioorthogonality, fast kinetics and biocompatibility.[1] Especially *s*-tetrazines emerged as powerful precursors for IEDDA reactions with strained alkenes and alkynes.[1] However, it still remains a challenge to incorporate *s*-tetrazines into macromolecules and current techniques mainly rely on amide bond formation of a carboxylic acid derived tetrazine based precursor and an amine.[2] Not only the site of labelling is thus strictly limited to amines, but the presence of a the bulky linker usually has a negative effect on the physiochemical properties and the three-dimensional structure of the moiety attached to the *s*-tetrazine.[3]



Herein, we report the synthesis of 3-halogenated, monosubstituted *s*-tetrazines and demonstrate their potential as precursors for the installation of a minimal tetrazine unit selectively into natural products and proteins.[4] Additionally, chemoselective probes are synthesized for the labelling of thiols in cell extracts in the context of targeted natural product isolation and in living cells.[4]

[1] B. L. Oliveira, Z. Guo, G. J. L. Bernardes, *Chem. Soc. Rev.* **2017**, *46*, 4895–4950.
[2] W. D. Lambert, Y. Fang, S. Mahapatra, Z. Huang, C. W. am Ende, J. M. Fox, *J. Am. Chem. Soc.* **2019**, *141*, 17068–17074.
[3] T. Cañeque, S. Müller, R. Rodriguez, *Nat. Rev. Chem.* **2018**, *2*, 202–215; M. Baalmann, M. J. Ziegler, P. Werther, J. Wilhelm, R. Wombacher, *Bioconj. Chem.* **2019**, *30*, 1405–1414.
[4] Simon D. Schnell, Lukas V. Hoff, Advaita Panchagnula, Simon Sieber, Anthony Linden, Karl Gademann, ChemRixv DOI: 10.26434/chemrxiv.10518857.v1.

Antibiotic Delivery via Disulfide Exchange

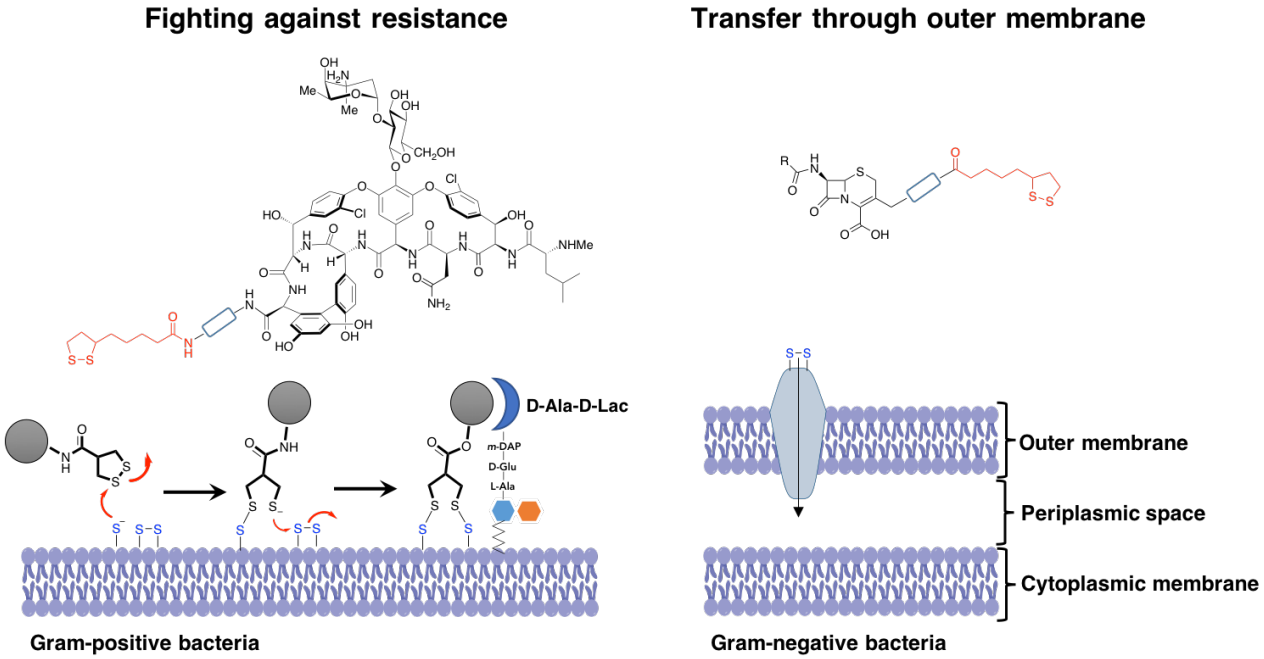
Inga Shchelik and Karl Gademann

University of Zurich, Winterthurerstrasse 180, 8057, Zurich, Switzerland
inga.shchelik@uzh.ch

There is a strong need for the development of new antibacterial agents, due to an increase in the number of pathogenic bacteria, including resistant species. However, the number of approved antibiotics in the market has been decreasing over the decades. One of the reasons includes that potential antibiotics often fail to reach the target,[1] in particular for Gram-negative bacteria. Therefore, several strategies have been developed for antibiotic delivery based on nanomaterials (self-therapeutic agents and carriers for antimicrobial cargo)[2] and modified living organisms.[3] However, there remains great interest in finding delivery strategies based on novel molecular mechanisms.

A number of dynamic covalent chemistry strategies have already been exploited, which can self-assemble, respond and dispart in a controlled manner in mammalian cells.[4] One of them includes the utilization of cell surface thiols to increase cellular uptake.[5,6,7] Gram-positive and Gram-negative cells also contain thiol groups in their cell envelopes, which emerges as a potential target for dithiol-mediated uptake strategy.

In the pursuit of this goal, our group selected two antibiotics to be derivatized. Vancomycin and cephalosporin are two well-known and broadly used inhibitors of bacterial cells wall biosynthesis.[8] The aforementioned antibiotics were modified with dithiol-containing aliphatic chains and/or lipoic acid.[6] All the obtained compounds were tested against Gram-negative and Gram-positive bacteria, including MRSA and vancomycin resistant Staphylococcus aureus (VRSA). Additionally, we studied the mechanism of action by microscopy to visualize Gram-negative bacteria mixed with fluorophore labelled dithiol derivatives.



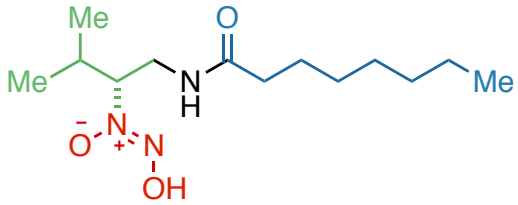
[1] M. F. Richter, P. J. Hergenrother, *A. N. Y. Acad. Sci.*, **2019**, *1435*, 18–38.
[2] A. Gupta, S. Mumtaz, C.-H. Li, I. Hussain, V. M. Rotello, *Chem.Soc.Rev.*, **2019**, *48*, 415-427.
[3] I. P. Kerschgens, K. Gademann, *ChemBioChem*, **2018**, *19*, 439–443.
[4] S. Ulrich, *Acc. Chem. Res.*, **2019**, *52*, 510−519.
[5] A. G. Torres, M. J. Gait, *Trends in Biotechnology*, **2012**, *30*, 4-8.
[6] Q. Laurent, M. Berthet, Y. Cheng, N. Sakai, S. Barluenga, N. Winssinger, S. Matile, *ChemBioChem,* **2019**, *20*, 1–6.
[7] T. Li, S. Takeoka, *Int. J. of Nanomed.*, **2014**, *9*, 2849-2861.
[8] P. Sarkar, V. Yarlagadda, C. Ghosh, J. Haldar, *Med. Chem. Comm.*, **2017**, *8(3)*, 516–533.

Target Investigation of the Diazeniumdiolate Fragin

Simon Sieber,[1] Christian Jenul,[2] Christoph Daeppen,[1] Anugraha Mathew,[2] Dominic Höpfner,[3] Leo Eberl[2], and Karl Gademann[1]

[1]Department of Chemistry, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland
[2]Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zürich, Switzerland
[3] Novartis Institutes for BioMedical Research, Novartis Campus, 4056, Basel, Switzerland
simon.sieber@uzh.ch

To date, only 200 natural products containing a nitrogen-nitrogen bond have been isolated.[1] These compounds encompass in a wide range of biological activities and can be categorized in 16 main classes. One of these classes contains the unique diazeniumdiolate functional group that has been found in only a few natural products.[1] Fragin (**1**), isolated in 1967 from *Pseudomonas fragi,* is a member of this class and was found to possess diverse biological activities, such as antibacterial, antifungal, growth inhibitory and anticancer activities.[2-4] During our genomic analysis of the pathogenic bacteria *Burkholderia cenocepacia* H111, we identified the gene cluster responsible for the production of fragin (**1**).[5] A multidisciplinary approach was designed to investigate the biochemical properties of this natural product.



Fragin (**1**)

SAR study

Target identification

To initiate our investigations into its biological activity, an enantioselective total synthesis of fragin (**1**) was designed and performed affording sufficient amount of the natural product. Additionally, several derivatives were proposed and synthesized in order to interrogate the effect of each moiety on the biological effect of fragin (**1**). Finally, the antifungal target of fragin (**1**) was investigated using a genome-wide profiling approach on the model organism *Saccharmoyces cerevisiae*.

[1] L. M. Blair, J. Sperry, *J. Nat. Prod.* **2013**, *76*, 794–812.
[2] A. Murayama, S. Tamura, *Agric. Biol. Chem.* **1970**, *34*, 130–134.
[3] S. Tamura, A. Murayama, K. Hata, *Agric. Biol. Chem.* **1967**, *31*, 758–759.
[4] A. Murayama, S. Tamura, *Agric. Biol. Chem.* **1970**, *34*, 122–129.
[5] C. Jenul, S. Sieber, C. Daeppen, A. Mathew, M. Lardi, G. Pessi, D. Hoepfner, M. Neuburger, A. Linden, K. Gademann, L. Eberl, *Nat. Commun.* **2018**, *9*, 1297.

In silico designed IGFR2-specific peptides as targeting agent for activated hepatic stellate cells

Florian Weber[1,2], Tommaso Casalini[3,4], Gina Valentino[1,2], Lorine Brülisauer[1], Nico Andreas[5], Andreas Koeberle[6], Thomas Kamradt[5], Alessandro Contini[7], Paola Luciani[1,2]

[1] Department of Pharmaceutical Technology, Friedrich Schiller University Jena, Germany; [2] Present address: Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland; [3] Institute of Mechanical Engineering and Material Technology, Department of Innovative Technology, SUPSI, Manno, Switzerland; [4] Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland; [5] Institute of Immunology, Jena University Hospital, Jena, Germany; [6] Department of Pharmaceutical Chemistry, Friedrich Schiller University Jena, Germany; [7] Dipartimento di Scienze Farmaceutiche - Sezione Chimica Generale e Organica "A. Marchesini", Università degli Studi di Milano, Milano, Italy

florian.weber@dcb.unibe.ch

The lack of accurate and easily applicable methods for the diagnosis of liver fibrosis, a chronic pathology characterized by an accumulation of extracellular matrix released by activated hepatic stellate cells (HSC), has been a major limitation for clinical management in liver diseases. The identification of biomarkers specific for alterations of liver microstructure, combined with a non-invasive optical imaging modality, could guide the clinicians towards a timely therapeutic strategy. In the present study, structural information of insulin-like growth factor 2 receptor (IGF2R), a protein overexpressed on activated HSCs, was used for an experimental *in silico* screening of novel specific peptide ligands starting from the IGF2R/IGF2 complex. Molecular dynamic simulations led to the identification of a putative peptide sequence containing the most relevant amino acids for the interaction (IGF2 E12-C21). The Residue Scan tool, implemented in the Molecular Operating Environment (MOE) software, was then used to optimize the binding affinity. The designed peptides and their associated scrambled sequences were fluorescently labelled and their binding affinity to LX2 cells, a model for activated human HSCs [1], was tested by flow cytometry and confocal microscopy. HepG2 cells (model for human polarized hepatocytes) and HEK293 cells (human embryonal kidney cells) were used as negative controls. The unlabelled sequences were tested *in vitro* for their proteolytic resistance by HPLC. All peptides were characterized by an equilibrium dissociation constant (K_D) ≤ 13 μM. Furthermore, all sequences have shown a 2- to 4-fold increase in binding for LX2 cells compared to HepG2 or HEK293. A significant superior binding efficiency of the targeting peptides compared to their scrambled sequences could be observed. Serum stability of the peptides in 50% (v/v) FBS in PBS at 37 °C showed a proteolytic stability of about 60%-80% after 24 h. The increased binding of the *in silico* identified peptides towards LX2 cells compared to the negative controls and the satisfactory proteolytic stability makes them ideal candidates for potential targeting ligands on the surface of lipid-based nanocarriers.

[1] G. Valentino, C. Zivko, F. Weber, L. Brülisauer, P. Luciani, *Pharmaceutics*, **2019** *11,* 12.

Mapping the neighborhood – a BioID-based approach to identify potential interaction partners of hexose-6-phosphate dehydrogenase

Michael Weingartner[1], Maria Tsachaki[1], Thomas Bock[2] and Alex Odermatt[1]

[1]Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland
[2]Proteomics Core Facility, Biozentrum, University of Basel, 4056 Basel, Switzerland

Background: Hexose-6-phosphate dehydrogenase (H6PD) is the only verified source of NADPH in the lumen of the endoplasmic reticulum. It provides reducing equivalents to 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) for activation of glucocorticoids. H6PD plays a role in the control of cancer cell proliferation and migration by its impact on the unfolded protein response, calcium homeostasis and redox regulation. Beside a verified interaction between H6PD and 11β-HSD1 there are currently no other interacting partners known. The objectives of this work were to search for potential interaction partners and regulators of H6PD.
Methods: Recombinant H6PD linked to a promiscuous biotin ligase was expressed in MDA-MB 231 breast cancer cells and used to generate biotinylated proteins vicinal to H6PD (BioID). Qualification and quantification of biotinylated proteins was achieved by mass spectrometry. Potential candidates were selected and validated by knockdown and co-immunoprecipitation experiments.
Results: AGR2, a member of the protein disulfide isomerase (PDI) family, was identified as an interacting partner of H6PD. Knockdown of AGR2 using siRNA led to increased H6PD protein levels at different time points in the two breast cancer cell lines MDA-MB 231 and MCF7. Co-immunoprecipitation confirmed the interaction between AGR2 and H6PD.
Conclusion: Modulation of gene expression using siRNA showed an effect of AGR2 on H6PD protein levels. However, the detailed mechanism of interaction remains unknown. Further experiments are needed to reveal whether AGR2 is involved in the correct folding of H6PD by disulfide bond modulation.

Peptide dendrimers as delivery systems for nucleic acids

S. J. Zamolo, J-L. Reymond, T. Darbre
Department of Chemistry and Biochemistry
University of Bern, Freiestrasse 3, 3012 Bern

Gene therapy is a powerful technique that allows the treatment of many diseases at a molecular level through the introduction of genetic materials into cells. However, the transfection procedure is often very challenging, due to the lack of efficient delivery systems.[1]
Peptide dendrimers/lipid hybrid systems were showed to be efficient transfection reagents for DNA [2] and siRNA into HeLa cells [3]. Recently, these compounds have also been successfully applied in oligonucleotides delivery [4].
We are now exploring a new library of third generation peptide dendrimers in order to perform transfection of plasmid DNA coding for CRISPR-Cas9. The optimization of hydrophilicity, hydrophobicity and the introduction of non-natural amino acids in the structure allowed us to obtain systems displaying high pDNA transfection efficiency in absence of helper lipid. In particular, biological experiments showed high transfection efficiency -measured as GFP expression by FACS analysis- low cytotoxicity and low immunogenicity.
Furthermore, pDNA transfection experiments performed on 3D cellular spheroids showed promising transfection efficiency and low cytotoxicity, properties necessary for potential in vivo applications.

References:

[1] U. Lachelt, E. Wagner, *Chem. Rev.* ***2015****, 115, 11043-11078.*
[2] A. Kwok, G. Eggimann, J. L. Reymond, T. Darbre, F. Hollfelder, *ACS Nano,* ***2013****, 7, 4668-4682.*
[3] A. Kwok, G. Eggimann, M. Heitz, J. L. Reymond, F. Hollfelder, T. Darbre, *ChemBioChem.* ***2016****, 17, 1-8.*
[4] O.Saher, C. Rocha, E. Zaghloul, O. Wiklander, S. Zamolo et al., *Eur. J. Pharm. Biopharm.,* ***2018****, 132, 29-40.*

Profiling of extracellular vesicles from lipid-treated hepatic stellate cells

Cristina Zivko[1,2], Gina Valentino[1], Gregor Fuhrmann,[2] Paola Luciani[1]

[1]Institute of Pharmacy, Friedrich Schiller University Jena, Otto Schott Straße 41, Jena, Germany
Present: Department of Chemistry and Biochemistry, Freiestrasse 3, 3012 Bern, Switzerland
[2]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), 66123 Saarbrücken, Germany

cristina.zivko@dcb.unibe.ch

The activation of human hepatic stellate cells (HSCs) is a driving factor of liver fibrosis.[1] We evaluated differences in the extracellular vesicles (EVs) shed by HSCs in their different phenotypical states. Untreated HSCs were compared to their activated and quiescent counterparts. Successful purification by size exclusion chromatography was confirmed by a distinct resolution between EVs and protein aggregates as seen by bicinchoninic acid (BCA) assay. Differently treated HSCs produced EVs in comparable amounts and size distributions as determined by nanoparticle tracking analysis (NTA). Quantile subtraction of the distribution curve obtained from untreated cells shows that activated HSCs produce smaller EVs (80-150 nm) more prominently than quiescent cells. Electron microscopy imaging confirmed the polydispersity in the samples. Lipid-based antifibrotic formulations were developed and screened for their potential application on HSCs. Our results create an important basis for the potential non-invasive detection of liver fibrosis and evaluation of treatment response.

[1] JE Puche, Y Saiman, SL Friedman, *Compr. Physiol.*, **2013**, *3*, 1473–1492.