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Abstract Book

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Welcome Letter

Honourable guests and conference participants!

It is my pleasure to address you on this important scientific event – a conference dedicated to achievements in discovery of new drugs. The COVID-19 pandemic has reaffirmed the importance of developing new treatments to tackle unexpected challenges. The pandemic has also left its impact on well-known chronic diseases. However, we can be certain – new drugs have provided enormous progress in treating diseases that were previously fatal.

Drug discovery is a unique field, and we can be proud that Latvia can provide the entire research lifecycle in this area – from design of new molecules to clinical trials. The Latvian Institute of Organic Synthesis is a flagship institution in discovering new drugs. It always provides the opportunity to meet many young researchers that understand their mission to improve both the quality of research and the quality of human life. Alongside excellent research team leaders and specialists in various fields, they are reaching for a common goal – overcoming all of the challenges of creating new drugs.

The Institute successfully participates in European Union Framework Programme projects, implementing projects ranging from research, to regional development, to twinning and teaming projects, thereby proving that Latvian drug discovery researchers are capable of collaborating at a high level both in Europe and the world.

The Republic of Latvia has supported investment in drug discovery infrastructure since 2004, resulting in the development of new, purpose-suited facilities and the installation of world-class equipment. The Ministry of Education and Science has very recently confirmed a commitment to support the development of a Biopharmaceutical Research Centre with 15 million *euro* from EU Structural Funds, provided the excellence centre proposal is successful in Horizon Europe Teaming call. Drug discovery and biopharmaceuticals are an especially important priority of Latvia's smart specialisation strategy, and they will always hold an important place among the priorities of the Ministry.

Drug discovery is both an obstacle course and a marathon. My sincere thanks to all the researchers whose long-term efforts have helped invent new drugs, saving many lives!

I wish you a successful conference, many new ideas and initiatives for the development of new drugs, thereby fulfilling global sustainability goals and gifting all of our lives with more years and greater health.

State Secretary for the Ministry
of Education and Science
Līga Lejiņa

Selenium in drug discovery: a blessing and a curse

Pavel Arsenyan

Latvian Institute of Organic Synthesis, Aizkraukles 21, LV1006, Riga, Latvia
pavel@osi.lv

Refinery production of selenium worldwide is about 2700 tons annually. 17% of it is used in nutrition and cosmetics, the rest – in the production of various materials. Over the past 20 years, scientific studies have clearly demonstrated that selenium is an irreplaceable microelement with essential properties for human health. Selenium is an active component of GPx, which is an enzyme involved in cell redox homeostasis. Selenium has antioxidant properties and it is crucial in physiological and pathological processes linked to increased reactive oxygen species generation. Selenium is needed for the proper functioning of the immune system, as a key nutrient it inhibits HIV progression to AIDS and decreases a risk of cardiovascular diseases. Indeed, relationships between the level of selenium in the daily diet and the risks of developing various types of cancers have been established. However, the ain negative issue about selenium is a narrow range between therapeutic and toxic doses.

Selenium-containing compounds used in industry are quite toxic, e.g. LD50 of widely used nutrient – sodium selenite – varies from 8.1 to 12.1 mg/kg, which is comparable with potassium cyanide toxicity. In the last decades, introduction of selenium into biologically active molecules have attracted increasing attention. Some compounds exhibit excellent properties as antioxidants, redox modulators, antitumor and antihypertensive agents in preclinical studies. Unfortunately, from thousands of synthesized and studied selenium-containing compounds not a single one has been approved as a drug so far.

Herein, the development of novel methodologies for the synthesis of fused selenophenes, isoselenazolium and indolizinium salts will be discussed with the aim to find the right direction for the future research in the elaboration of drug candidates.

Acknowledgements
Financial support from ERDF project Nr. 1.1.1.1/19/A/016 is gratefully acknowledged.

Activity-enhanced antimicrobial peptides

Fredrik Björkling

We have identified a class of bacteria-penetrating peptide constructs, which upon conjugation to selected antimicrobial peptides (AMPs) enhance the original activity up to 32-fold. Recently, we found that such modification of tridecaptin analogues (a subtype of AMP) provides compounds with potent broad-spectrum activity against Gram-negative bacteria without concomitant mammalian cell toxicity in HepG2 cells or hemolysis.

The frontrunner compound (CEP709) has high antibacterial potency (minimal inhibitory concentrations below 1 μg/ml) and low cellular toxicity. CEP709 is active against multi-drug resistant (MDR) Gram-negative bacteria of the ESKAPE pathogen group, including *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, which are a major cause of life-threatening infections. In addition, CEP709 has excellent stability in mouse serum (with a half-life of about 10 h), is well tolerated in mice (up to 10–20 mg/kg upon subcutaneous administration), and exhibits predominantly renal elimination, showing some accumulation in kidneys and liver. Finally, we have demonstrated *in vivo* efficacy of CEP709 in mouse peritonitis/sepsis models (with *E. coli*, *K. pneumoniae*, and *P. aeruginosa*) as well as in a thigh infection model (with *E. coli*). Altogether, CEP709 is suggested to be a good drug candidate for further development.

Industry academia collaboration in AMR R&D: Does it take two to tango?

Jürgen Brem

Janssen: Pharmaceutical Companies of Johnson & Johnson, Belgium

Antibiotics are arguable the cornerstone of modern medicine by combating infectious disease and extending life expectancy for the last 100 years. Diseases such as pneumonia, sepsis, neonatal meningitis and urinary tract infection are now easily treated and wound infections from surgery and dentistry can be prevented. The World Health Organisation warns that without effective antibiotics, the success of major surgery and cancer chemotherapy would be extremely compromised.

The development of a new drug or therapy from basic science proposal to the 'bench to clinic' stage is normally a slow endeavor and may benefit of close collaborations between industry and academy.

Currently some of the major pharmaceutical companies are no longer investing in the development of low-profit antibiotics, but the pipeline for new antibiotics is dramatically running dry. However, Janssen is an active partner of innovative global networks of academics and/or industry partners, aiming to tackle antibiotic resistance.

The talk will cover past learnings, present overview, and potential future paths of the AMR R&D.

Structure-based optimization of an anticancer compound

Dolbois A., Bedi R. K., Bochenkova E., Müller A., Moroz-Omori E. V., Huang D., Caflisch A.

University of Zürich, Switzerland
caflisch@bioc.uzh.ch

N^6-methyladenosine (m^{6}A) is the most frequent of the 160 RNA modifications reported so far. Accumulating evidence suggests that the METTL3/METTL14 protein complex, part of the m^{6}A regulation machinery, is a key player in a variety of diseases including several types of cancer, type 2 diabetes, and viral infections. The talk will focus on a protein crystallography-based medicinal chemistry optimization of a METTL3 hit compound that has resulted in a 1400-fold potency improvement and an IC_{50} of 5 nM for the lead compound **22** (also called **UZH2**, Figure 1). The series has favorable ADME properties as physicochemical characteristics were taken into account during hit optimization. **UZH2** shows target engagement in cells and is able to reduce the m^{6}A/A level of polyadenylated RNA in MOLM-13 (acute myeloid leukemia) and PC-3 (prostate cancer) cell lines.[1]

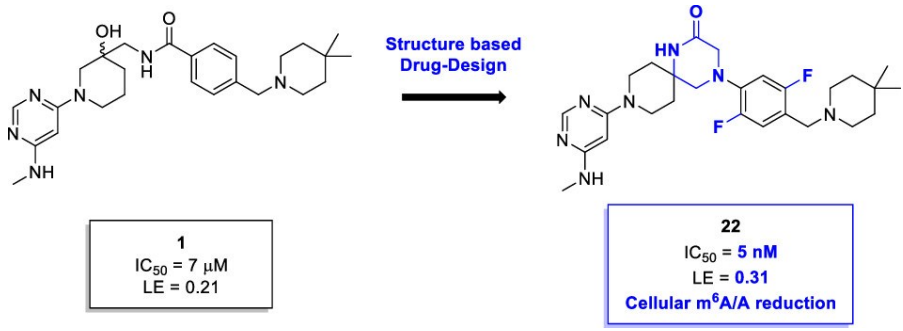

Figure 1. Protein structure-based optimization of a micromolar hit into a low nanomolar inhibitor of METTL3.

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Nanomedicine for glioblastoma therapy

Valentin Cena[1,2]

[1] CIBER, Instituto de Salud Carlos III, 28031 Madrid, Spain
[2] Unidad Asociada Neurodeath, Facultad de Medicina, Universidad de Castilla-La Mancha, 02006 Albacete, Spain
valentin.cena@gmail.com

Glioblastomas (grade IV astrocytoma) are the most common malignant primary brain tumors in adults and are among the most aggressive and difficult-to-treat cancers.[1] RNAi has a high potential to be useful in therapeutics since it is able to knockdown proteins involved in the pathogenesis of different diseases,[2] by targeting cellular mRNA.[3] Clinical trials have already shown RNAi effectivity in some diseases such as hereditary transthyretin amyloidosis[4] and acute intermittent porphyria.[5] The therapeutic approach to GBM that we propose is based on the use of specific siRNA to knock-down proteins involved in GBM cells proliferation and survival. Several steps are required for a nanoparticle to be potentially effective in glioblastoma therapy: bind siRNA, protect it from degradation, lack toxicity against neurons and astrocytes, being able to knock down specific proteins in glioblastoma cells in vitro, and be able to cross the blood-brain barrier and deliver siRNA to the brain parenchyma

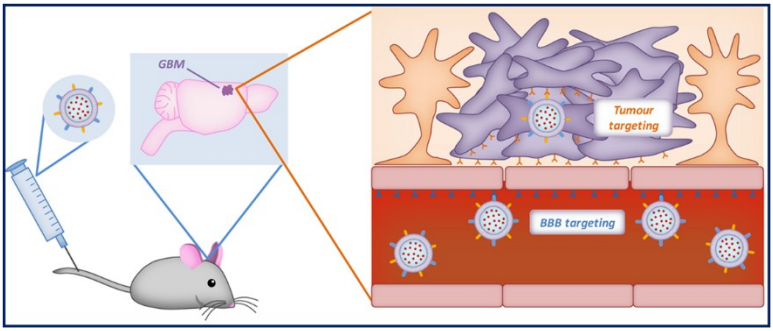

Figure 1. Use of nanomedicine in glioblastoma therapy.

Acknowledgements
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Testing repurposed drugs for neurodegeneration *via* mitochondrial profiling

Lisa Chakrabarti

Our interest is in mitochondrial biology and how it is different according to tissue, age and where there is pathology. Mitochondrial dysfunction is a recognised feature of neurodegenerative disease and of the ageing process. We have used mass spectrometry-based proteomics and lipidomics approaches to delineate the biochemical composition of mitochondria in different paradigms including disease, ageing and exercise. Though the snapshots of mitochondrial biology and chemistry are informative there is still more to understand about the way these molecules modulate the production of energy in the form of ATP. Mitochondrial physiology can be useful as a readout for testing drugs for their ability to normalise metabolic processes in disease. We have been using high-resolution respirometry to evaluate repurposed drugs in human cells, tissues and cell lines. There are opportunities to incorporate cell metabolism evaluation for repurposed ‘known to be safe drugs’ which will speed their progression into the clinic.

Hydroxalogs

David Crich

University of Georgia, Department of Pharmaceutical and Biomedical Sciences,
Department of Chemistry, and Complex Carbohydrate Research Center, Athens, GA 30602, USA.
David.Crich@uga.edu

This lecture will trace the evolution of the concept of trisubstituted hydroxylamines, or hydroxalogs, as novel isosteres for use in medicinal chemistry.[1] The talk will cover the origins of the concept as an isosteric replacement for glycosidic bonds and its extrapolation to natural product analogs. It will continue with a discussion of the development of new reactions for trisubstituted hydroxylamine synthesis, particularly by N–O bond formation, before discussing metabolic stability, influence on "drug-like properties", and ongoing applications in drug design.

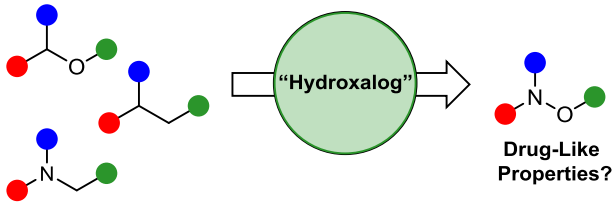

Figure 1. The Hydroxalog Concept.

Acknowledgements
This work is supported by the NIH (GM 144753).

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From crystallographic fragment screen to preclinical candidates: open science discovery of new antivirals

Daren Fearon, The COVID Moonshot Consortium

Diamond Light Source, Didcot, United Kingdom,
Research Complex at Harwell, Didcot, United Kingdom
Daren.fearon@diamond.ac.uk

The development of novel, low cost and globally available antiviral therapeutics remains an essential goal for the current SARS-CoV-2 pandemic. Furthermore, future pandemics could be prevented with easily deployable broad spectrum oral antivirals and open knowledge bases that accelerate novel antiviral discovery.

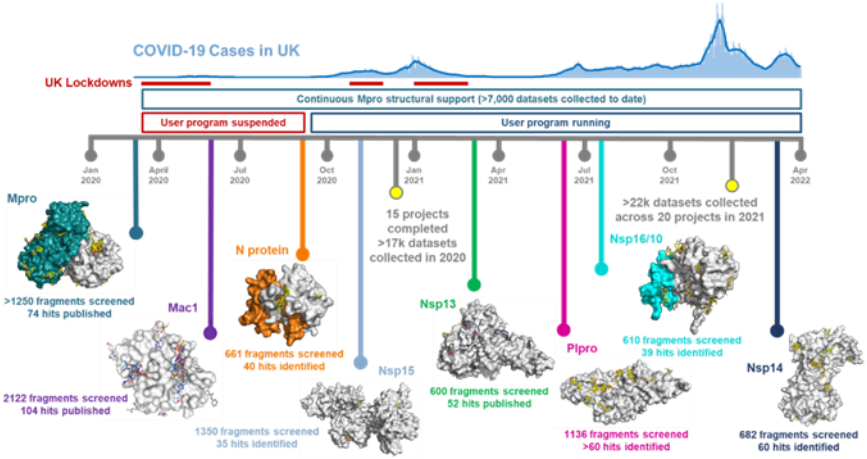

Figure 1. Timeline of SARS-CoV-2 fragment screens with Xchem.

To identify starting points for the development of such therapeutics, the XChem team at Diamond Light Source, in collaboration with international colleagues, has performed large crystallographic fragment screens against 8 key SARS-CoV-2 protein targets including the Main protease[1] and the Nsp3 macrodomain.[2]

This work identified numerous starting points for the development of potent antiviral therapeutics as exemplified by the COVID Moonshot – a fully open science structure enabled drug discovery campaign targeting the SARS-CoV-2 main protease.[3] By leveraging crowdsourced medicinal chemistry design, high throughput structural biology, machine learning and exascale molecular simulations, we discovered a novel scaffold that is differentiated to current clinical candidates in terms of toxicity and pharmacokinetics liabilities, and developed it into orally bioavailable inhibitors with clinical potential within 2 years.

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Discovery of potent Gram-negative antibacterials targeting leucyl-tRNA synthetase

Paul W. Finn[1], Jekaterina Bolsakova[2], Michael Charlton[1], Michael Dawson[1], Grace Edmund[1], Liene Grigorjeva[2], Guillaume Hoffmann[3], Kristaps Jaudzems[2], Aigars Jirgensons[2], Andres Palencia[3], Dace Rasina[2], Marija Skvorcova[2], Janis Veliks[2]

[1] Oxford Drug Design, Oxford, UK
paul.finn@oxford drug design.com
[2] Latvian Institute of Organic Synthesis, Riga, Latvia
[3] INSERM, Grenoble, France

Leucyl-tRNA synthetase is a member of the aminoacyl-tRNA family of bacterial enzymes. This target family is clinically validated: Mupirocin is an inhibitor of isoleucyl-tRNA synthetase that has been successfully developed and marketed for the treatment of skin infections since the mid 1980's. However, mupirocin lacks significant Gram-negative activity and cannot be used systemically. As yet, not aminoacyl-tRNA synthetase inhibitor with a Gram-negative spectrum of activity has been developed.

In seeking a leucyl-tRNA synthetase inhibitor with such a spectrum, Oxford Drug Design has combined its computational platform with structural biology to design novel chemotypes. These have been designed as binders to the catalytic site of the enzyme, maintaining potent inhibition while using a small binding footprint, to minimize the frequency of resistance. Compounds with frequency of resistance of the order of 2×10^{-9} have been identified; significantly better than previous compounds binding to the enzyme's editing site. Resistant organism with mutations in the protein sequence of the leucyl-tRNA synthetase have not yet been observed. Mutations in the gene promoter lead to low-level resistance. The main resistance mechanism observed has been up-regulation of yhhY, an acetyl transferase that inactivates inhibitors via acetylation. Ongoing work has identified strategies to design out this liability.

The current compounds have activity against Gram-negative pathogens, including strains resistant to existing antibiotic classes. No mechanism-based cross resistance has been seen. The current lead compounds have good exposure in mice and have shown activity in multiple mouse models of infection in a dose dependent manner, including via oral administration. Optimization is ongoing to improve antibacterial activity and spectrum, further understand the driver for efficacy and optimize in vivo efficacy. If successful, we believe that such a compound would be valuable additional treatment option for infections caused by resistant Gram-negative organisms.

New heterocyclization and C–H functionalization methods

Vladimir Gevorgyan[1,2]

[1] University of Texas at Dallas
vlad@utdallas.edu
[2] University of Texas Southwestern Medical Center
vladimir.gevorgyan@utsouthwestern.edu

Heterocycles are ubiquitous structural units, broadly found in bioactive molecules and drugs. Often, synthesis of multisubstituted heterocycles via known methods is a challenging task. Thus, novel mild and general protocols, which help solving this problem are exceedingly desirable. To this end, we developed several new multicomponent coupling reactions, novel cycloisomerization and migratory cycloisomerization, and transannulation methods, which allow for a quick assembly of densely-substituted monocyclic and fused heterocyclic motifs.

The most abundant fragments of vast majority of organic molecules, including heterocycles, are C–H bonds. Until several decades ago, unactivated C–H bonds were considered inert, and thus functionalization of organic molecules relied on functional group interconversion. Lately, functionalization of various types of C–H bonds, operating by different mechanisms have been developed. Our group developed several new transition metal-catalyzed and transition metal-free approaches for selective functionalization of C(sp^2)–H and C(sp^3)–H bonds.

The scope of these and related transformations will be demonstrated and the mechanisms will be discussed.

Structure-based design of photoswitchable ligands targeting enzymes and receptors

Pau Gorostiza[1]

[1] Institute for biengineering of Catalonia (ICREA, IBEC, BIST, CIBER)
pau@icrea.cat

The large number of photoswitchable biomolecules discovered and developed in recent years covers a great variety of cellular functions like catalysis of metabolic processes, cytoskeletal polymerization and motors, nucleic acids dynamics, intracellular signaling and perhaps most dazzlingly membrane excitability, which has been at the focus of optogenetics and photopharmacology. The dream of precisely and remotely photocontrolling every aspect of the cell's workings in intact tissue appears within reach and offers the promise of interrogating complex cellular processes to discover their molecular mechanisms. Recent and ongoing projects at IBEC focused on photopharmacology will be reviewed, including the development and applications of freely diffusible and tethered photoswitchable ligands of ionotropic and G protein-coupled receptors. The design takes advantage of available structural information about the protein targets and their pharmacology. These molecular tools allow spatiotemporal control of endogenous proteins in single neurons, and of emerging activity in the brain, including cortical waves.

Redirecting the peptide cleavage: novel cathepsin B inhibitors with inversely oriented warheads

Michael Gütschow

Pharmaceutical Institute, University of Bonn, Germany
guetschow@uni-bonn.de

The typical feature of peptidic inhibitors of cysteine proteases includes an N-capped peptide structure bearing an electrophilic warhead (e.g., an aza-nitrile, as established in our group[1,2]) in place of the scissile peptide bond. A linker can direct a carboxylic group to the S' region to allow for an advantageous salt bridging with the histidine residues of a special structural feature of cathepsin B, the occluding loop, thus enhancing cathepsin B selectivity.[3] Human cathepsin B is a cysteine protease of significant therapeutic importance.

We have designed cathepsin B inhibitors with dipeptide portions directed towards the occluding loop and equipped with fine-tuned, inversely oriented warhead structures which are cleaved upon the action of the active site cysteine leading to irreversible inhibition. The peptidic inhibitors undergo a redirected cleavage and the primed part of the inhibitor remained covalently attached to the protease, an opposite situation when compared to the doctrinal cysteine protease-catalyzed peptide cleavage. The formation of such a complex was confirmed by X-ray crystal structure analysis. Kinetic data at four human cathepsins obtained for an extended series of around 200 representatives of this chemotype demonstrated their selectivity for cathepsin B allowed to draw detailed structure-activity relationships.

The exciting mode of action involved the interplay with the occluding loop of cathepsin B and the attack of the active site cysteine at the inactivator's warhead by a crucial enzyme-catalyzed deprotonation step. Based on the tailored structure of the new inhibitors and in continuation of our previous studies,[4,5] the design of linker-connected bifunctional probes will also be discussed.

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Structural virology for drug design

B. Martin Hällberg

Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden
Martin.Hallberg@ki.se

SARS-CoV-2 is the etiologic agent of COVID-19, currently causing a devastating pandemic for which pharmacological interventions are urgently needed. The virus enters host cells through an interaction between the viral spike glycoprotein and the angiotensin-converting enzyme 2 (ACE2) receptor. Directly preventing this interaction presents an attractive possibility for suppressing SARS-CoV-2 cell entry. From the start of the COVID-19 pandemic, my group worked in collaboration with other groups around the world to develop therapeutic neutralizing antibodies first as a short-to-medium term approach to tackle COVID-19 in the clinic and later as a complement to vaccination for the immunocompromised and for clinical emergency use for variants of concern for which broad communal immunity is lacking. We have been able to structurally characterize a multitude of binders that broadly neutralize SARS-CoV-2 using cryo-EM.[1–5] This structural information has then been fed-back to develop even better neutralizing antibodies (Fig. 1) or guide efforts in relation to newly discovered variants of concern[5]. Hence, using the recent developments of cryo-EM and new antibody development methods our efforts provide an example on how we with structurally guided analysis and design of biologics can quickly respond to pandemics such as COVID-19 in order to minimize their devastating effects on human health and society.

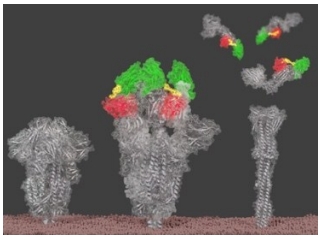

Figure 1. Structurally guided linkage of nanobodies with different epitopes.[3]

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Synthesis of bioactive marine natural products

Bengt Erik Haug

Department of Chemistry and Centre for Pharmacy, University of Bergen, Norway,
bengt-erik.haug@uib.no

The breitfussins,[1–3] which have been isolated from the Arctic marine hydrozoan *Thuiaria breitfussi,* are natural products that comprise a unique indole-oxazole-pyrrole framework not found in any other natural products. Due to the high ratio of heavy atoms to protons, the structures of the initially isolated breitfussin **A** and **B** could only be unambiguously confirmed through total synthesis.[2] Extensive investigation into the biological activity of the breitfussins, has revealed that several members of this class of natural products display nanomolar cytotoxic activity against cancer cell lines. Further investigations have attributed this activity to inhibition of protein kinases. Thus, the indole-oxazole-pyrrole framework of the breitfussins represents a new kinase inhibitor scaffold, with IC_{50} and K_d values in the nanomolar range when tested against a panel of protein kinases. Our synthetic efforts toward breitfussin **A**–**D** will be discussed.

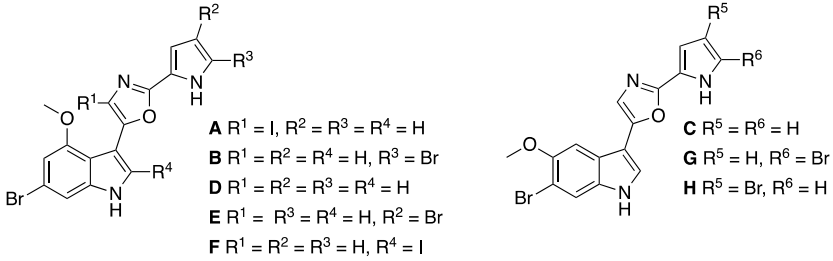

Figure 1. The breitfussin family of natural products.

Acknowledgements
This project has received funding from UiT The Arctic University of Norway, the University of Bergen and the Research Council of Norway.

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Bioavailability and biotransformation of polyphenolic compounds: does the gut microbiome play a lead role?

Nina Hermans

Research group NatuRA (Natural Products & Food Research and Analysis),
University of Antwerp, Belgium
nina.hermans@uantwerpen.be

Natural products offer a rich source of inspiration for potential new lead compounds against diseases for which adequate treatment or prevention is lacking. Nevertheless, many natural products act as prodrugs which are biotransformed and activated after oral administration. Over the past decade, it has been shown that the gut microbiota play a significant role in the biotransformation of many natural compounds.

The colonic biotransformation of compounds by the gut microbiome can be studied in *in vitro* models, as they allow dynamic and multiple sampling over time.

We developed and validated an *in vitro* gastrointestinal platform including a gastrointestinal dialysis model with colon phase (GIDM-Colon) and bioanalytical strategies to investigate and elucidate gastrointestinal biotransformation of natural (or synthetic) compounds.[1]

More specifically, bioavailability issues of natural polyphenolic compounds, including chlorogenic acid,[1] which is an important polyphenolic antioxidative constituent of coffee, and olive phenolic compounds,[2] will be illustrated.

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Development of orthosteric inhibitors of RAD51:BRCA2 interaction

Marko Hyvonen

University of Cambridge
mh256@cam.ac.uk

BRCA2 controls RAD51 recombinase during homologous DNA recombination (HDR) through eight evolutionarily conserved BRC repeats, which individually engage RAD51 *via* the FxxA motif. To enable fragment-based and structure-guided approaches, we developed a monomeric surrogate system based on thermostable archaeal RadA. Using this, we identify fragments binding to the FxxA binding site. Using SAR from peptides binding to this site and extensive medicinal chemistry programme, we developed CAM833, an orthosteric inhibitor of RAD51:BRC interaction with a Kd of 366 nM. CAM833 competes with BRC4 (the highest affinity BRC repeat) binding to RAD51 and dissolves RAD51 oligomers, which rely on similar interaction of its own FxxA motif. In cells CAM833 diminishes formation of damage-induced RAD51 nuclear foci and inhibits RAD51 molecular clustering, CAM833 potentiates cytotoxicity by ionizing radiation, augmenting 4N cell-cycle arrest and apoptotic cell death. It also works in combination with poly-ADP ribose polymerase (PARP)1 inhibitors to suppress growth in BRCA2-wildtype cells through simultaneous inhibition of homologous recombination and non-homologous end-joining. Thus, chemical inhibition of the protein-protein interaction between BRCA2 and RAD51 disrupts HDR and potentiates DNA damage-induced cell death, with implications for cancer therapy.

Diarylethenes as promising drug candidates for photopharmacology

O. Babii[1], S. Afonin[1,2], T. Schober[1,3], I. V. Komarov[1,4,5]

[1] Lumobiotics GmbH (Germany)
[2] Karlsruhe Institute of Technology (Germany)
[3] University of Cambridge (United Kingdom)
[4] Enamine Ltd. (Ukraine)
[5] Taras Shevchenko National University of Kyiv (Ukraine)
igor.komarov@knu.ua

The idea of using reversibly photoisomerizable biologically active compounds as drugs has been discussed in scientific literature for several decades, but only recently was put on reliable experimental ground. One of the known photoisomerizable chemotypes, diarylethenes, demonstrated promising pharmacodynamic and pharmacokinetic characteristics in structural context of cyclic peptidomimetics. The progress which the anticancer diarylethene-derived peptidomimetics have made toward clinical application (Fig. 1) will be presented.

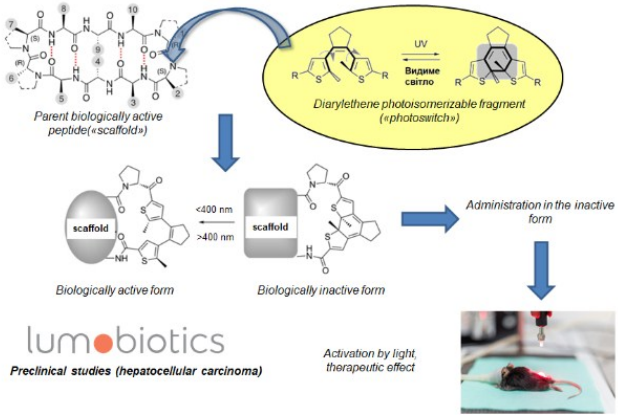

Figure 1. Progress in development of diarylethene-based anticancer drugs.

Acknowledgements
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Cardiometabolic effects of new classes of antidiabetic agents: a way back to cardiomyocytes

Ilze Konrade, Prof., Dr.med.

Department of Endocrinology, Riga East University Hospital, Riga, Latvia
Department of Internal Diseases, Riga Stradiņš University, Riga, Latvia (I.K.)
ilze.konrade@rsu.lv

Insulin resistance-induced abnormalities in glucose and fat homeostasis are hallmarks of type 2 diabetes mellitus (T2DM). These metabolic disturbances are followed by cellular dysfunction and the development of diabetic cardiomyopathy leading to heart failure. Cardiovascular disease remains the leading cause of death in patients with T2DM. Over the past five years, a wave of cardiovascular outcome trials for both glucagon-like peptide-1 receptor agonists (GLP-1RA) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors have been initiated, and both GLP-1RA and SGLT-2 inhibitors have been shown to reduce cardiovascular risk in patients with T2D. As a result, these drugs, originally approved for use as antihyperglycemic agents, have now been established as useful for their cardiovascular benefits, also in nondiabetic populations. SGLT2 inhibitors exert hypoglycaemic effects by inhibiting glucose reabsorption at the proximal convoluted tubules, causing glycosuria, natriuresis and volume contraction. The impressive reduction of heart insufficiency can be commonly attributed to another mechanism of action, inhibition of sodium-loaders in the plasma membrane (NHE-1) affecting intracellular cardiomyocyte sodium homeostasis. Potentiation of GLP-1 action through selective GLP-1 receptor activation by agonists improves endothelial function, augments ventricular contractility, enhances myocardial glucose uptake, and exerts cytoprotective and metabolic actions on blood vessels and cardiomyocytes, making GLP-1RA drugs essential for the prevention of atherogenesis. In conclusion, antidiabetic drugs protect cardiomyocytes and possess cardiometabolic effects in both patients with T2DM and nondiabetic populations.

Effects of exercise on energy metabolism – focus on mitochondrial adaptations

Steen Larsen

Xlab & Center for Healthy Aging, Department of Biomedical Sciences,
University of Copenhagen, Denmark
& Clinical Research Centre, Medical University of Bialystok, Poland

It is well known that exercise is important for a healthy life. Lack of exercise has many negative effects on the metabolism. In the talk focus will be on how exercise or lack of exercise impacts glucose and lipid metabolism. Furthermore how the mitochondria are affected by exercise is also importance and will be discussed. It is well known that exercise improves glucose as well as lipid metabolism, but exercise does also improve mitochondrial function.

A part of the talk will also focus on how commonly used drugs as for example statins affect a normal exercise adaptation. This is of great importance, since many people are using statins to lower cholesterol levels in plasma.

The effects of exercise on energy metabolism with a special focus on the mitochondria will be discussed. Furthermore, it will also briefly be discussed how different drugs potentially affect a training adaptation.

Rational design of a SMARCA2 selective and orally bioavailable VHL-recruiting PROTAC

R. McLennan[1], C. Kofink[2], N. Trainor[1], B. Mair[2], S. Wöhrle[2], M. Wurm[2], N. Mischerikow[2], M. Roy[1], G. Bader[2], P. Greb[2], G. Garavel[2], E. Diers[1], C. Whitworth[1], V. Vetma[1], K. Rumpel[2], M. Scharnweber[2], J. Fuchs[2], T. Gerstberger[2], Y. Cui[3], G. Gremel[2], P. Chetta[2], S. Hopf[2], N. Budano[2], J. Rinnenthal[2], G. Gmaschitz[2], M. Mayer[2], M. Koegl[2], A. Ciulli[1], H. Weinstabl[2], W. Farnaby[1]

[1] Center for Targeted Protein Degradation, School of life Sciences, University of Dundee, Scotland, UK
[2] Boehringer Ingelheim RCV GmbH & Co KG, 1121, Vienna, Austria
[3] Boehringer Ingelheim Pharma GmbH & Co KG, 88397 Biberach, Germany
rzmclennan@dundee.ac.uk

Cancer focused Proteolysis-targeting Chimera (PROTAC) drug discovery offers an attractive modality to target as-yet undrugged oncoproteins for patients with limited or no treatment options. Herein we describe a rational approach to designing PROTACs with oral bioavailability in mind, highlighting the use of ternary co-crystal structures to guide linker composition and exit vector placement. This led to the design of compounds that presented higher potency and suitable PK properties culminating in a tool compound, ACBI2, exhibiting selectivity towards SMARCA2 over its closely related paralogue SMARCA4. ACBI2 will soon be made available via the opnMe.com open innovation portal and will provide a blueprint to arrive at oral efficacy for bifunctional drug modalities.

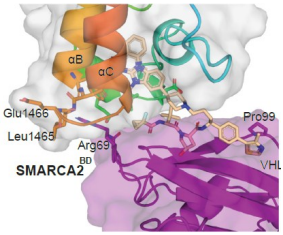

Figure 1. Ternary Complex of VCB:compound-6:SMARCA2.

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Small molecule targeted RNA degraders – RNA modifications, SARS-CoV-2 and beyond

Sigitas Mikutis, Gonçalo J. L. Bernardes

Yusuf Hamied Department of Chemistry, University of Cambridge, sm2029@cam.ac.uk

Recent advances in targeted nucleic acid degradation and editing methods have revolutionized both basic and translational sciences. However, currently available technologies such as siRNA and CRISPR-Cas systems are nucleic acid-based thus have numerous inherent drawbacks. To circumvent these issues, we developed novel chemical small molecule systems to degrade RNA in a targeted manner.

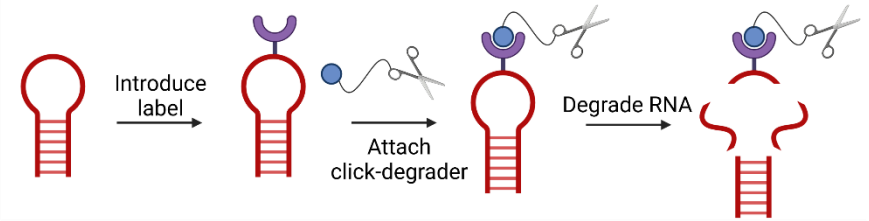

Figure 1. CLICK-Seq approach to targeted RNA degradation. It exploits metabolic hijacking strategy and click chemistry to degrade a subset of cellular RNA.

In the CLICK-Seq approach, a metabolic hijacking strategy is combined with click chemistry to label RNA with biorthogonal handles and degrade it (Fig. 1).[1] We exploited this approach to interrogate the substrates and functions of two RNA methylases, expanding the list of types and identities of methylated RNA species. Altogether, the developed approaches expand the toolkit of methods to manipulate RNA and suggest novel therapeutic strategies.

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Approaches towards innovative antibiotics from microbes

Rolf Müller

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)
yannic.nonnenmacher@helmholtz-hips.de

The global rise of antimicrobial resistance, mainly due to the mis- and overuse of antibiotics, is one of the most pressing issues of our time. To counteract this development, novel resistance-breaking antibiotics are urgently needed.[1] Historically, the vast majority of antibiotics have been derived from microbial natural products. As compared to traditional bacterial producers, such as actinomycetes and bacilii, myxobacteria have been studied less extensively and thus harbor a large potential for the discovery of entirely new natural product scaffolds exhibiting promising bioactivities.[2]

I will discuss recent results from our laboratory regarding the identification of novel bioactive natural products from microbes based on different approaches. Following a bioactivity-guided screening approach, we identified the cystobactamids: a novel class of myxobacterial gyrase inhibitors active against a broad panel of Gram-negative and other multidrug resistant pathogens.[3] Another exciting class of myxobacterial antimicrobials are the chlorotonils. These chlorinated macrolides do not only show broad antibacterial activity against Gram-positive germs, but also exhibit promising antimalarial activity in the nanomolar range.[4] The class of darobactins efficiently targets a protein located in the outer membrane of Gram-negative bacteria. I will show how we combined genetic engineering approaches with heterologous expression to improve the production yield of the compound and gain access to novel derivatives with improved activity.[5]

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Multi-targeted chemotherapeutics hitting G-quadruplex foldings and cancer-related human carbonic anhydrases

Alessio Nocentini[1], Antonio Randazzo[2], Paola Gratteri[1], Claudiu Supuran[1]

[1] Neurofarba Department, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy
[2] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy
e-mail: alessio.nocentini@unifi.it

Drug combinations to hit multiple biological targets has proved to be a successful pharmacological strategy in limiting the onset of tumor resistance, but more effective therapeutic approaches are urgently needed. The use of hybrid-based multi-target ligands is gaining relevance for the treatment of multi-factorial diseases such as cancer, providing an improved pharmacokinetic profile, reduced risk of drug interactions, and a better treatment adherence compared to combined therapies.[1] Here we propose hybrid chemotherapeutics hitting two highly promising antitumor targets in the medicinal chemistry landscape: G-quadruplex (G4) structures, a non-canonical DNA fold present in regulatory regions of some oncogenes or in telomeres,[2] and human carbonic anhydrases (hCAs) IX and XII, transmembrane isoforms overexpressed in a *plethora* of solid tumors.[3] The molecular hybrids were designed to contain a coumarin or sulfonamide CA inhibitor (CAI) scaffold and a berberine core as G4 ligand, connected by 1,2,3-triazole based linkers. The ability of the newly synthesized compounds to inhibit the hCAs IX and XII and to bind and stabilize G4 structures *in vitro* will be presented, as well as preliminary data over the in cell biological action of the most effective derivatives.

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Chemical contributions to fighting and preventing prevalent diseases

Jule P. Dietz, Tobias Lucas, Matthias Krumb, Till Opatz*

Joahnnes Gutenberg University, Department of Chemistry,
Duesbergweg 10-14, 55128 Main, Germany
opatz@uni-mainz.de.

Heterocycles play a key role in the machinery of life and it is not surprising that heterocyclic compounds are also privileged structural elements found in most pharmaceuticals. Economic alternative syntheses of important antiinfectives (pyrimidines, nucleosides, HIV integrase inhibitors – see Figure 1) developed in the course of the "Medicines for All" initiative of the Bill and Melinda Gates Foundation will be presented along with an emerging concept involving a synthetic vaccine.[1–5]

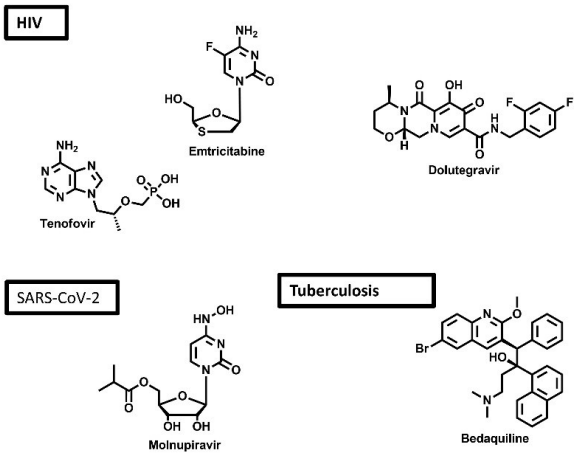

Figure 1. Antiinfectives for which alternative syntheses have been developed.

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ABC transporter blood-brain barrier function in neurodegenerative and neuroinflammatory diseases – from basic research to treatment

Jens Pahnke[1,2,3]

[1] Department of Pathology, Section of Neuropathology,
Translational Neurodegeneration Research and Neuropathology Lab (www.pahnkelab.eu),
University of Oslo and Oslo University Hospital, Sognsvannsveien 20, 0372 Oslo, Norway
[2] LIED, PahnkeLab, University of Lübeck and University Medical Center Schleswig-Holstein,
Ratzeburger Allee 160, 23538 Lübeck, Germany
[3] Department of Pharmacology, Faculty of Medicine, University of Latvia,
Jelgavas iela 4, 1004 Rīga, Latvia
jens.pahnke@medisin.uio.no

The current understanding of neurodegenerative diseases focuses on the deposition of toxic peptide species as larger aggregates in the brain of effected individuals. However, patients with sporadic neurodegenerative diseases do not overexpress these peptides. Moreover, they are hampered in removing the physiologically produced peptides, e.g. Aβ and α-synuclein. The blood-brain barrier plays a prominent role in the active and effective removal of metabolites and also in the efflux of toxic peptides from the brain into the blood stream.

Here, ABC transporters were discovered by us and others to have a fundamental role in the homeostasis of the brain's environment. In 2011, we described a new ABC transporter (ABCC1) that increases Aβ in the brain of knock-out animals by 12-14fold. Since then, we could show that the activation of ABC transporters can be used as a diagnostic tool and a new treatment option for the elderly with Alzheimer's disease and other neurodegenerative diseases.

Towards the development of selective modulators of cadherin-mediated cell-cell adhesion

Emilio Parisini

Latvian Institute of Organic Synthesis
emilio.parisini@osi.lv

Cadherins are transmembrane calcium-dependent cell adhesion proteins that mediate cellular adherens junction formation and tissue morphogenesis. Loss of cadherin-mediated adhesion has been implicated in many different steps of tumor progression such as invasion and migration, and is strongly related to cell–cell detachment and metastasis. Altered expression profiles of epithelial E-cadherin (CDH1) and neuronal N-cadherin (CDH2) have often been observed in cancer cells, most notably in the context of the epithelial-to-mesenchymal transition (EMT) process that occurs during cancer progression. Despite its tumor repressor role in the majority of carcinomas, in epithelial ovarian cancer (EOC) cells E-cadherin shows a high level of expression during tumor progression and facilitates EOC cell proliferation. Due to the protein's extreme flexibility, the rational design of small cadherin homo-dimerization inhibitors is difficult. We determined the crystal structure of an E-cadherin extracellular fragment in complex with a peptidomimetic compound (FR159) that partially inhibits cadherin homophilic adhesion. The structure revealed an unexpected binding mode and allowed the identification of a druggable cadherin interface. Based on this crystal structure, we identified small molecule inhibitors (AS9 and AS11) that specifically modulate E-cadherin-mediated cell-cell adhesion at micromolar concentrations. We also determined the crystal structures of the complex between E-cadherin and three different small molecule inhibitors identified *via* a FBDD approach. These three fragments bind to a different pocket of the protein also at micromolar concentrations. The two sets of inhibitors appear to be able to act synergistically.

Systems biology-derived network treatments for traumatic brain injury and post-traumatic epilepsy – lessons learned

Asla Pitkänen

University of Eastern Finland, Kuopio, Finland

Approximately 70 million individuals are estimated to suffer a TBI each year. TBIs account for about 10% of acquired structural epilepsy. The risk of post-traumatic epilepsy (PTE) increases as the severity of the TBI increases. Despite over 20 favorable preclinical proof-of-concept trials investigating more than a dozen different interventions in animal models of PTE, there are currently no available treatments for patients at risk of epileptogenesis after TBI. We have developed a pipeline for the discovery of transcriptomics-derived disease-modifying therapies and used it to validate treatments *in vitro* and *in vivo* that could be repurposed for TBI treatment. In the first step, we apply *in silico* LINCS analysis to identify candidate treatments modulating the TBI-induced transcriptomics networks. Second, we test the "top hits" in neuron-BV2 microglial co-cultures, using tumour necrosis factor α as a monitoring biomarker for neuroinflammation, nitrite for nitric oxide-mediated neurotoxicity and microtubule associated protein 2-based immunostaining for neuronal survival. Then, based on (a) therapeutic time window *in silico*, (b) blood-brain barrier penetration and water solubility and (c) anti-inflammatory and neuroprotective effects *in vitro,* we choose the test compounds for validation in a lateral fluid-percussion model of TBI in rats. The *in vivo* phase is critical to detect favorable anti-epileptogenic and disease-modifying effects, but also to reveal adverse events and unexpected functional impairments in behavioral tests or electroencephalogram. Our pipeline provides a rational stepwise procedure for evaluating favorable and unfavorable effects of systems-biology discovered compounds that modulate post-TBI transcriptomics.

Medicines for millions of patients

David Rees

Astex, Cambridge UK
david.rees@astx.com

In this lecture I share personal anecdotes from three drug discovery projects, sugammadex an anaesthetic reversal agent from Organon Scotland, and ribociclib and erdafitinib, both oncology drugs arising from Astex UK collaborations with Novartis and Janssen respectively. These drugs have been used to treat millions of patients. The learnings from this research highlight innovation, teamwork, and collaborations.

Astex has pioneered fragment-based drug discovery (FBDD) screening ultra-small compounds, which means a super high hit rate of low affinity but ligand efficient binders. Elaboration into much higher affinity, selective leads requires design based on hundreds of X-ray crystal structures and 'hand crafted' chemical synthesis to incorporate additional protein binding groups.

Sugammadex utilises chemical chelation as a novel concept to reverse the anaesthetic effects of Organon's steroid-derived neuromuscular blocking (NMB) drugs.

Teamworking within departments and across disciplines runs through these projects and kept them alive during the dark times when progress was stalled. In addition to teamworking, Astex has been fortunate to have had research collaborations with different organisations, all internationally leading in their own areas.

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MedChem-relevant *gem*-difluorocycloalkane building blocks and their impact on physicochemical properties

Ryabukhin S. V.[1–3]*, **Melnykov K. P.**[1,2], **Holovach S.**[1,3], **Herasymchuk M.**[1,2], **Volochnyuk D. M.**[1–3], **Grygorenko O.O.**[1,2]

[1] Enamine Ltd, 23 Matrosova str., 01103, Kyiv, Ukraine
[2] National Taras Shevchenko University of Kyiv, 60 Volodymyrska str., 01601, Kyiv, Ukraine
[3] Institute of Organic Chemistry of NASU, 5 Murmanska str., Kyiv, Ukraine
s.v.ryabukhin@gmail.com

Fluorinated cycloalkane building blocks are useful structural motifs that become increasingly important in various areas, and most of all in drug discovery and agrochemistry. Recently, we elaborated chemical routes to the compounds types **1**–**11**, which can be promising for the further industrial scale-up. These investigations, as well as pioneering lab-scale approaches to the C7 **1**–**3** types compounds and cyclobutanes type **10** building blocks, will be discussed in the report.

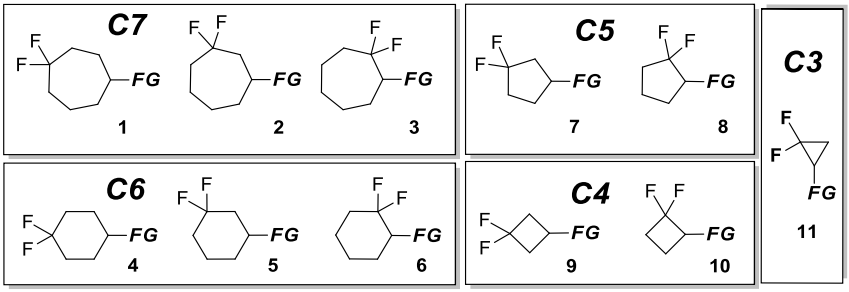

Figure 1.

Also, the effect of *gem*-diflurination on acidity/basicity (pK_a), lipophilicity (LogP), aqueous solubility (S_w), and metabolic stability (intrinsic clearance, CL_{int}) of functionalized C3–C7-cycloalkanes is established and compared to those of non-fluorinated and acyclic counterparts. All these investigations will be discussed in the presentation.

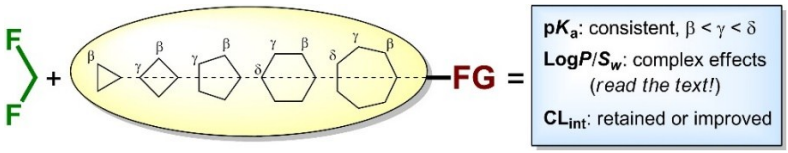

Figure 2.

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Medicinal plants in Latvia: traditional knowledge and future perspectives

Inga Sile[1,2], Valerija Krizhanovska[1,2], Arta Kronberga[3], Ilva Nakurte[4], Ieva Mezaka[4], Osvalds Pugovics[1], Solveiga Grinberga[1], Maija Dambrova[1,2]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
inga.sile@farm.osi.lv (I.S.), valerija@osi.lv (V.K.), osvalds@osi.lv (O.P.),
solveiga@osi.lv (S.G.), maija.dambrova@farm.osi.lv (M.D)
[2] Riga Stradins University, Latvia
[3] Field and Forest, SIA, Cēsis County, Latvia
arta.kronberga@fieldandforest.lv (A.K.)
[4] Institute for Environmental Solutions, Cēsis County, Latvia
ilva.nakurte@vri.lv (I.N.), ieva.mezaka@vri.lv (I.M.)

In recent years, interest in natural bioactive compounds has increased considerably, especially in the food, cosmetic, and pharmaceutical industries. Documentation of historical knowledge on the use of medicinal plants inspires new ideas for the practical applications. Our aim was to analyze traditional knowledge on the past use of medicinal plants and to evaluate the potential for the domestication of nine species of medicinal and aromatic plants (MAP): *Primula veris*, *Galium odoratum*, *Daphne mezereum*, *Tussilago farfara*, *Pulsatilla pratensis*, *Convallaria majalis*, *Glechoma hederacea*, *Chaledonium majus* and *Alchemilla* spp.

Data on plant species and their applications were collected from the records of Latvian folk medicine, the Archives of Latvian Folklore. Phytochemical analysis of selected plant extracts was carried out using liquid chromatography-mass spectrometry techniques to compare the chemical composition between populations growing in the wild and cultivated conditions. The impact of growing conditions on the biological activity of plant material was assessed using various *in vitro* and *ex vivo* methods.

In folklore materials, 211 plant taxa were identified. The selected MAP species were mainly mentioned to treat pain and cough, while *C. majus* was mentioned as a treatment for various skin diseases. Changes in the chemical composition of selected MAP species were observed between wild and cultivated samples. For example, the total content of alkaloids in extracts prepared from cultivated *C. majus* was significantly higher than that of wild samples. The composition of cultivated *G. hederacea* extracts was found to depend on the stage of plant development; however, the main factor that affected the chemical composition and biological activity was the accession rather than the place of growth. The extracts of *C. majus* and *D. mezereum* possess selective toxicity to different types of cancer cells.

As a result, the data obtained allow us to make decisions about potential of commercial growing and select the most promising populations for domestication.

Acknowledgements
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Pharmacokinetics, biodistribution and passive tumor accumulation of non-opsonizing nanoparticles

Antons Sizovs[1,2]*, Rinalds Serzants[1,2,3], Baiba Svalbe[1,2], Irina Cesnokova[1,2], Gundega Stelfa[1,2,4]

[1] Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia
[2] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia
[3] Faculty of Pharmacy, Riga Stradins University, Riga, Latvia
[4] Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia
antons.sizovs@farm.osi.lv

Nanoparticles (NPs) have been extensively investigated for cancer therapy improvements, such as the reduction of systemic toxicity or the enhancement of drug accumulation in tumors as well as cancer diagnostics. The meta-analysis of data published over the last decade, however, revealed that the median efficiency of nanoparticles delivery to tumor sites is under 1%; additionally, NP disposition within the tumor is limited to periphery and NPs only reach 2% of cancerous cells. These problems can be largely attributed to rapid non-specific opsonization of NP resulting in formation of a protein corona which leads to poor pharmacokinetic properties and has lead to many ambiguous conclusions regarding delivery efficiency dependence on physiochemical properties of NP. We have developed a series of non-opsonizing polymers and nanoparticles with various sizes and used them to determine pharmacokinetics, biodistribution and tumor accumulation efficiency dependence on NP size in mice. Our data demonstrates that non-targeted NP accumulation in tumors is not a rapid process and long circulation times are essential to maximize it. Furthermore, tumor selectivity increases with a decrease in nanoparticle size and reaches maximum for NPs under 20 nm. We will present our findings and discuss how developed materials will be used to further nanomedicine.

Acknowledgements
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Exploring uncharted pharmacological space to uncover drug targets of the future

Sven Marcel Stefan[1,2]*, Katja Stefan[1], Jens Pahnke[1–3], Vigneshwaran Namasivayam[2,4]

[1] Department of Pathology, University of Oslo, Norway
[2] LIED, University of Lübeck, Germany
[3] Department of Pharmacology, University of Latvia, Riga, Latvia
[4] Department of Pharmaceutical and Cellbiological Chemistry, University of Bonn, Germany
s.m.stefan@medisin.uio.no

The discovery of structurally distinctive pharmacological agents with innovative mode of action is the major obstacle in medicinal chemistry today. Unfortunately, the pharmacological space is mostly uncharted, leaving numerous pharmacological targets undruggable. Polypharmacology holds the key to unlock under-explored target structures as potential pharmacological targets of the future. The recently released novel drug discovery tool 'Computer-aided Pattern Analysis' ('C@PA')[1,2,3] linked novel drug discovery with the exploration of yet undruggable pharmacological targets. C@PA identified conserved substructural features of bioactive agents, such as (non)aromatic poly-(hetero)cycles and functional groups, which establish polypharmacology (Figure 1). This discovery provided a new perspective in the development of structurally novel bioactive agents addressing a yet completely uncharted territory of pharmacological space.

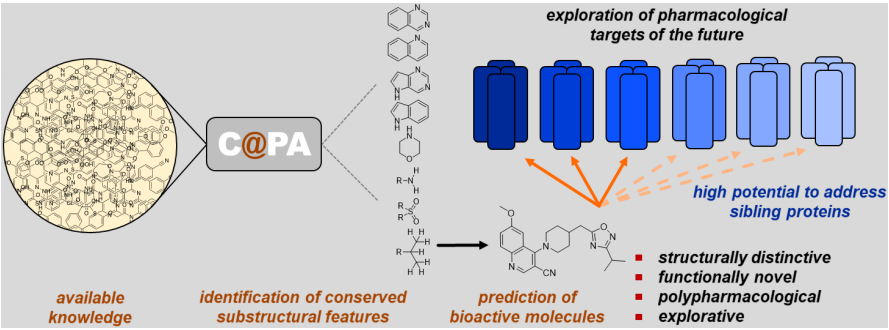

Figure 1. Schematic workflow of Computer-aided Pattern Analysis (C@PA).

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Precision targeting of drugs and nanoparticles with homing peptides

Tambet Teesalu

Laboratory of Precision and Nanomedicine, Institute of Biomedicine and Translational Medicine, Centre of Excellence for Translational Medicine, University of Tartu, Ravila 14b, 50411 Tartu, Estonia
Materials Department, Molecular, Cellular, and Developmental Biology Department, Physics Department, and Biomolecular Science and Engineering Program, University of California, Santa Barbara, California 93106, USA
tambet.teesalu@ut.ee

I will give a summary of our work on application of systemic homing peptides for precision delivery of drugs, imaging agents and nanoparticles in the context of cancer and other diseases.

Our laboratory is uses *in vivo* peptide phage display screens to identify homing peptides that bind to specific targets in the vasculature. Corresponding synthetic peptides are explored for targeting drugs, biologicals, and nanoparticles into tumors to increase their therapeutic index. I will discuss our preclinical and clinical efforts on development of peptide-guided diagnostic agents and therapeutics for the early detection and precision treatment of solid tumors (glioblastoma, and breast, prostate, ovarian, prostate, and colorectal carcinoma), and for treatment of neurological diseases.

Ancient survivors – conifer polyprenol studies for modern applications

Ilona Vanaga, Uģis Klētnieks, Baiba Jansone, Ruta Muceniece

Faculty of Medicine, University of Latvia
ilona.vanaga@lu.lv

Latvian pine (*Pinus sylvestris*) and spruce (*Picea abies*) tree needles contain unique natural substances that enable their sustainability during extreme weather conditions and can be utilized for the health benefits of humans.

Polyprenols are compounds derived from conifers that contain polyprenyl units with a hydroxyl group at the end of the chain[1] (Fig. 1). Since the 1980s polyprenols have been studied in pre-clinical studies mostly for gastroenterological effects, but nowadays, as research on polyprenols continues, their properties and potential applications have also been investigated *in vivo* against statin-induced myopathies[2,3] and in amateur athletes.[4]

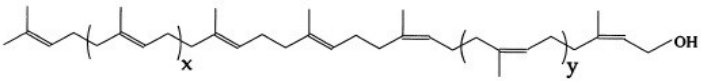

Figure 1. General formula of polyprenols.

Polyprenols as free substances have limited bioavailability due to high hydrophobicity and poor emulsification in the digestive tract. By reason of these limitations novel forms need to be developed to alter polyprenol biodisposition for a markedly improved delivery. Polyprenol liposomes[5] in soft gelatine capsules and cosmetic microemulsions have been prepared and studied in humans. An overview of the research results and various polyprenol applications are presented.

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Amino acids derived diazoketones – shelf stable reagents for heterocyclizations

Volochnyuk D. M.[1–3]*, Pendiukh V. V.[1,2], Yakovleva H. V.[1], Pashenko A. E.[1–3], Rozhenko A. B.[2], Ryabukhin S. V.[1–3]

[1] Enamine Ltd, 78 Chervonotkatska str., Kyiv, 02094, Ukraine
[2] Institute of Organic Chemistry, National Academy of Sciences of Ukraine, 5 Murmanska str., Kyiv, Ukraine
[3] National Taras Shevchenko University of Kyiv, 60 Volodymyrska str., Kyiv, Ukraine
d.volochnyuk@gmail.com.

α-Amino acids derived diazoketones (AADDK) are an important class of reagents in organic synthesis. Using natural proteinogenic α-amino acids as starting material leads to easy excess to chiral AADDK. Therefore the reagents could be considered as one of the instruments for chiral pool α-amino acids synthesis. In this research, we have elaborated the semi-industrial methodology for generating a solution of diazomethane in the organic phase in the flow reactor. Using the reactor an efficient and practical flow procedure for Boc-protected aminodiazaketones was developed After investigation of the thermal properties, as well as the shelf-life time of diazoketones, it was claimed that the compounds are good "shelf-keeping" reagents for the medicinal chemist.

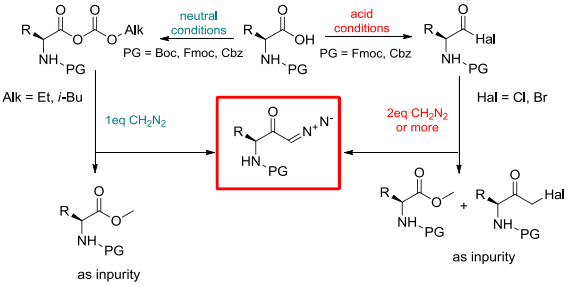
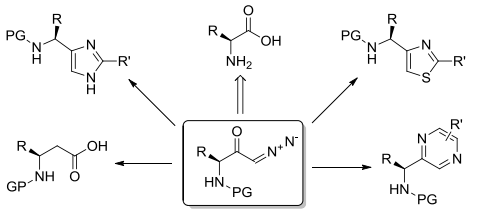


Figure 1.

The utility of diazoketones was demonstrated by their utilisation as haloketone's surrogates in different heterocyclisations to thiazoles, imidazoles, pyrazines, β-aminoacids etc.



Scheme 1.

Acknowledgements
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Multivalent approach to targeting carbonic anhydrases

Prof. **Jean-Yves Winum**

University of Montpellier, Pôle Chimie Balard Recherche
IBMM - UMR 5247 - 1919, route de Mende
34293 MONTPELLIER cedex 5, France

The zinc metalloenzymes carbonic anhydrases (CAs, EC 4.2.1.1) are considered as drug targets for several pathologies, and different inhibitors found clinical applications as diuretics, antiglaucoma agents, anticonvulsants, and anticancer agents/diagnostic tools. Their main drawback is related to the lack of isoform selectivity leading to serious side effects for all pathologies in which they are employed. Even if the potency of carbonic anhydrase inhibitors has been greatly improved in the last years, with inhibitors reaching inhibition constants in the femtomolar range, the selectivity issue remains important.

In this presentation, the multivalent approach to targeting carbonic anhydrases will be discussed. This new strategy may open new opportunities in the drug design of innovative isoform-selective carbonic anhydrases inhibitors with potential applications in cancer.

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Catalytic enantioselective 1,2-rearrangement

Jieping Zhu

Laboratory of Synthesis and Natural Products, Institute of Chemical Sciences and Engineering,
Ecole Polytechnique Fédérale de Lausanne, EPFL-SB-ISIC-LSPN, Switzerland
Jieping.zhu@epfl.ch

Rearrangement reaction is a broad class of organic transformation involving the migration of an atom or a group from one center (migration origin) to another (migration terminus) within the same molecule.[1] Such bond reorganization process affords a structural isomer of the original substrate allowing, in many cases, construction of the molecular frameworks not easily accessible by other approaches. The peculiarity of some representative 1,2-anionotropic rearrangements is that they could involve either a carbon cation intermediate or are reversible rendering the development of catalytic enantioselective version challenging. In this talk we will present our recent work on the development of catalytic enantioselective 1,2-anionotropic rearrangements and their applications in the synthesis of natural products as well as bioactive compounds.[2] A novel type of 1,2-dyotropic rearrangement involving Pd(IV) as one of the migrating groups and its potential implication in reaction design will also be discussed.[3]

Acknowledgements
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Metabolomic signature of Sigma-1 receptor knock-out mice

Liga Zvejniece[1], Baiba Svalbe[1], Gundega Stelfa[1,2], Baiba Zvejniece[1,3], Edijs Vavers[1,4], Maija Dambrova[1,4]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
[2] Latvia University of Life Sciences and Technologies, Jelgava, Latvia
[3] University of Latvia, Riga, Latvia
[4] Riga Stradins University, Riga, Latvia
liga@farm.osi.lv

The Sigma-1 receptor (Sig1R) has been intensively studied in the context of neurodegenerative and psychiatric diseases for several decades. In recent years the role of Sig1R in the pathogenesis and therapy of cardiometabolic, cancer and liver diseases has started to emerge. Metabolomics affords detailed characterization of metabolic phenotype and derangements that underlie diseases and helps to discover new therapeutic targets and biomarkers. Therefore, the aim of the present study was to assess the metabolomic profile of Sig1R knockout (KO) mice in plasma and brain tissue samples in order to further characterize the role of this receptor.

In the final data set, hierarchical clustering analysis showed clear plasma sample clustering between adult (6 months old) Sig1R KO and wild-type (WT) mice but not old animals (18 months old). Adult Sig1R KO mice demonstrated significantly decreased concentration of 70 and increased concentration of 12 metabolites in the blood plasma. Sig1R KO mice had a significantly decreased concentration of amino acids (15 out of 20) both essential and nonessential, short-chain acylcarnitines, lysophosphatidylcholines, phosphatidyl-cholines, sphingomyelins, ceramides, hexosyl-ceramides and significantly higher levels of histamine, anserine, carnosine in the blood plasma. The principal component analysis showed that deletion of Sig1R has more affected metabolites in blood plasma than in the brain at both ages. We identified 35 affected metabolic pathways in adult plasma from Sig1R KO mice. The identified pathways are associated with pathogenesis of different types of seizures, cardiovascular and lipid metabolism disorders.

In conclusion, we found for the first time that the Sig1R function is important for biosynthetic pathways of amino acids. Moreover, our findings suggest that Sig1R is involved not only in the regulation of central nervous system processes like depression, seizure, memory, but also cardiovascular function and lipid metabolism.

Acknowledgements
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4-Cyanamidobenzenesulfonamide derivatives: potentional neuropathic pain attenuating agents

Morteza Abdoli[1], **Raivis Žalubovskis**[2]

[1] Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia
[2] Latvian Institute of Organic Synthesis, Riga, Latvia
mortezaabdoli1987@gmail.com

Owing to the lack of specific medicines to management of neuropathic pain, much attention has been paid to the discovery of therapeutic compounds to prevention and treatment of this pain.[1] In this regards, carbonic anhydrases (CAs) have recently been identified as novel therapeutic targets for neuropathic pain syndrome.[2] Along this line and in continuation of our works in the development of selective CA inhibitors (CAIs),[3,4] herein we report for the first time an efficient synthetic strategy for the preparation of 4-cyanamidobenzenesulfonamide derivatives as a new class of isomer-selective inhibitors of carbonic anhydrase. The compounds were tested for the inhibition of brain-associated hCA VII isoform and three other cytosolic isoforms hCA I, II, and XIII.

Studies using the stopped-flow technique demonstrated that among those four investigated isoforms, hCA VII was the most susceptible to inhibition with the newly prepared sulfonamides, which showed K_{Is} ranging between 1.3 and 30.6 nM. Except, unsubstituted 4-cyanamidobenzenesulfonamide and derivatives substituted by short aliphatic chains, all other substitution patterns present in these compounds lead to highly effective hCA VII inhibitors. Among the investigated compounds, 4-[*N*-(4-bromobenzyl)-cyanamido]benzenesulfonamide seems to be the most selective hCA VII inhibitor, with K_{Is} of 2.4 nM. Interestingly, this compound showed 1.6, 7.8, and 3.4 folds more selective inhibitory activity against CA VII than hCA I, hCA II, and hCA XIII isoforms, respectively, compared to the standard drug (acetazolamide; AAZ). Therefore, this class of compounds may be considered as interesting starting points for the development of novel anti-neuropathic pain agents.

Acknowledgements
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Mechanism of PAP248-286 amyloid formation: an insight from molecular dynamics simulations

Nikhil Agrawal[1], Emilio Parisini[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, LV 1006, Riga, Latvia
nikhil.agrawal@osi.lv

HIV has infected more than 75 million people since its inception and, so far, has claimed the lives of more than 36 million people worldwide. The one very crucial natural factor that has been identified to play a key role in HIV transmission is fragments of the prostatic acidic phosphatase (PAP) protein. The PAP248-286 peptides aggregate and form amyloid fibrils termed Semen-derived enhancers of viral infection (SEVI) that capture HIV particles and strongly enhance the number of productively infected cells by promoting virion-cell attachment and fusion. To investigate the aggregation of PAP2482-86 peptides we have employed molecular dynamics simulations. Our data showed that PAP248-286 peptides aggregate to form both structured oligomers, i.e. containing a cross β –sheet structure, and amorphous aggregates, i.e. featuring an irregular structure. Overall, the study elucidates aggregation of PAP248-286 at the atomic level and opens new avenues for structure-based drug design against PAP248-286 peptides.[1]

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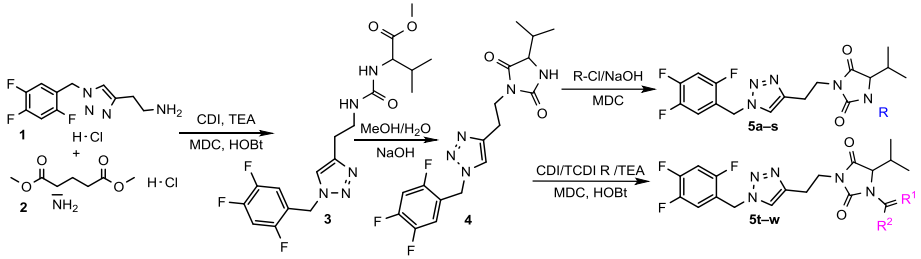
Trifluorobenzylimidazolidine-based DNA gyrase inhibitors for microbial infections

Manikandan Alagumuthu[1]

[1] Dept. of Microbiology, M S Ramaiah College of Arts, Science and Commerce, Bangalore-54, India
mailtomicromani@gmail.com

Up-surging challenge on the critical microbial resistance wants global attention.[1] We report a new class of trifluorobenzylimidazolidine-based DNA gyrase inhibitors evaluated as antimicrobial agents. Molecules **5a**–**s** substituted with R-Cl displayed an enhanced antibacterial activity and decreased hemolytic activity. On the other hand, a higher increment in antibacterial activity resulting in better selective antibacterial compounds **5t**–**w** materialized with R^1 and R^2 substitution (Scheme 1). The optimized lead compounds derived from SAR studies (structure–activity relationship) were **5g**,**h**,**p**,**s**,**v**. These compounds were active against various Gram-positive and Gram-negative bacteria at a low concentration (MIC ranged between 2.8–4.6 µg/ml) and displayed low toxicity toward mammalian cells (average HC_{50} = 902 µg/ml and average EC_{50} against HEK = 75 µg/ml). The maximum relative DNA gyrase inhibitory potential was 98.15 ± 2.18 with a lowest IC_{50} of 0.024 µg/ml which was nearly 100 times less than the lowest MIC obtained in this study. Furthermore, compounds **5h**,**s** were able to kill metabolically sedentary bacterial cells and eliminate pre-formed biofilms of MRSA. These compounds showed exceptional activity in a mouse model of skin infection with an average reduction of~5 log MRSA burden at 45 mg/kg dose without any sign of skin toxicity even at 225 mg/kg. Most prominently, they exposed potent efficacy in an *ex vivo* model of human skin infection (with a reduction of average 96.25 ± 1.28% MRSA burden at 40 µg/ml), which designates boundless potential of these compounds as effective antibacterial agents to treat skin infections.



Scheme 1. Route of synthesis of trifluorobenzylimidazolidine derivatives. Where, R = alkyl, aryl, alkysulfonyl, arylsulfonyl, alkyl carbonyl, Alkyl carbonate, ary carbonate and aryl carbonyl. Where, R^1 = oxygen or sulfur; R^2 = alkyl amine and substituted aryl amines.

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Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors

Anastasija Balašova[1,2], **Raivis Žalubovskis**[1,2]

[1] Latvian Institute of Organic Synthesis, 21 Aizkraukles St., Riga LV-1006, Latvia
[2] Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, 3/7 Paula Valdena St., Riga LV-1048, Latvia
balasova@osi.lv, raivis@osi.lv

Carbonic anhydrases (CAs) are a superfamily of metalloenzymes present across all kingdoms of life, as they catalyze reversible carbon dioxide hydration.[1] Inhibition of the CAs has pharmacological applications in many fields, such as anticancer agents, antiglaucoma, diuretics, antibacterial, anti-infectives, and many more.

Previously, in our research group, benzoxathiepine 2,2-dioxides **1** were designed and synthesized.[2] They demonstrated good inhibitory activity and selectivity of tumor-associated hCA IX and hCA XII. Extending our research, we decided to synthesize potential benzoxathiepine 2,2-dioxide **1** bioisosteres – benzoxaphosphepine 2-oxides **2**.

To better understand the structure–activity relationship, benzoxaphosphepine 2-oxide aryl derivatives **3** were synthesized using Suzuki–Miyaura cross-coupling. A series of benzoxaphosphepine 2-oxide acylamino derivatives **4** were synthesized to extend compound library.

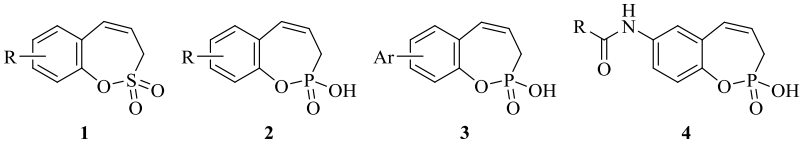

Figure 1. Structures of compounds **1**–**4**.

Acknowledgements
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Enzyme engineering of fructosyl peptide oxidase to widen its active site access tunnel and improve its thermal stability

Hajar (Nika) Estiri[1], Shapla Bhattacharya[1], Jhon Alex Buitrago[1], Linda Legzdiņa[1], Alfonso Gautieri[2], Emilio Parisini[1,2]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, Latvia
[2] Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133, Milano, Italy
emilio.parisini@osi.lv, alfonso.gautieri@polimi.it, shapla1502@outlook.com

Enzyme engineering is a tailoring process that allows the modification of naturally occurring enzymes by introducing partial modifications to their sequence and to their structural features to provide them with improved catalytic efficiency, stability, or specificity. Fructosyl peptide oxidases (FPOX), are flavoproteins that catalyse the oxidation of fructosyl amino acids to form glucosone, amino acid, and hydrogen peroxide. These enzymes find application in the management of diabetes, and specifically in the detection of glycated haemoglobin (HbA1c). However, naturally occurring FPOX are not able to detect HbA1c directly because these enzymes show no significant activity on intact proteins due to the buried active site and to the narrow tunnel that provides access to their catalytic pocket.

The aim of the project is to expand FPOX substrate range and thermal stability *via* rational design. In particular, our goals are 1) to design mutants that show a significantly wider and shorter access tunnel, relative to the wild-type (WT) enzyme and 2) to improve the thermal stability of the wild-type enzyme by generating variants that feature salt bridges, improved RMSF or disulphide bonds.

To this end, we applied a rational design approach whereby, using a computational filter involving both Rosetta design and molecular dynamics simulations, we screened a large library of possible candidate mutants. Upon *in silico* selection of the candidates with the best score, model validation was carried out in the lab, which involved the production of soluble proteins and their biophysical characterization. Validation was done using a combination of biophysical analyses and structural characterization *via* X-ray crystallography, which allows the acquisition of new sets of coordinates that may provide new starting points for further rounds of molecular engineering.

The most promising candidate genes were synthesized, subcloned into pET 15(b) or pET 17(b) vectors and BL21 star (DE3) and Shuffle T7 host cells for protein expression. Biophysical studies (thermal shift assay and circular dichroism) were used to measure the thermostability of enzymes. X-ray crystallography was used to determine the structure of the different variants. Enzymatic activity assays were used to evaluate the activity of new the enzymes toward different substrates.

The results indicate that our engineered enzymes, which feature either newly introduced disulphide bonds, salt bridges, or an improved RMSF, have better thermal stability than the natural enzyme. Biophysical characterization by thermal shift assay and circular dichroism show an increase in Tm to 60°C for the FPOX enzyme variants X02 and nX02 (leading to an increase of 8°C compared to the natural enzyme). Structural analysis of the obtained crystals (nX02, DO2, XO2, XO4, XO7) confirms the improved RMSF and the disulfide bond in these mutants. Enzymatic assays carried out on thermally stabilized enzymes show that one of the new variants (nX20) features nearly the same activity as the natural enzyme. We are currently investigating the activity of the nX02 variant toward larger substrates.

Acknowledgments
We thank the ERDF project BioDrug (No. 1.1.1.5/19/A/004) and the Latvian Council of Science (grant No. lzp-2020/2-0013 to E.P.) for financial support.

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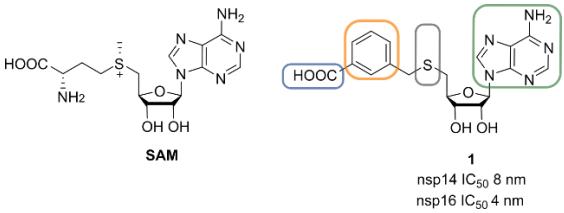
Design and synthesis of 3-(adenosylthio)methyl benzoic acid derivatives inhibiting SARS-CoV-2 methyltransferases

Olga Bobiļeva[1], Raitis Bobrovs[1], Evelīna Elva Sirmā[1], Iveta Kaņepe[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, Rīga LV-1006, Latvia
olga_bobileva@osi.lv

Coronaviruses have mechanisms to protect their genome. A series of self-encoded nonstructural proteins (NSP) construct 5'-end RNA cap structure which consists of 7-methylguanosine connected to the nucleoside of the RNA through a triphosphate bridge and a methylated 2'-*O*-group of the first nucleotide. The cap structure ensures efficient translation and replication of the virus and protects viral RNA from antiviral response of the host cell. SARS-CoV-2 methyltransferases NSP14 and NSP16 in complex with NSP10 employ S-adenosyl-methionine (SAM) as a methyl donor to methylate the cap structure. Capping enzymes are considered to be valid antiviral targets, as their inhibition induces antiviral response.[1]

Recently we have discovered nanomolar SARS-CoV-2 methyltransferases inhibitor 3-(adenosylthio)methyl benzoic acid (**1**) based on SAM methionine modifications.[2] In present work, we explore different scaffolds of structure **1** guided by computational docking. Carboxylic acid bioisosteric replacement, benzene moiety decoration with additional substituents, adenosine modifications, and sulfur replacement strategies were used for the compound design resulting in improved inhibitory activity and cell membrane permeability.



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Identification of 5-sulfosalicylic acids as threonyl-tRNA synthetase inhibitors

Raitis Bobrovs[1], Karīna Ermansone[1], Marija Skvorcova[1], Jekaterina Boļšakova[1], Larisa Varačeva[1], Aigars Jirgensons[1]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
raitis.bobrovs@osi.lv

Malaria remains one of the world's most devastating disease, with staggering social and economic consequences in tropical and subtropical areas. Malaria is caused by the *Plasmodium* parasite that is transferred by *Anopheles* mosquitos. While there are drugs available to treat the malaria, the spread of drug-resistant parasite strains drives the researchers to look for drugs with new way of action that can inhibit the resistant malaria parasite strains. Protein translation is a promising pathway to target; therefore, here we aim to identify threonyl-tRNA synthetase (ThrRS) inhibitors as a potential antimalarials.

Potential ThrRS inhibitors were identified *via* high throughput virtual screening (HTVS), where 16 compounds showed low micromolar activity (>50 μM). 5-phenyl-sulfamoylsalicylic acid (IC_{50} = 13.1 μM) was selected as a hit compound for further optimization due to synthetic accessibility and moderate ligand efficiency (LE = 0.34). Molecular docking suggested that salicylic acid group of the compound is interacting with a binding site coordinated zinc ion, whereas phenyl sulphonamide group is located in the ATP binding subsite. More than 100 hit analogues were synthesized or purchased from commercial vendors, and their activity was tested in enzymatic assay. Modifications at the salicylic acid functionality were not tolerated, and lead to inhibition potency decrease, indicating the essential role of this group. Modifications at the phenyl sulphonamide group, however, had only minor impact on the compound activity. Despite all our efforts, the original hit compound remained one of the most active compounds within this series, and no significant increase in binding potency was achieved.

Acknowledgements
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A donor-acceptor Stenhouse adduct active in neuronal $GABA_ARs$ and photoswitchable in water

Rossella Castagna[1,2], Galyna Maleeva[1,3], Carlo Matera[1,4], Pau Gorostiza[1,3,5]

[1] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Baldiri Reixac 10-12, 08028 Barcelona, Spain
[2] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga 1006, Latvia
[3] Network Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBERBBN), Spain
[4] Department of Pharmaceutical Sciences, University of Milan, 20133 Milan, Italy
[5] Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
rossella.castagna@osi.lv

Donor-acceptor Stenhouse adducts are a rapidly emerging class of photochromic compounds whose conformation can be efficiently switched using visible and near-infrared light. Over the last year, the switching mechanism of DASA has been extensively studied. However, the use of these compounds in water has been hampered by a spontaneous and irreversible conversion to a non photoactive form.

The difficulty of using DASAs in water is even more disappointing since they are native red absorbing photoswitching, quality that is widely appreciated in the field of photopharmacology. This field combines a pharmacological approach with the use of light to enable spatio-temporal control of biological processes and drug action. To date, photopharmacology has been extensively used to manipulate biological activity at the cellular level by targeting ion channels, G protein-coupled receptors, enzymes and protein –protein interactions.[1,2]

In order to reduce phototoxicity of light used in photopharmacology, low-energy light (i.e. red light) should be used (less scattering in tissue and deeper penetration in the body) and red-absorbing photodrugs need to be designed.

To this end, here, we present the molecular design of a novel γ-aminobutyric acid type A receptors ($GABA_ARs$) ligand derivative based on the DASA scaffold that displays photochromic properties with red light and is active in neuronal $GABA_ARs$.[3]

Acknowledgements
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Dendritic nanoparticles to enhance the antibacterial properties of lysozyme and endolysin against gram-negative bacteria

Kinga Skrzyniarz[1], Javier Sanchez-Nieves[2], Michał Arabski[1], Zuzana Drulis-Kawa[3], Karol Ciepluch[1]

[1] Division of Medical Biology, Jan Kochanowski University, Uniwersytecka Street 7, Kielce 25-406, Poland
karol.ciepluch@ujk.edu.pl
[2] Universidad de Alcalá (UAH), Dpto. de Química Orgánica y Química Inorgánica, Campus Universitario, E-28871 Alcalá de Henares, Madrid, Spain
[3] Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wrocław, Wrocław, Poland

Multidrug-resistant (MDR) pathogens are an established and growing worldwide public health problem. The MDR gram-positive and gram-negative bacteria with a broad spectrum of virulence factors cause serious damage to host tissues and efficiently evading the immune system response. The antibacterial agents currently in clinical development are mostly derivatives of well-established antibiotic and they are affected by pre-existing cross-resistance, which may reduce their efficiency in critical ill patients. Nowadays, several strategies have been studied to overcome multidrug resistance including bacteriophages, antimicrobial proteins (lysins), stimulators of immune system or membrane permeabilizator (e.g. antimicrobial peptides and nanoparticles). Antimicrobial proteins, like lysozymes produced by animals or bacteriophage lysins, enable the degradation of bacterial peptidoglycan (PG) and, consequently, lead to bacterial cell lysis. However, the activity of those enzymes is not satisfactory against gram-negative bacteria because of the presence of an outer membrane (OM) barrier. Therefore, the complexation of nanoparticles with phage-derived endolysin and lysozyme can improve their antibacterial properties against gram-negative bacteria. The nanoparticles can be complexed with endolysin or lysozyme, where nanoparticles act as permeabilizers of the bacterial outer membrane (OM) and thus can lead to strengthening bactericidal activity of antimicrobial protein responsible for the degradation of peptidoglycan PG. This helps to create a new tool to fight with multidrug resistance bacteria.

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Mass spectrometry proteomics analysis of ABCA7-deficient Alzheimer's disease mice reveal new diagnostic and treatment targets

Anna Maria Górska[1], Irene Santos[1], Jens Pahnke[1,2,3]

[1] Department of Pathology, Section of Neuropathology, www.pahnkelab.eu, University of Oslo and Oslo University Hospital, Oslo, Norway
[2] LIED, University of Lübeck, Lübeck, Germany
[3] Department of Pharmacology, Faculty of Medicine, University of Latvia, Rīga, Latvia
a.m.gorska@medisin.uio.no

Alzheimer's disease (AD) is the fifth leading cause of death for adults aged 65 years and older, and the sixth leading cause of death for all adults. In 2019 more than 55 million people worldwide were affected by AD and other forms of dementia, and according to the World Health Organization (WHO) the number is expected to rise to approximately 140 million by 2050. AD is currently defined on the basis of amyloid-β (Aβ) plaque and tau neurofibrillary tangle deposition within the neocortex, the biochemical and cellular changes in the brain remain incompletely understood. Mass spectrometry (MS)-based proteomics is a powerful technique, as it allows a simultaneous identification and quantification of proteins in complex biological samples such as brain tissue. Therefore, we used a well-established APP-transgenic mice model of AD (APPPS1-21) with increased human Aβ levels in the brain. We created a unique mouse model combining APPPS1-21 mice with ATP-binding cassette, sub-family A, member 7 (ABCA7)-deficient mice (*ABCA7*ko).

In 2010, a genome-wide association study (GWAS) identified ABCA7, as a novel risk gene of AD. Therefore, we wanted to characterize if the presence of functional ABCA7 and/or increased Aβ deposition influences the proteomic pattern in APPPS1-21 mice, and if the observed changes are associated with early and late stages of the disease. Targeted approaches aiming on disease mechanisms associated with the ABCA7 transporter could be a promising approach for drug and biomarker development.

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LC–MS/MS analysis of omega-3 polyunsaturated fatty acids and their acylcarnitines in blood plasma

Gukālova Baiba[1], Grīnberga Solveiga[1], Križanovska Valērija[1,2], Sevostjanovs Eduards[1], Dambrova Maija[1,2], Liepiņš Edgars[1]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
[2] Department of Pharmaceutical Chemistry, Riga Stradins University, Latvia
baiba.gukalova@osi.lv, solveiga@osi.lv, valerija@osi.lv, eduards@osi.lv, maija.dambrova@farm.osi.lv, ledgars@farm.osi.lv

The health benefits of omega-3 polyunsaturated fatty acids (PUFAs) are widely recognized in cardiometabolic disease management. Reduced risk of cardiovascular disease and type 2 diabetes, as well as other beneficial effects on health, are observed when dietary saturated FA are replaced with PUFAs.

The aim of this study was quantitative determination of PUFAs (free acids) and PUFAC (respective acylcarnitines) in mouse and healthy volunteer blood plasma using LC-MS/MS method.

Samples were collected from low-density lipoprotein receptor knock-out mice fed for 16 weeks PUFA-rich high fat diet to induce the development of atherosclerosis and healthy volunteers who received 10 ml of PUFA-rich fish oil for 5 days during the meal. A simple protein precipitation extraction (PPE) with ACN/MeOH (3:1, v/v) was used to extract plasma samples and the resulting extracts were analyzed using reversed phase UPLC–MS/MS. The mobile phase consisted of gradient elution of 0.1% formic acid in water and ACN at a flow rate at 0.4 ml/min. Separation was achieved on an Acquity UPLC BEH C18 column. Detection was performed in the multiple reaction monitoring (MRM) mode, using an electrospray ion source. Our method had a run time of 6 min for PUFAs and 8 min for PUFAC. The calibration curves were linear over a 98-fold concentration range, with correlation coefficients (R^2) greater than 0.997.

PUFA-rich diet significantly increased DHA and EPA (5 and 38 times, respectively), respective acylcarnitine levels (12 and 23 times, respectively) and prevented the development of atherosclerosis in mice. In healthy volunteers, PUFAC levels increased only 1.1–2.5 times (n=5). After 10 years of regular fish oil intake PUFAC levels (n=1) were 6 times higher than the baseline level of other volunteers.

PUFA intake induces a similar increase in blood plasma concentrations of PUFAs (free acids) and PUFAC (respective acylcarnitines). Novel methods of PUFAs and PUFAC quantitative determination could be used in clinical studies to validate PUFA and PUFAC plasma levels as promising markers of PUFA intake and cardiac content.

1,2,3-Benzoxathiazine 2,2-dioxides as inhibitors of tumor-related carbonic anhydrases IX and XII

Jekaterīna Ivanova[1], **Raivis Žalubovskis**[1,2]

[1] Latvian Institute of Organic Synthesis, 21 Aizkraukles St., LV-1006, Riga, Latvia
[2] Institute of Technology of Organic Chemistry, Riga Technical University, 3/7 Paula Valdena St., LV-1048, Riga, Latvia
katja@osi.lv

Carbonic anhydrases (CA) are zinc metalloenzymes that catalyze CO_2 conversion to bicarbonate anion (Figure 1).

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

Figure 1.

hCAs (human CAs) are involved in a plenty of physiological processes including respiration, digestion, pH regulation, etc.[1,2]

Fifteen different CA isoforms have been identified and characterized in human so far.[3] In fact, most efforts in the last few decades have been focused on the tumor-associated isoforms (hCA IX and XII) that were shown to possess an important role in hypoxic tumor physiopathology and thus validated as biomarkers and therapeutic targets for various cancer types. Their inhibition has been related to the reduction of primary tumor growth, inhibition of invasion and metastasis, and a reduction in the cancer stem cell population.[4]

1,2-Benzoxathiine 2,2-dioxides are reported as very active and selective CA IX and CA XII inhibitors.[5] Here we present the synthesis and biological evaluation of nitrogen containing analogues of 1,2-benzoxathiine 2,2-dioxides, namely derivatives of 1,2,3-benzoxathiazine 2,2-dioxide (Figure 2).

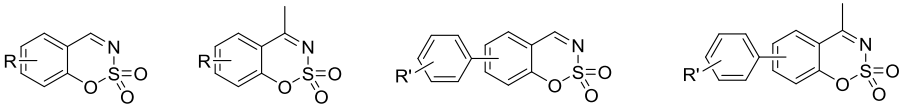

Figure 2.

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Hepatoprotective properties of natural substances versus well known drugs *in vitro* assays

Kaspars Jēkabsons[1], Jana Namniece[1], Annija Ušacka[1], Ruta Muceniece[1]

[1] Faculty of Medicine, University of Latvia, Riga, LV-1004, Latvia
kaspars.jekabsons@lu.lv

Non-alcoholic fatty liver disease (NAFLD) is a growing burden on the global health system and is estimated to affect approximately 25% of the population. Hepatocyte damages result in the death of hepatocytes and, consequently, the level of various liver enzymes and metabolites are altered, indicating the anomaly in metabolism and excretion of xenobiotics from the body. There are no specific medicines yet. Diet and active lifestyle are recommended. Plant phenolics have been studied extensively to provide scientific rationale behind their potential usage against various human ailments. There are a few effective herbal preparations like Liv-52, silymarin and essential phospholipids against hepatic complications. Until now, the main drugs for the treatment of NAFLD in clinics are lipid regulating agents such as statins; however, they have side effects and aggravate the deposition of lipids in the liver, leading to serious liver injury.[1]

We have studied the hepatoprotective/hepatotoxic effects of various drugs and bilberry genus berry pomace extracts on human liver HepG2 cells using *in vitro* assays of cell viability, lipid accumulation, oxidative stress, and acetylcholinesterase activity. HepG2 cells have a low metabolic capacity, so we assumed that the obtained results refer to the studied substances and not to their metabolites. Liver steatosis model was obtained by adding oleic and palmitic acids to the cell medium for the 24–48 h. All tested extracts and compounds at concentrations not influencing the cell viability were added simultaneously with lipids. The results of the study show that berry extracts, silymarin, metformin, simvastatin, atorvastatin, paracetamol, neostigmine and several other drugs reduce lipid accumulation in the cells. Additionally, we found that HepG2 cells are good model to assess acetylcholinesterase (AChE) activity and proved that known AChE inhibitors neostigmine, donepezil and galantamine, as well as the berry extracts and the silymarin inhibit AChE activity. The data support the recent hypothesis that AChE inhibitors may not only act on the CNS but also protect the liver from damage caused by other substances. Altogether our data suggest that natural substances can exhibit other effects in addition to their known antioxidant capacity as well as in clinics known drugs have multifaceted effects in the liver cells.

Acknowledgements
The study was supported by University of Latvia Foundation and Mikrotīkls Ltd. scientific grant No 2269.

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Plasmid identification and plasmid-mediated antimicrobial gene detection in Norwegian isolates

Ambreen Kauser[1], Ekaterina Avershina[1], Rafi Ahmed[1]

[1] Inland Norway University of Applied Sciences, Post box 400 2418 Elverum, Norway
ambreenkauser63@gmail.com

Antimicrobial resistance (AMR) is considered a potential threat to global health. Norway has had a low prevalence of resistant bacteria. But in recent years there has been an increase in resistant bacteria including *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Traditionally, clinical microbiology has used culture-based techniques to determine antimicrobial susceptibility and resistance profiles, but now whole genome sequencing for antibiotic susceptibility (WGS-AST) has emerged as a potential alternative.

We aimed to investigate the prevalence of antimicrobial resistance genes and plasmids in WGS of 111 clinical Norwegian isolates of *E. coli*, *K. pneumoniae*, and *A. baumannii*, to identify correlations between phenotypic and genotypic resistance in the isolates, which are related to antibiotic resistance to β-lactam, aminoglycosides, fluoroquinolone, trimethoprim, tetracycline, and phenicol.

The most occurring drug class was β-lactam antibiotic with TEM (38%) in *E.coli*, SHV (67%) in *K. pneumoniae*, and OXA (100%) and TEM (45%) gene families in *A. baumannii*. *In silico* detection of plasmids with *Brooks et al* database showed plasmid p2_000837 as prominent plasmid 12% *E.coli* isolates. There were four plasmids (pIB_NDM_1, p2_W5-6, pCHL5009T-102k-mcr3, pVir_020022) in 2% *K. pneumoniae* isolates which were also shared with *E. coli*. Only one plasmid (pHZ23-1-1) was confirmed in 9% of *A. baumannii* isolates. PLSDB detected Plasmid A and plasmid 4 with the maximum percentage in *E.coli* (10%) and *K. pneumoniae* isolates (4%). In *E. coli* and *K. pneumoniae*, the presence of incompatibility groups was observed; IncFIB (64 and 27%), Col156 (74 and 27%), IncFII (43 and 15%), while IncHI-1B(pNDM-MAR) (12%) were present only in *K. pneumoniae*.

A total of 75 isolates had resistance to the tested β-lactam antibiotics, out of which 63 had the corresponding resistance genes (ampC, SHV, CTX-M, TEM, LEN, OXA). Only 11 *E.coli* and one *K. pneumoniae* isolates were found to have resistance genes and the plasmids on the same node to confirm plasmid mediated resistance.

This study demonstrates the utility of WGS in defining resistance elements and highlights the diversity of resistance within the selected isolates to further the diagnostics and therapeutics for the treatment of the relevant infections.

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Development of lactam-based inhibitors of SARS-CoV-2 M^{pro}

Linda Kinena[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, LV-1006, Latvia
linda@osi.lv

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted an urgent need for the development of new antiviral drugs. The main protease (M^{pro}) of SARS-CoV-2 plays an important role in viral replication and has become an attractive drug target for virus inhibition. The active site of SARS-CoV-2 M^{pro} contains Cys145 and His41 which allows the use of structural subunits for covalent binding to the thiol of a cysteine residue ("warheads") in the active site of cysteine proteases.[1]

In our design of inhibitors considerably less chemically reactive and non-toxic lactams and their structural analogs were chosen as "warheads" for covalent inhibition of SARS-CoV-2 proteases M^{pro}. β- And γ-lactam subunit serves as the warhead for the covalent modification of cysteine proteases with the mechanism of action involving the cleavage of the β-lactam ring by cysteine residue in the active site of the enzyme (Figure 1).

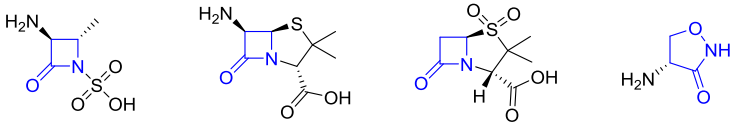

Figure 1. Representative examples of β- and γ-lactams used in the design of inhibitors.

For chosen β- and γ-lactams IC_{50} values for M^{pro} inhibition were determined using fluorescence resonance energy transfer (FRET) assay.

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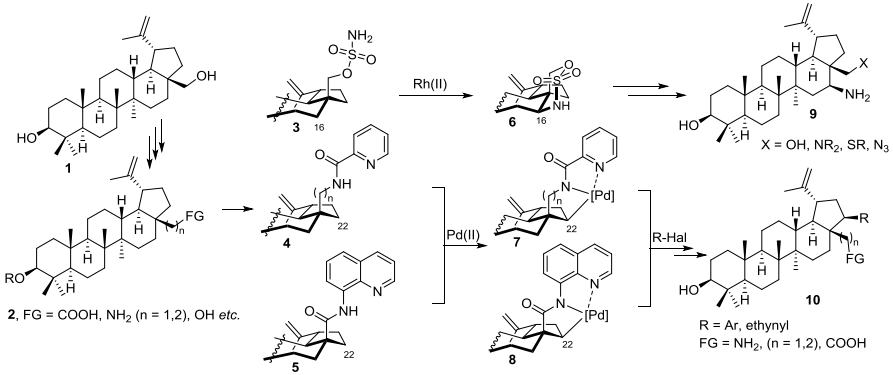
C–H activation of pentacyclic triterpenoids

Vladislavs Kroškins[1], Jevgeņija Luginina[1], Kristiāns Jankovičs[1], Māris Turks[1]

[1] Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, P. Valdena str. 3, Riga 1048, Latvia
vladislavs.kroskins@rtu.lv

Betulin **1** is pentacyclic triterpenoid natural product that is observed as secondary metabolite in more than 200 different types of plants. Betulin and its derivatives exhibit several important pharmacological properties such as antitumor, anti-inflammatory, antiparasitic, and antiviral activities.[1] The aim of this work is to obtain novel biologically active betulin derivatives by C–H functionalization at C(22) and C(16). For this purpose, precursors **3**, **4**, **5** bearing different directing groups were obtained.



8-Sulfamate ester **3** was used for *Du Bois* γ-C–H bond amination *via* formation of oxathiazinane **6**.[2] Intermediate **6** can be further converted into differently functionalized compounds **9** through the ring opening reactions.

8-Aminoquinoline amide **5** and picoline amides **4** were combined successfully with aryl halogenides and haloalkynes in the *Daugulis* C–H activation conditions.[3]

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Induction of pyroptosis to enhance antibacterial effect of antimicrobial proteins

Dorota Kuc-Ciepluch[1], Karol Ciepluch[1], Zuzanna Drulis-Kawa[2], Michał Arabski[1]

[1] Division of Medical Biology, Jan Kochanowski University, Uniwersytecka Street 7, Kielce 25-406, Poland
[2] Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wroclaw, Wroclaw, Poland
dorota.kuc.ciepluch@gmail.com

The increase in antibiotic resistance of Gram-negative bacteria leads to the search for new alternative strategies against these microorganisms. The important problem of drugs with clinic potential is the overcome the bacterial outer membrane by them. In this study, the permeabilization of the outer membrane during a natural process called pyroptosis was tested. Pyroptosis is an immune response of eukaryotic cells to bacterial cell components, mainly lipopolysaccharide (LPS) leading to the secretion of protein gasdermin D,[1,2] which might cause perforation of the bacterial cell membrane and would allow the influx of antibacterial agents as recombinant phage endolysin. This enzyme disrupts bacterial cells *via* the degradation of peptidoglycan.

The immune response of the THP1-Null2 cell line using different types of *Pseudomonas aeruginosa* LPS was tested. The studies showed that LPS induces pyroptosis cell death in monocytes, leads to gasdermin D cleavage, and release interleukins outside the cells. The level of induction strictly depends on the type of used LPSs. Next, collected supernatant was used to permeabilize the outer bacteria membrane of *Pseudomonas aeruginosa* strains to enhance the antimicrobial effect of lysozyme and phage-derived endolysin. Permeabilization of the bacterial membrane caused by gasdermin D allows antimicrobial proteins to reach the peptidoglycan, and as a result, inhibits the growth of bacteria. The knowledge gained can help to overcome the growing problem of multidrug resistant bacteria.

Acknowledgements
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X-ray structures of carbonic anhydrase IX and XII in complex with sulfonamide-based inhibitors

Janis Leitans[1], Andris Kazaks[1], Raivis Zalubovskis[2], Kaspars Tars[1]

[1] Latvian Biomedical Research and Study Center, Ratsupites 1, Riga, Latvia, LV 1067
[2] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, Latvia, LV 1006
janis.leitans@biomed.lu.lv

Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc-containing metalloenzymes which catalyze the reversible reaction of carbon dioxide and a water molecule forming bicarbonate ion and hydrogen ion. CAs are widespread in many different organisms and responsible for many vital processes in mammals. Some of the CAs are therapeutic targets, as their enzymatic activity is associated with many disorders.[1]

Human carbonic anhydrase isoforms IX and XII (hCA IX and hCA XII) are expressed in a limited number of normal tissues, while they are significantly overexpressed in many solid tumors.[2] Selective hCA IX and hCA XII inhibitors can be used to slow down tumor cell spread or as a biomarker for specific tumor types.[3]

Available structural data of the protein–inhibitor interactions are essential for rational drug design. We managed to co-crystallize hCA IX and hCA XII in complex with sulfonamide inhibitors, resulting in high-resolution X-ray structures. Data can be used for rational design of selective hCA IX and hCA XII inhibitors.

Acknowledgements
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Stereoselective olefination with sterically demanding Julia-Kocienski reagents: total synthesis of oxoprothracarcin, oxotomaymycin, and boseongazepine B

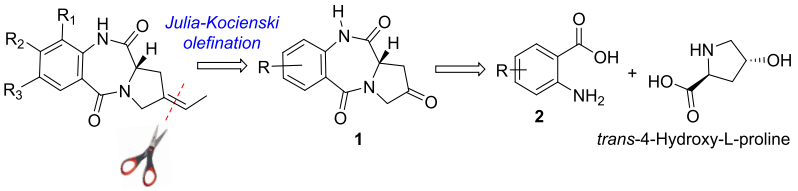
Zigmārs Leitis[1], Guna Sakaine[1], Gints Šmits[1]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia, LV-1006
zigmars@osi.lv

Pyrrolo[1,4]benzodiazepines (PBD) are a broad family of natural products possessing considerable anticancer activity owing to their ability to covalently bonding through N-2 of guanine in the minor groove of DNA.[1]

Several PBD members possess an *E*-configured C2 alkylidene group in the pyrrolidine ring, the configuration of which plays a crucial role in the cytotoxic properties of these compounds.[2] Although several total syntheses of these natural products have been published, a stereoselective introduction of the alkylidene substituent still possesses a considerable challenge. Within our preliminary studies,[3] we have shown that a late-stage olefination is a convenient approach to synthesize these natural products.

Herein we report our studies on the Julia–Kocienski olefination of N-unprotected PBD triones **1**, including the development of novel reagents, optimization of reaction conditions, and determining the olefination stereochemistry determining factors. The necessary triones **1** can be easily synthesized in 2 steps starting from readily available *trans*-4-hydroxy-L-proline and the corresponding anthranilic acids **2**.



Oxoprothracarcin (R_1 = R_2 = R_3 = H)
Oxotomaymycin (R_1 = H, R_2 = OH, R_3 = OMe)
Boseongazepine B (R_1 = OMe, R_2 = R_3 = H)

Scheme 1. Retrosynthetic analysis of PBD natural products.

Acknowledgements
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Synthesis of tetrazole fused pyrido-pyrimidines

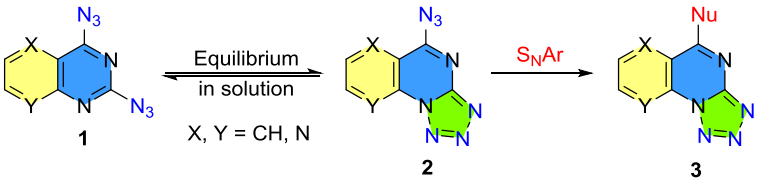
Kristaps Leškovskis[1], Irina Novosjolova[1], Māris Turks[1]

[1] Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, P. Valdena Str. 3, Riga, LV-1048, Latvia
kristaps.leskovskis@rtu.lv

Pyrimidine-fused heterocycles are privileged scaffolds that attract great interest due to their biological properties.[1] Modification and refinement of such scaffolds is a promising strategy for the development of novel drugs. Recently a new class of tetrazole-fused pyridopyrimidines have evaluated as antidepressants and epilepsy drugs.[2]

From synthesis perspective, heterocycles with azido-azomethine structural entity are interesting due to present dynamic azide tetrazole equilibrium in solution phase.[3] The equilibrium can be shifted towards one or other tautomer by altering ambient conditions such as solvent polarity and temperature. Thus, azide tetrazole ring–chain tautomerism is known to influence S_NAr reactivity and regioselectivity.[4]

Herein we describe efficient and straightforward synthesis method of fused tricyclic tetrazolopyridopyrimidines (Scheme 1). We discovered that diazido substrate **1** undergoes azide–tetrazole equilibrium which directs S_NAr to take place at the C-5 position displacing residual azide as a leaving group.



Scheme 1. Synthesis of tetrazolopyridopyrimidines.

Acknowledgements
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Substrate-based inhibitors of malarial subtilisin-like serine protease containing boronic acid warhead

Elina Lidumniece[1], Chrislaine Withers-Martinez[2], Michael J. Blackman[2], Aigars Jirgensons[1]

[1] Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia
[2] Malaria Biochemistry Laboratory, The Francis Crick Institute, London NW1 1AT, United Kingdom
elina.lidumniece@inbox.lv

Malarial subtilisin-like serine protease (SUB1) is a multifunctional processing protease with a significant role in egress of merozoites by activation of a cascade of proteolytic events (Figure 1), leading to rupture of human red blood cell (RBC).[1] Inhibition of SUB1 can prevent parasite replication and disease progression rendering this enzyme as an attractive drug target.

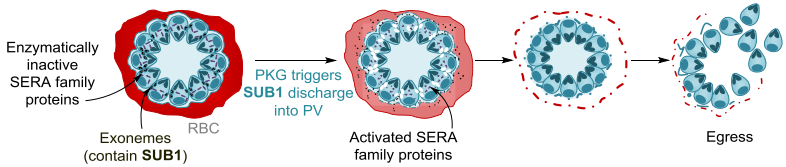
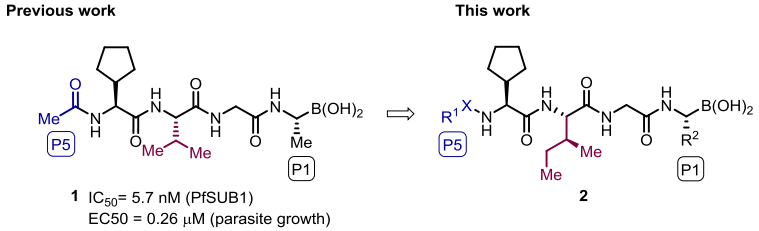


Figure 1. The merozoite egress from red blood cell.

Our previous research led to boronic acid based inhibitor **1** with nanomolar PfSUB1 inhibitory potency and remarkable inhibition of parasite egress in RBC assay[2] (Scheme 1). In this work we report the results of SAR studies of boronic acid based inhibitors **2** depending on the *N*-capping groups (P5 position) and the side chains at P1 position. The compounds were synthesised and evaluated for their PfSUB1 inhibitory activity compared to the parent inhibitor **1** (Scheme 1).



Scheme 1. Development of peptidic boronic acid containing inhibitors.

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Starvation pretreatment affects results of chemical screening when using auxotrophic budding yeast *Saccharomyces cerevisiae*

Zile A.[1], Ozolina Z.[1], Liepins J.[1]

[1] Institute of Microbiology and Biotechnology, University of Latvia
janis.liepins@lu.lv

Many biochemical processes and regulations are well preserved across eukaryotes – from fungi to mammals. Budding yeast *Saccharomyces cerevisiae* is a popular molecular model of eukaryotic cells, also widely used for screenings and humanized. For example, purine synthesis or glycolysis pathway in *S. cerevisiae* can be functionally replaced by human analogues.[1,2]

S. cerevisiae is often used as a primary screen to test chemical–genetic interactions of the compound library. Typically, stationary phase cells or exponentially growing cells are used in these screens. However, cell reaction to the given compounds does not depend only on its chemical structure and gene of interest, but also on physiological status of the yeast cells which might also depend on gene deletions this strain carries.

We explored effects of mitochondrial inhibitors (Na-valproate, metformin, methotrexate, antimycin A) on growth of purine synthesis knockouts after cultivated in complete or in purine or nitrogen deficient media. We observed that depending on pretreatment sensitivity to inhibitors differ. Moreover, purine starved auxotrophic cells exhibited higher fitness than prototrophic cells when cultivated with antimycin A. Interestingly, ODmax and sensitivity to methotrexate differs in *ade4* strain when differently treated (complete media or starved for nitrogen or purine).

Our findings might be useful when using yeast as a model for purine auxotrophic organisms, like intracellular parasites (*Leishmania spp.*, *Toxoplasma spp.*, *Plasmodium spp.*).[3] Intracellular parasites undergo specific life cycle when shifting environments from poor (salivary glands of insects) to nutrient rich (human erythrocytes). By changing cultivation conditions of the yeast, we are able to simulate different life stages of the parasite and thus be able to test specific compounds targeted for specific stage of parasites life.

Acknowledgements
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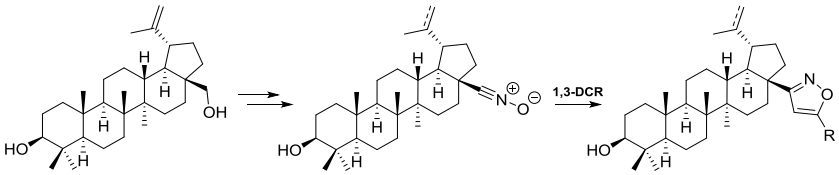
Application of betulin-derived nitrile oxides in the construction of cytostatic lupane-type triterpenoid–isoxazole conjugates

Jevgeņija Lugiņina[1], Martin Linden[2], Māris Bazulis[1], Viktors Kumpiņš[1], Anatoly Mishnev[3], Siegfried R. Waldvogel[2], Māris Turks[1]

[1] Faculty of Materials Science and Applied Chemistry, RigaTechnical University, P. Valdena Str. 3, Riga 1007, Latvia
[2] Department of Chemistry, Johannes Gutenberg University Mainz, Duesbergweg 10–14, Mainz, 55128, Germany
[3] Latvian Institute of Organic Synthesis, Aizkraukles Str. 21, Riga, 1006, Latvia
maris.turks@rtu.lv

Betulin is a naturally occurring pentacyclic triterpenoid that is found in the bark of birch tree, with content up to 35% of bark dry weight. Research reveals, that betulin and its semisynthetic derivatives possess wide spectrum of biological activity.[1]



Betulin lupane-nitrile oxide lupane-isoxazole hybrids

Here we preset a method for the preparation of novel lupane-type triterpenoid–isoxazole conjugates using successful combination of electro-organic synthesis and conventional approaches.[2] The exceptionally stable and isolable nitrile oxides allowed creation of isoxazole library by 1,3-dipolar cycloaddition reactions with various alkynes. Some of the title conjugates exhibit cytostatic properties against breast cancer cell line MCF7, glioblastoma multiform cell line U-87 MG and lung carcinoma cell line A549 with growth inhibition (GI_{50}) concentrations up to 11 μM, while being harmless to immortalized human fibroblasts hTERT (GI_{50} > 100 μM).

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Synthesis of $^{13}C/^{15}N/^{2}H$ isotopycally labeled fluorinated amino acids and their precursors for applications in structural biology and drug discovery

Ansis Maleckis[1], Elwy H. Abdelkader[2], Iresha D. Herath[2], Gottfried Otting[2]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, Latvia
maleckisansis@gmail.com
[2] ARC Centre of Excellence for Innovations in Peptide & Protein Science,
Research School of Chemistry, Australian National University, Canberra, ACT 2601, Australia
gottfried.otting@anu.edu.au

Expedient syntheses of fluorinated leucines, valines and alanine and their $^{13}C/^{15}N/D$ isotopologues were developed. Readily available and inexpensive reagents such as $NaBD_4$, carbon-^{13}C dioxide and sodium azide-1-^{15}N were used as isotope sources (Figure 1). Obtained fluorine-containing amino acids are valuable building blocks for peptide drugs as well as exceptionally advantageous NMR handles in structural biology and medicinal chemistry. Preliminary results indicate that synthesized amino acids are good substrates for cell-free protein expression.

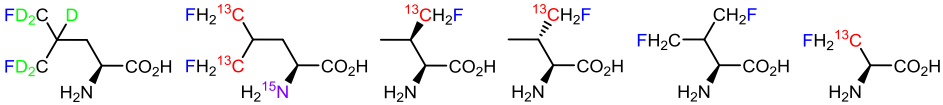

Figure 1. Synthesized leucines, valines and alanine.

In addition, syntheses of indoles labeled with $^{13}C–^{1}H$ and $^{13}C–^{19}F$ spin pairs were developed to investigate their use as tryptophan surrogates for *in vivo* and *in vitro* protein expression (Figure 2). In all syntheses, carbon-^{13}C dioxide was used as an inexpensive ^{13}C isotope source and indole carbocycle was constructed with ruthenium-mediated ring-closing metathesis. Successful cell-free incorporation of the synthesized labeled tryptophans into protein enabled high-resolution and high-sensitivity NMR experiments. Highly sensitive and responsive $^{13}C–^{1}H$ and $^{13}C–^{19}F$ spin pairs make the synthesized indoles particularly valuable reporters for NMR-based drug discovery methods.

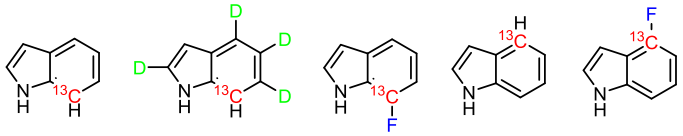

Figure 2. Synthesized indoles containing $^{13}C–^{1}H$ or $^{13}C–^{19}F$ spin pairs.

Acknowledgements
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Synthesis of C^4-substituted-7-azabicyclo[2.2.1]heptane-1-carboxylic acids

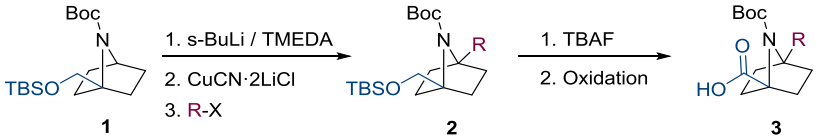
Mērija Agnese Meiberga[1,2], Krista Jaunsleine[1], Ilga Mutule[2]

[1] University of Latvia, Jelgavas iela 1, Riga, LV-1004
[2] Latvian Institute of Organic Synthesis, Aizkraukles iela 21, Riga, LV-1006
merija.agnese.meiberga@osi.lv

Conformationally rigid cyclic α-amino acids have been widely used in the design of peptides and peptidomimetics. By fixing a molecule in the biologically active conformation, it may increase efficiency and selectivity of its interaction with receptors. 7-Azabicyclo[2.2.1]heptane-1-carboxylic acid (Ahc) has already been incorporated in various peptide models mostly as conformationally constrained proline analogue.[1]

Herein we report the synthesis of previously unreported 7-azabicyclo[2.2.1]heptane-1-carboxylic acid derivatives **3** which are substituted at C(4)-bridgehead position, thus providing access to rich variety of new Ahc derivatives.

The key intermediate used in the synthesis is TBS protected amino alcohol **1**. Lithiation allows convenient functionalization of the C(4) bridgehead position and transmetallation of organolithium intermediate with CuCN·2LiCl complex allows the use of broad scope of electrophiles R-X.



Scheme 1. General scheme for synthesis of C(4) substituted Ahc.

Amino acid precursors **2** are deprotected and oxidized to give bicyclic α-amino acids **3**.

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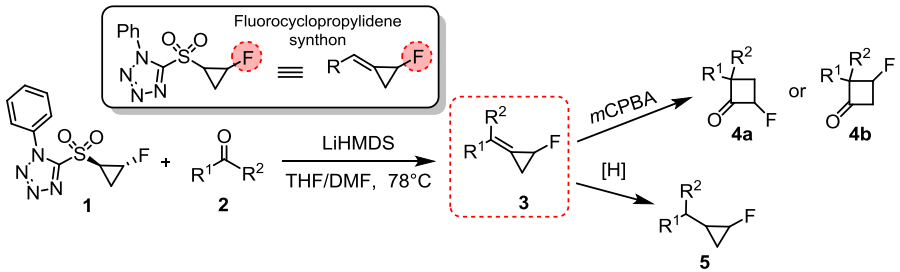
Synthesis of fluorocyclopropylidenes *via* Julia–Kocienski olefination

Renate Melngaile[1], **Janis Veliks**[1]

[1] Latvian Institute of Organic Synthesis
renate.melngaile@osi.lv

The fluorocyclopropyl group presented in the drug molecule plays on important role as a bioisostere which possesses the ability to increase its activity and bioavailability.[1] There is proof that the presence of a fluorocyclopropyl group in a known drug molecule indeed enhances its activity parameters in comparison to the original drug.[2]

Herein we demonstrate synthesis and application of less studied[3] compounds – monofluorocyclopropylidenes (Scheme 1) – which gives opportunity to introduce fluorocyclopropyl moiety into carbonyl group containing substrates – aldehydes, ketones – potential drug molecules.



Scheme 1. Synthesis and application of fluorocyclopropylidenes.

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Effect of molecules initially released from damaged murine bones on MG63 and NIH3T3 cells

Anastasija Metlova[1], Irina Česnokova[1], Antons Sizovs[1]

[1] Latvian Institute of Organic Synthesis
anastasija.metlova@farm.osi.lv, antons.sizovs@farm.osi.lv

Stimulation of bone fracture healing in many cases is essential for complete patient recovery. Understanding of the bone healing process could yield novel targets for pharmacological intervention in non-union cases but is far from complete. Here we investigate cellular response to the molecules released directly after the bone damage using inexpensive and 3R-compliant *in vitro* model. We report that extracts from damaged murine bones increase proliferation of osteoblast-like cells that are responsible for the rebuilding of bone tissue without affecting their metabolic activity.

Bone extracts were prepared from isolated femurs and tibias of mice. Isolated bones were further cleaned, crushed or left intact, incubated in serum-free cell growth medium for 1 h, filtered and applied to MG63 and NIH3T3 cell lines. Quantification of the cell number and MTT assays were performed after 1, 3 and 7 days of incubation.

During 7 days extracts from damaged tibias and femurs positively influenced proliferation of MG63 cells (Fig. 1). Extracts from fractured femurs significantly increased the number of NIH3T3 cells within 7 days. No significant changes in metabolic activity were observed in either cell line.

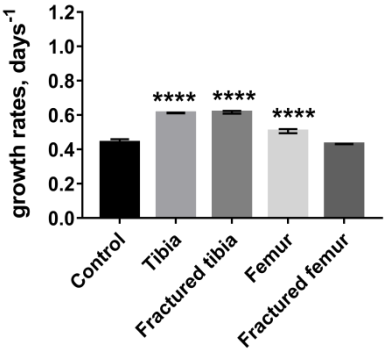

Figure 1. Proliferation rate of MG-63 osteoblast-like cells was influenced by the extract from murine bones. n=5, ANOVA, followed by Dunnet's test; ****($p<0.0001$).

Based on the obtained results, we speculate that bone extracts can be fractionated and growth-stimulating molecules and their targets can be identified to serve as drug candidate and drug target in complicated non-union fractures.

Accelerating small molecule drug discovery by a powerful collaborative approach

Tanja Miletić for the EU-OPENSCREEN consortium

EU-OPENSCREEN ERIC, Robert-Rössle-Str. 10, 13125 Berlin, Germany
tanja.miletic@eu-openscreen.eu

Chemical compounds, which are selected for their ability to exert a specific biological effect on cellular targets, represent versatile chemical probes in basic research to advance our understanding of pathologies at the molecular and cellular level and to validate novel drug targets. At the same time, bioactive compounds represent starting points for the development of new effective therapeutics. In fact, the majority of marketed drugs today are small chemical molecules. Despite the benefits, however, the discovery of these compounds requires significant efforts in terms of state-of-the-art facilities, expertise (e.g., in assay development or medicinal chemistry) and resources (e.g., comprehensive compound collections), which are often unavailable to most academic researchers.

As the European Research Infrastructure for Chemical Biology and early Drug Discovery, EU-OPENSCREEN (www.eu-openscreen.eu) supports researchers with the aim to accelerate drug discovery efforts in an open-access setting through collaborations with international researchers from academia and industry.[1] The consortium brings together ca. 30 scientific partners from universities and public research institutions in 10 European countries. The Latvian Institute of Organic Synthesis is the Baltic partner institute in the EU-OPENSCREEN initiative.

EU-OPENSCREEN uses various types of compound collections: a collection of approx. 2500 known bioactives and a 2500-compound diversity collection, both for assay validation and pilot screening experiments; a diversity collection with ca. 100 000 commercial compounds; a growing collection of compounds which have been submitted by academic chemists; and a fragment-library with 968 fragments and 88 so-called "minifrags". The fragment library is jointly used in fragment-based screening campaigns by EU-OPENSCREEN partners (including the Latvian Institute of Organic Synthesis in Riga) and structural biology groups of the Instruct-ERIC/ iNEXT-Discovery initiative allowing researchers to perform structural screens and subsequently use medicinal chemistry expertise to progress fragment hits. Here we will present how researchers can access EU-OPENSCREEN screening and medicinal chemistry services and its compound collections and benefit from the collaborative multidisciplinary effort in the discovery of novel therapeutics.

Acknowledgements
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Identify new biological activities of your compounds

Victoria Mora[1], Bahne Stechmann[1], Robert Hamel[1], Wolfgang Fecke[1], Osvalds Pugovics[2], Aigars Jirgensons[2]

[1] EU-OPENSCREEN, Robert-Rössle-Str. 10, 13125 Berlin, Germany
victoria.mora@eu-openscreen.eu
[2] Latvian Institute of Organic Synthesis, Aizkraukles street 21, LV-1006, Riga, Latvia

Compounds synthesized by academic chemists represent a rich, untapped source for novel chemical diversity. In many cases, the possibilities of chemists to systematically test these compounds against a variety of drug targets are limited. For biologists, who develop suitable assays, these compounds are often not readily accessible.

The Latvian Institute of Organic Synthesis is the Baltic partner institute in the EU-OPENSCREEN initiative. EU-OPENSCREEN is a consortium of approx. 30 academic partner institutes across 10 European countries, which support chemical biologists to implement their discovery projects by providing access to high-throughput screening platforms, screening collection and hit-to-lead optimisation support.

In order to make the invaluable chemistry of local chemistry groups accessible to a broader scientific community and to allow chemists to systematically screen their compounds for novel bioactivities, EU-OPENSCREEN offers chemists the opportunity to make their compounds available, in a regulated and transparent framework, to a wider community of biologists, who screen these compounds in suitable bioassays. By doing so, chemists can expose their compounds to a broad range of different biological/drug targets to screen for unknown bioactivities of their compounds, which would otherwise not be feasible in individual one-to-one-collaborations. Once a compound has been identified as an active hit compound, a research collaboration between the chemist (who submitted the compound) and the biologist (who developed the bioassay) can be initiated.

Here we will present how compounds from the Latvian Institute of Organic Synthesis are made accessible through EU-OPENSCREEN, and explain the procedure and benefits for other Baltic chemists to submit their compounds to uncover the hidden bioactivities of their compounds.

Safety of G2-S16 polyanionic carbosilane dendrimer as possible HIV-1 vaginal microbicide

Alba Martín-Moreno[1,2], Rafael Ceña-Diez[1,2], María Jesús Serramía[1,2], José Luis Jiménez[1,2], Rafael Gómez-Ramirez[3], Mariángeles Muñoz-Fernández[1,2]

[1] Sección Inmunología, Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón
[2] Laboratorio de Inmunobiología Molecular, Instituto de Investigación Sanitaria Gregorio Marañón
[3] Networking Research Center of Bioengineering, Biomaterials and Nanomedicine (CIBER BBN)
mmunoz.hgugm@gmail.com

The UNAIDS objective for 2020 was 500 000 new HIV-1 infections per year. However, the latest annual reported data confirmed 1.7 million new HIV-1 infections in that year. Those data evidence the need for new prevention strategies and prophylactic treatments. This prevention crisis occurred in spite of the knowledge and availability of efficient prevention strategies. The G2-S16 dendrimer is a microbicidal polyanionic carbosilane dendrimer currently being tested for topical vaginal application,[1] which has been shown to be efficient in the prevention of HIV-1 infection. However, safety tests were lacked. For this purpose, we injected intravenously G2-S16 dendrimer to CD1 mice, thereby analyzing the hemogram, blood biochemical markers of systemic damage, accumulation in the organs and organ-tissue damage in heart, spleen, kidney, liver, and brain. This work shows that even if the G2-S16 dendrimer penetrates the epithelial tissue, it does not cause vaginal irritation or tissue damage.[2] Moreover, the i.v. injection of the G2-S16 dendrimer did not cause a damaging effect on the studied organs and it did not modify the hemogram or the biochemical plasma markers. In conclusion, the G2-S16 dendrimer has a very good safety profile, indicating that this molecule can be a very safe and efficient vaginal microbicide

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Visible light photoredox catalysis for radical fluoromethoxylation

Nagarajan Ram Kumar[1], **Janis Veliks**[1]

[1] Latvian Institute of Organic Syntheis, Riga, Latvia
ram.kumar.nagarajan@osi.lv

Incorporation of the mono-fluoromethyl group can profoundly influence the physicochemical properties of organic molecules, offering a promising strategy for the discovery of novel pharmaceutical agents.[1] We have developed nitrogen based redox active reagents for the generation of fluoromethoxy radical under photoredox catalysis.[2] Direct fluoromethoxylation of unfunctionalized C(sp^2) centres is achieved for the synthesis of complex β-fluoromethoxy alcohols/amides and α-fluoromethoxy ketones. These complex fluoromethoxylated products which are inaccessible until now, may serve as useful building blocks or fragments in synthetic and medicinal chemistry both in academia and industry.

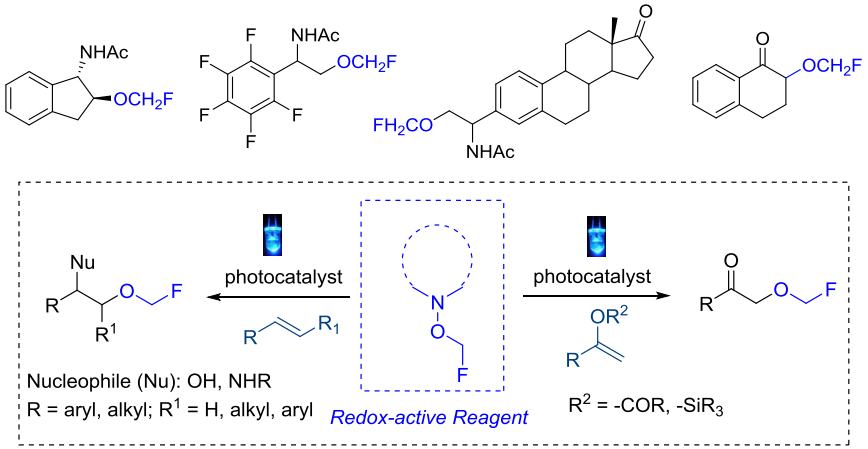

Figure 1. Selected examples of fluoromethoxylated compounds.

Acknowledgements
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Mesenchymal stem cell-derived extracellular vesicles as cisplatin carriers in lung cancer-on-a-chip model

Karina Narbute[1], Roberts Rimsa[2], Artis Galvanovskis[1], Janis Plume[1], Arnita Spule[2], Fēlikss Rūmnieks[1], Gatis Mozolevskis[2], Arturs Abols[1,2]

[1] Latvian Biomedical research and study center, Ratsupites Str.1, Riga, LV-1067
[2] CellboxLabs, LTD.
karina.narbute@biomed.lu.lv

Lung cancer is the most common cause of death with more than 50% lethal prognosis in the first year after diagnosis and the 5-year survival rate less than 18%. Although several treatments are available, they often leave the patients struggling with unwanted side effects. Several studies suggest that mesenchymal stromal cells (MSC) derived extracellular vesicles (EVs) have several advantages to become modern drug delivery systems, however current *in vitro* methods are not well suited for EV delivery tests from blood vessels to cancer tissues. Therefore, our aim is to test whether MSC derived EVs can improve cisplatin, a platin-based chemotherapy drug, effect and improve cell tolerance by testing it in lung cancer on a chip and lung on a chip *in vitro* model.

Cisplatin was used at physiologically relevant concentration and compared with cisplatin loaded EVs, EVs without cisplatin and negative control. Tissue integrity, cell viability, apoptosis, cell migration and cytokine assay were compared between lung on a chip and lung cancer on a chip developed from stable and primary cell lines. Currently we are in a process of obtaining results from these experiments, however the preliminary results suggest of EV ability protect lung tissue integrity based on cascade blue experiments in comparison to cisplatin alone. Final results will be presented at the conference.

Acknowledgements
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Structure-based design and characterization of cyclic peptides interfering with platelet aggregation

Kanin Wichapong[1], Johana Hrdinová[1,2], Delia I. Fernández[1], Bogac Ercig[1,2], Bibian Tullemans[1], Jan Voorberg[2], Johan Heemskerk[1,3], Gerry A. F. Nicolaes[1]

[1] Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands
[2] Department of Molecular and Cellular Hemostasis, Sanquin-Academic Medical Center, 1011 LZ Amsterdam,
[3] Synapse Research Institute, Kon. Emmaplein 7, 6214 AC Maastricht, The Netherlands
g.nicolaes@maastrichtunversity.nl

von Willebrand factor (VWF) is a plasma protein that circulates in a compact form unable to bind platelets. Upon shear stress, conformational changes occur that expose the VWF A1 domain, allowing binding of VWF to the platelet glycoprotein Ib-V-IX (GPIb chain). To better understand the role of this interaction in cardiovascular disease, molecules are needed that can specifically interfere with the opened VWF A1 domain interaction with GPIb. Therefore, we used an in house developed protocol for in silico drug discovery[1] to design stable cyclic peptides that can interfere with the platelet-binding of the VWF A1 domain. Several peptides were designed and in silico tested, after which two peptides with the lowest predicted binding free energy were synthesized: the monocyclic mono-vOn Willebrand factoR-GPIb InTerference (ORbIT) peptide and bicyclic bi-ORbIT peptide. Interference of the peptides in the binding of VWF to GPIb-V-IX interaction was retained by flow cytometry in comparison with the blocking of anti-VWF A1 domain antibody CLB-RAg35. In collagen and VWF-dependent whole-blood thrombus formation at a high shear rate, CLB-RAg35 suppressed stable platelet adhesion as well as the formation of multilayered thrombi. Both peptides phenotypically mimicked these changes, but they were less potent than CLBRAg35. After structure-based in silico optimization, an improved peptide named opt-mono-ORbIT (28 amino acids) was synthesized. Opt-mono-ORbIT was characterized by an increased inhibitory activity under flow. In conclusion, our structure-based design of peptides resulted in physiologically effective peptide-based inhibitors, even for convoluted complexes such as GPIb-VWF A1.

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Synthesis and biological evaluation of potent thioredoxine reductase inhibitors

Anna Nikitjuka[1], Raivis Žalubovskis[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles Str. 21, Riga, Latvia
anna@osi.lv

Physiological and pathological functions of thioredoxine reductase (TrxR) system in cellular processes have been extensively investigated.[1] Available evidences approve that dysregulation of TrxR results in various human diseases, especially cancer.

An increasing number of TrxR inhibitors which are in clinical trials for the treatment of different types of cancers has been reported so far.[2] However, construction of the specific inhibitors of TrxR over other related enzymes (e.g. glutathione reductase) remains a challenge.

One of our research direction, is synthesis of small, structurally diverse products to define a new and selective class of Trx inhibitors. Herein, we present a library of santamarine[3] derivatized compounds, possessing a potential Michael acceptor moiety. The preliminary biological activity results proved the obtained library to be specific inhibitors unless the mechanism of action and stability is still under investigation.

Acknowledgements
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Targeting type II classical cadherins using fragment-based and peptidic approaches

Teodors Pantelejevs[1], **Martins Kalnins**[1], **Emilio Parisini**[1]

[1] Biotechnology Group, Latvian Institute of Organic Synthesis,
Aizkraukles 21, LV-1006, Riga, Latvia
teodors.pantelejevs@osi.lv

Cadherins are calcium-dependent adhesion molecules that mediate contacts between cells at adherens junctions. Different tissues express different cadherins subtypes, ensuring adhesion specificity. Classical cadherins contain an elongated extracellular portion consisting of five immunoglobulin-like extracellular (EC) domains, of which the outermost N-terminal domains EC1 and EC2 mediate the protein-protein interaction (PPI) between cadherin protomers on opposing cells, called trans-dimerisation. A central feature of this interaction is the strand-swapping of one or two tryptophan residues between the EC1 subunits, in type I and type II cadherins, respectively (Figure 1). Type II cadherins, such as cadherin-11 and VE-cadherin, are potential therapeutic targets in a range of conditions, including metastatic cancer, arthritis and fibrosis.

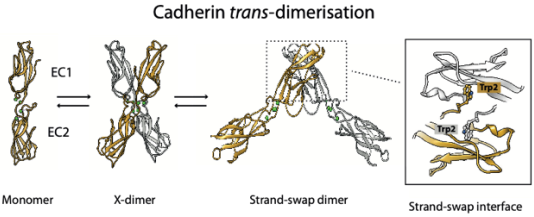

Figure 1. Cadherin *trans*-dimerisation.

In this work we aim to inhibit the strand-swap interaction of classical type II cadherins. For this we have engineered constructs of the EC1-EC2 fragment with a more accessible Trp pocket for detecting weak interactions. In a fragment-based approach, we have utilized differential scanning fluorimetry as a primary screening method, which was then followed by biophysical and crystallographic validation. The resulting fragments can serve as starting points for lead-like compound design. In a parallel approach, we use affinity selection of cyclic peptides to identify potent peptidic binders. For this, we harness the combinatorial diversity of peptide libraries displayed on filamentous bacteriophage.

Acknowledgements
The work is funded by the ERDF grant Nr. 1.1.1.5/21/A/002 “STARCAT: Selective targeting of Cadherin trans-dimerisation”. We acknowledge the Swiss Light Source synchrotron facility for providing X-ray beam time (proposal 20201060).

Synthesis of new AcrAB-TolC efflux pump inhibitors

Laura Paunina[1], Cristina Durante Cruz[2], Marina Madre[1], Tania Szal[3], Päivi Tammela[2], Aigars Jirgensons[1], Björn Windshügel[3], Jānis Veliks[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia
[2] Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland
P. O. Box 56 (Viikinkaari 5E), FI-00014, Helsinki, Finland
[3] Discovery Research ScreeningPort, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Schnackenburgallee 114, 22525 Hamburg, Germany
laurapaunina@gmail.com

Bacterial resistance to the existing classes of antibiotics is one of the most important challenges for the future healthcare system and bacterial cells efflux pumps play an important role for this internal drug resistance. To reduce the ability of the efflux pumps binding to medication substrates, the molecules called efflux pump inhibitors are used to rejuvenate the antibiotics activity by binding to the efflux pump protein.[1]

In the framework of the project, it was hypothesized that AcrAB-TolC efflux pump outer membrane protein TolC in Gram-negative *E. coli* bacteria cells could represent an attractive drug target. Therefore, analogues of a new class of TolC inhibitors have been synthesized to identify structure-activity relationships (SAR) (Figure 1).

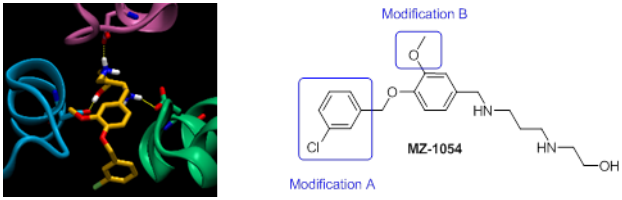

Figure 1. Predicted binding mode of hit compound MZ-1054 and TolC protein.

Acknowledgements
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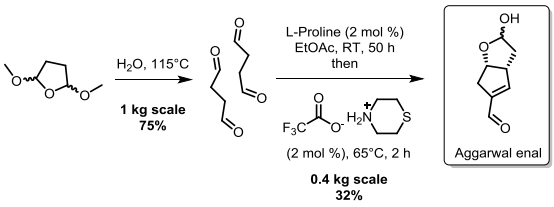
Scale-up development of Aggarwal enal bicyclic intermediate

Andrejs Pelšs[1], **Kirill Shubin**[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia
a-pelss@osi.lv

Prostanoids are important class of potent lipid mediators that are involved in the regulation of many biological processes such as inflammation, pain response and fever. This class of compounds has found wide-spread use as pharmaceuticals for the treatment of several diseases including pulmonary arterial hypertension and glaucoma (4.5 billion EUR global market). Recently a multitude of modern and short syntheses of various prostanoids were reported, rejuvenating this historically rich synthesis field. Remarkably short seven step synthesis of $PGF_2\alpha$ reported by Aggarwal group in 2012 has good potential for industrialization.[1]

Herein, we report the results of scale-up investigation of enantioselective two step route to Aggarwal enal bicyclic intermediate using extensively reoptimized reaction conditions.[2] Kilogram scale synthesis of succinaldehyde starting material was developed. Safety assessment of this volatile, unstable and polymerization prone compound was performed revealing recommended handling guidelines. Challenging organocatalytic dimerization of succinaldehyde was achieved on hectogram scale. The transfer from magnetically stirred small scale reactions to mechanically stirred large scale reactions in reactor required the finding of appropriate proline catalyst crystalline form.



Scheme 1. Scale-up route to Aggarwal enal.

Acknowledgements
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Bacteriophage-derived double-stranded RNA exerts anti-SARS-CoV-2 activity *in vitro* and in golden syrian hamsters *in vivo*

Vaivode Kristine[1], Verhovcova Irina[1], Skrastina Dace[1], Petrovska Ramona[1], Kreismane Madara[1], Salmina Kristine[1], Lapse Daira[1], Rubene Diana[1], Kalnina Zane[1], Pjanova Dace[1]

[1] Latvian Biomedical Research and Study Centre, Ratsupites street 1, k-1, Riga LV-1067, Latvia
dace@biomed.lu.lv

Bacteriophage-derived dsRNA, known as Larifan, is a nationally well-known broad-spectrum antiviral medication. This study aimed to ascertain the antiviral activity of Larifan against the novel SARS-CoV-2. The antiviral activity of Larifan against SARS-CoV-2 *in vitro* was measured in human lung adenocarcinoma (Calu3) and primary human small airway epithelial cells (HSAEC) using cytopathic effect assay, viral RNA copy number detection by digital droplet PCR (ddPCR) and infectious virus titration in Vero E6 cells. The antiviral effect of Larifan *in vivo* was detected in the SARS-CoV-2 infection model in golden syrian hamsters. Larifan (5 mg/kg) was administered either subcutaneously or intranasally twice before and after virus infection with a 24-hour interval between doses. The viral RNA copies and infectious virus titre were detected in animal lungs on days three and five post-infection. Histopathology of lungs was analyzed as well. Larifan inhibited SARS-CoV-2 replication *in vitro*. Viral RNA copy numbers in the supernatant of Calu3 cells dropped from 9.6×10^6 to an average of 1.4×10^6 ($p = 0.0296$). Titre of the infectious $7.0\log_{10}$ virus measured in Vero E6 cells also dropped significantly to an average of $4.5\log_{10}$ ($p = 0.0286$). A reduction in viral RNA copy number was also observed in HSAEC, especially when Larifan was added before infection ($p = 0.0218$) with a drop from 4.1×10^5 to 2.3×10^4. Larifan also markedly reduced virus numbers in infected hamsters' lungs on day three and five post-infection, with a more pronounced effect after intranasal administration reaching a drop by $2.7\log_{10}$ on day three and $2.0\log_{10}$ on day five ($p = 0.0032$). The administration of Larifan also reduced the amount of infections virus titer in the lungs ($p = 0.0039$) by $4.3\log_{10}$ and $2.8\log_{10}$ at day three and five post-infection, respectively. Improvements in the infection-induced pathological lesion severity in the lungs of animals treated with Larifan were also demonstrated by histological analyses. The inhibition of SARS-CoV-2 replication *in vitro* and the reduction of the viral load in the lungs of infected hamsters treated with Larifan alongside the improved lung histopathology suggests a potential use of Larifan in controlling the COVID-19 disease also in humans.

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Development of dihydropyridine-arginine hybrids for glioblastoma therapy

Martins Rucins[1], Martins Kaukulis[1], Irene Rodriguez-Clemente[2,3], Cristina de la Torre[2,3], Karlis Pajuste[1], Arkadijs Sobolevs[1], Aiva Plotniece[1], Valentin Ceña[2,3]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
[2] CIBER, Instituto de Salud Carlos III, Madrid, Spain
[3] Unidad Asociada Neurodeath, Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain
aiva@osi.lv

NANO4GLIO project aims to establish the bases for a new treatment approach for glioblastoma. For this purpose, we will use nanoparticles as carriers siRNA and an anticancer drug.

The aim of the work: 1) design and synthesis of 1,4-dihydropyridine-arginine hybrid molecules (Figure 1); 2) characterization of liposomes; 3) studies of biological activities; 4) evaluation of the structure-activity relationships.

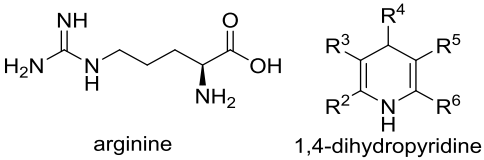

Figure 1. The main fragments for design of hybrid molecules.

Our initial studies showed that cationic 1,4-dihydropyridines (1,4-DHP) with dodecyl-oxycarbonyl substituents demonstrated high transfection efficiency *in vitro*.[1,2] Positively charged guanidine group of arginine contributes to the membrane penetration ability.[3] Synthetic methodology includes 'click' chemistry.[4]

Studies were resulted in the 22 original 1,4-DHP-arginine hybrid molecules with variation of amount of arginine (Arg) moieties and their positions at 1,4-DHP cycle. The nanoparticles were aimed to transfect siRNA into glioblastoma cell lines within the objectives of the NANO4GLIO project. Each nanoparticle was tested for the ability to bind siRNA, protect it from RNAse-mediated degradation, intrinsic toxicity on glioblastoma cells as well as on primary neuronal cultures and astrocytes that share the anatomical space with glioblastoma cells in patients. The performance of the nanoparticles on these different tests was dissimilar allowing a structure-activity relationship analysis. These screening tests allowed to select three 1,4-DHP-Arg hybrids for more detailed biological studies.

Acknowledgements
This work was supported by the EURONANOMED3 project "Nanomedicine for glioblastoma therapy" (NANO4GLIO).

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A dihydropyridine–arginine hybrid with high transfection efficiency in glioblastoma cells

Irene Rodriguez-Clemente[1,2], Cristina de la Torre[1,2], Martins Rucins[3], Martins Kaukulis[3], Karlis Pajuste[3], Arkadijs Sobolevs[3], Aiva Plotniece[3], Valentin Cena[1,2]

[1] CIBER, Instituto de Salud Carlos III, 28031 Madrid, Spain
[2] Unidad Asociada Neurodeath, Facultad de Medicina, Universidad de Castilla-La Mancha, 02006 Albacete, Spain
[3] Latvian Institute of Organic Synthesis, Riga, Latvia
Irene.RClemente@uclm.es, valentin.cena@gmail.com

Interference RNA (RNAi) is a powerful physiological mechanism that is active in most cells regulating a significant number of cell functions. siRNA is a synthetic compound that mimics endogenous RNAi mechanisms and that by selectively knocking down certain proteins involved in tumoral cells proliferation and survival could be effective to interfere with cancer cell survival. However, siRNA requires an effective vector for complexing it, preserving its stability and preventing their degradation until they are delivered to the target cells. We have studied the transfection abilities of a dihydropyridine–arginine hybrid. The aim was to establish if the nanoparticle could protect siRNA from RNAse-mediated degradation, being non-toxic for neurons and astrocytes and being able to markedly reduce the intracellular levels of both p42-MAPK and Rheb, two key proteins for proliferation and survival of glioblastoma cells.

The nanoparticle showed to a very promising compound to become a scaffold to be developed as an agent able to deliver siRNA to glioblastoma cells since it was able to bind siRNA and protect it from degradation, the nanoparticle was not toxic by itself for astrocytes and was able to decrease the levels of the protein targets, p42-MAPK and Rheb, to about 10 to 20% for the original values (Figure 1).

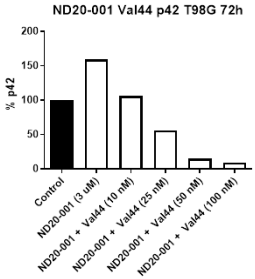

Figure 1. Knock down of p42-MAPK by specific siRNA.

Acknowledgements
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Novel 1-R-tri(*p*-F-benz)silatranes

Vitalijs Romanovs[1], **Edvins Kulins**[1], **Viatcheslav Jouikov**[2]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
vitalijs.romanovs@inbox.lv
[2] UMR 6226 ISCR, University of Rennes, 35042 Rennes, France
vjouikov@univ-rennes1.fr

Silatranes of the type X-Si(OCH_2CH_2)$_3$N are known to have a palette of diverse biological and pharma-related activities.[1] Much less is known about their derivatives with aryl-fused lateral chains, tribenzsilatranes X-Si(OC_6H_4)$_3$N; one of the reasons being their poor solubility and specific redox activity akin to that of triarylamines.

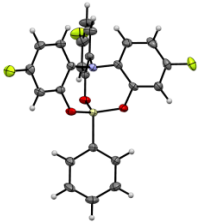

Figure 1. X-Ray structure of phenyl tri(*p*-F-benz)silatrane.

Expanding the families of silatranes with potentially interesting biological activity, we designed and synthetized a new family of Si-substituted tribenzsilatranes with the aromatic side chains F-substituted at *p*-position to N. The novel silatranes have been synthetized from organotriacetoxysilanes (R = CH_2CH_2CN, *t*-Bu, Ph, Tol) and the nitrilotris(*p*-F-*o*-phenols) preliminary prepared by (1:2) Ullmann condensation of the corresponding *p*-F-*o*-anizidine and *p*-F-*o*-iodoanisole.

In view of correlations of biological and redox activity of Si-containing derivatives,[2] the electrooxidation of the new silatranes has been considered revealing the formation of persistent cation radicals which exist as bi-stable species, in contrast to the starting fluorinated aminotriphenol. This feature is ascribed to the specific intramolecular N→Si dative interaction in these systems under the steric constraint imposed by the rigid tribenzo atrane cage.

Acknowledgements
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Humanized ABCA7 mice – a new Alzheimer's disease risk gene model for treatment development

Irene Santos[1], Pablo Bascuñana[1], Jens Pahnke[1,2,3]

[1] Department of Pathology, Section of Neuropathology, www.pahnkelab.eu, University of Oslo and Oslo University Hospital, Oslo, Norway
[2] LIED, University of Lübeck, Lübeck, Germany
[3] Department of Pharmacology, Faculty of Medicine, University of Latvia, Rīga, Latvia
i.s.g.sanz@medisin.uio.no

Adenosine-triphosphate-(ATP)-binding cassette (ABC) transport proteins are membrane-bound transport proteins that are ubiquitously present in the human body. They play a major role in determining the distribution of intrinsic and xenobiotic drugs between intra- and intercellular compartments. Many ABC transporters have been identified as key players in neurological disorders such as Multiple sclerosis, Hungtington's disease, or Alzheimer's disease (AD). AD is the most common form of dementia in the world and it is characterized by brain atrophy as a consequence of the deposition of amyloid-β (Aβ) peptides and tau protein hyperphosphorylation and aggregation. Since 2011, several studies have strongly suggested a link between genetic polymorphism of *Abca7* and AD reason why this ABC transporter is now considered an important genetic determinant for late-onset AD. However, the precisely role of ABCA7 in the development and progression of the disease is not yet known, as well as its relationship with amyloid processing and clearance. For this reason, our group has developed the first humanized ABCA7 (floxed) murine model with the main objective not only to understand the pathology but also of the future design of treatments based on the pharmacological modulation of this transporter. For this purpose, we have crossbred our human *ABCA7*$^{-/-}$ mice with the APPPS1-21 model and followed the progression of the disease from an early stage (50 days old) when amyloid is starting to aggregate, to an advanced state (200 days old). We have found that APPPS1-hA7ko animals present more insoluble Aβ42 at 100 and 200 days of age in comparison to APPPS1-21 mice. Even also the heterozygote group also showed more insoluble Aβ42 levels which could correlate with the fact most of the patients with the *Abca7* polymorphism are heterozygous. Nevertheless, there are no differences in soluble Aβ42 concentration on plasma at 100 and 200 days which would indicate that ABCA7 is not directly involved in amyloid transport outside the CNS. The increase in Aβ42 levels in APPPS1-hA7ko group also correlates with an increase in the number and coverage of Iba-1 and GFAP glia-positive cells in the cerebral cortex (only in the males but not in the females) which might suggest a link between the impairment in the phagocytic activity of the glial cells and the formation of the amyloid plaques.

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Role of enhanced permeability-retention effect in TNBC cancer with stealth nanoparticles

R. Serzants[1,2], B. Svalbe[1,2], G. Stelfa[1,2,3], I. Cesnokova[1,2], A. Sizovs[1,2]

[1] Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Aizkraukles iela 21, Riga, Latvia
[2] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Kalku iela 1, Riga, Latvia
[3] Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, K. Helmana Street 8, Jelgava, Latvia
rinaldsserzants@gmail.com

Triple-negative breast cancer (TNBC) is a form of very aggressive breast cancer with a poor short-term prognosis. First-line treatment is based on aggressive chemotherapy that has serious side effects. Nanoparticles (NPs) can reduce the severity of side effects by selectively delivering therapeutics to tumors. Nanomedicine aims to take advantage of the enhanced permeability retention (EPR) effect to reduce the systemic effects of chemotherapy. It is generally accepted that EPR is based on size-selectivity of leaky tumor vasculature, allowing NPs to preferentially accumulate in tumors. The majority of studies describing EPR ignore the rapid opsonization of NPs which leads to often dramatic changes in NP sizes and surface properties. For this reason, the optimal NP size for exploiting the EPR effect remains unknown. Here, we prepared heavily PEGylated NPs with 18, 29, 54 nm hydrodynamic diameters to study EPR dependence on size and to explore the limitations of EPR effect in TNBC. First, using fluorescence correlation spectroscopy, we demonstrated that our NPs do not undergo opsonization in mouse serum for at least two weeks. In the absence of opsonization all tested NPs avoided rapid clearance, elimination half-lives were inversely dependent on NP size and reached an unprecedented value of 3.0 days for 18 nm NP. NPs also showed wide biodistribution in healthy Balb/c mice. In the presence of TNBC tumors, elimination half-lives were only slightly decreased, revealing that tumors do not work as NP ‘sink’ by the action of EPR. Nevertheless, tumor was the major accumulation site for all tested particles. Our data demonstrate that NP selectivity towards TNBC decreases as NP size increase. Liver/tumor NP concentration ratios for 18, 29, 54 nm NPs were 0.15, 0.40, 0.76, respectively. Obtained data suggests that our NP can be used to investigate tumor permeability and potentially serve as tool for cancer diagnostics.

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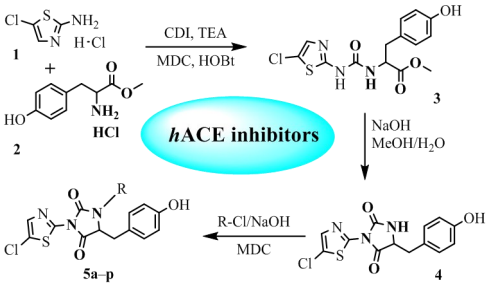
Chlorothiazolyl–imidazolidine based CVD modulators *via h*ACE inhibition

Manisha Shah, Manikandan Alagumuthu[1], Sivakumar Arumugam

[1] School of Biosciences and Technology, Vellore Institute of Technology, Vellore-632014, India
[2] Dept. of Microbiology, M S Ramaiah College of Arts, Science and Commerce, Bangalore-54, India
siva_kumar.a@vit.ac.in

We report a three-step synthesis of 3-(5-chlorothiazol-2-yl)-5-(4-hydroxybenzyl) imidazolidine-2,4-dione derivatives **5a**–**p** in search of effective cardiovascular disease (CVD) modulators (Scheme 1). In step one, methyl [(5-chlorothiazol-2-yl)carbamoyl] tyrosinate (**3**) was obtained from the reaction between 5-chlorothiazol-2-amine hydrochloride (**1**) and 2-(2-amino-3-methoxy-3-oxopropyl)-5-hydroxybenzene-1-ylium hydrochloride (**2**). In step 2, compound **3** undergone cyclization to obtain the parent compound 3-(5-chlorothiazol-2-yl)-5-(4-hydroxybenzyl)imidazolidine-2,4-dione (**4**). In step 3, compounds **5a**–**p** obtained by substituting various R-Cl entities. All compounds were obtained in moderate to good yield (72–92%) and characterized by NMR, FTIR, HRMS etc. All compounds were docked into the active site of the human angiotensin converting enzyme (*h*ACE, PDB ID: 1O86).[1] The ACE inhibitors, through the mechanism of blocking the activation of the renin-angiotensin system, interfere with atherogenesis, constructively remodel the left ventricle (LV) and arteries,[2] and possibly will improve the prognosis of atherosclerosis patients.[3] Based on the molecular mechanistic values (binding energy (BE) range –8.21 to –10.92 kcal/mol), compounds **5a** (–9.6), **5d** (–9.4), **5j** (–10.92) and **5n** (–9.8) were screened for further *in vitro* and *in vivo* studies. All values dominated the standard drug we used (Lisinopril, –7.30). Further, **5j** and **5n** were used for the *in vitro*, nitric oxide (NO) radical scavenging potential, *h*ACE inhibition, platelet aggregation test, and thrombolytic effect. The promising results obtained for these compounds indicate that these can be the candidate lead drugs to treat atherosclerosis and so they can be the future CVD modulators via *h*ACE inhibition mechanism.



Scheme 1. Route of synthesis of 3-chlorothiazolyl–imidazolidine derivatives.

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Transcriptome and SARS-CoV-2 biological network directed analysis to overcome various mutational and treatment limitations

Om Prakash Sharma[1], **Vinay Chandil**[1]

Innoplexus AG, Frankfurter Str. 27, 65760 Eschborn, Germany
om.sharma@innoplexus.com

To date, a total of 4256 interventional clinical studies are reported and 8 drugs have been approved by FDA for Emergency Use Authorization for covid-19. However, treatment benefits and frequent mutation are still a matter of concern. In order to improve therapeutic effects and minimise future covid-19 mutational driving infections, we have developed a research graph called CovInt (a network of covid-19) that includes all biological molecules associated in the network with their directionalities collected from publicly available and patient-derived multi-omics datasets.

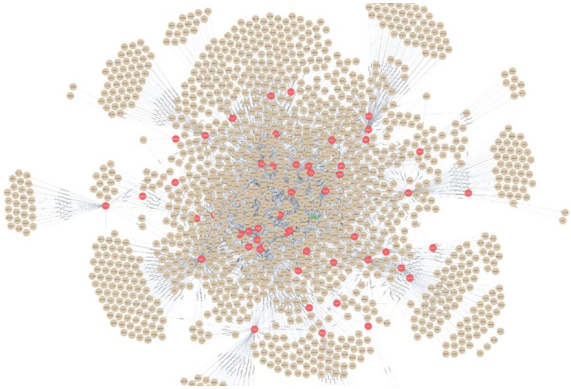

Figure 1. A snapshot of the sub-network of CovInt. Molecules in green are highlighted as pathways and protein–protein interactions (PPI's) are shown as brown in colour.

We further explored this network to identify and triangulate the key proteins, metabolic pathways and associated risk factors that can regulate moderate to severe covid-19 infections.

Acknowledgements
We thank Gunjan Bhardwaj, CEO and Founder of Innoplexus AG to support this research Work.

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Development of new 1,2-dihydropyridine derivatives as prospective antimicrobials and delivery systems

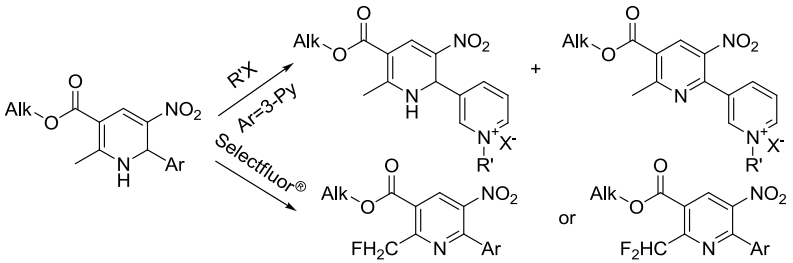
Karlis Pajuste[1], Davis Lacis[1], Ernests Tomass Auzins[2], Martins Rucins[1], Mara Plotniece[3], Nadiia Pikun[1], Aiva Plotniece[1,3], Janis Liepins[2], Arkadijs Sobolevs[1]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
[2] Institute of Microbiology and Biotechnology, University of Latvia, Riga, Latvia
[3] Faculty of Pharmacy, Riga Stradiņš University, Riga, Latvia
arkady@osi.lv

Introduction of fluorine or additional lipophilic alkyl substituents into molecules of 1,4- or 1,2-dihydropyridines (DHP) or pyridines (Py) and other heterocycles will provide a possibility to influence their properties and biological activity.[1–3]

The aim of the work is: synthesis of 1,2-DHP and Py derivatives with fluorine atoms or lipophilic moieties; characterization of formed nanoparticles; studies of antibacterial activity and toxicity; evaluation of the structure–activity relationships.

Synthesis of 1,2-DHP derivatives was performed using reported methods.[4] Modifications of 1,2-DHP were performed in two ways (Scheme 1). Fluorination leads to the fluoro/difluoromethyl substituted derivatives. Quaternization with alkyl halides forms cationic compounds with additional alkyl moieties.



Scheme 1. Structural modifications of 1,2-dihydropyridines.

To estimate toxic potential of the substances, we tested their effects on the growth of 6 different microbial species. Two dissect toxicity effects of the compounds – two test modes were applied: acute test on the bacteria with following recovery and growth test to record compound "chronic" effects on the living cells.

Acknowledgements
This work was supported by the PostDoc project No1.1.1.2/VIAA/3/19/587 (K. Pajuste), ERDF project No.1.1.1.1/19/A/147 (E. T. Auzins and J. Liepins) and the SPRINGBOARD project (funding from the EU Horizon 2020 Research and Innovation Programme under grant agreement No 951883).

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Iron-catalyzed fluoromethylene transfer

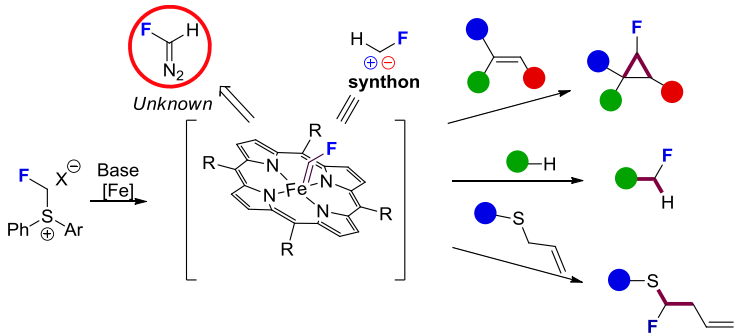
Artūrs Sperga[1], **Jānis Veliks**[1]

[1] Latvian Institute of Organic Synthesis
arturs.sperga@inbox.lv, janis.veliks@osi.lv

Synthesis of fluorine-containing molecules is of great interest due to its unique properties and vast application in pharmaceuticals, agrochemicals and materials.[1]

Fluoromethylsulfonium salts are emerging as synthetic equivalents of flouromethylene synthon.[2] However, their reactivity in transition metal catalyzed reactions is unexplored. Therefore, it is important to research the potential of metal-catalyzed fluoromethylsulfonium salt reactions to expand the borders of fluorinated molecule's synthesis.

Herein, we wish to report first iron-catalyzed fluoromethylene transfer from sulfonium salt (Scheme 1).[3]



Scheme 1. Reactivity of iron-catalyzed fluoromethylene transfer.

The developed method allows cyclopropanation of large variety of alkenes. Additionally, this approach can be extended to insertion and rearrangement reactions.

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Rational drug discovery of novel ABCA1 and ABCA7 modulators applying pattern-analysis approaches

Katja Stefan[1], Vigneshwaran Namasivayam[2,3], Irene Santos[1], Jens Pahnke[1,2,4], Sven Marcel Stefan[1,2]

[1] Department of Pathology, Section of Neuropathology, www.pahnkelab.eu, University of Oslo and Oslo University Hospital, Oslo, Norway
[2] LIED, University of Lübeck, Lübeck, Germany
[3] Department of Pharmaceutical and Cellbiological Chemistry, Pharmaceutical Institute, University of Bonn, Bonn, Germany
[4] Department of Pharmacology, Faculty of Medicine, University of Latvia, Rīga, Latvia
katja.stefan@medisin.uio.no, jens.pahnke@medisin.uio.no, s.m.stefan@medisin.uio.no

Genetic variant and genome-wide association studies uncovered the ABC transporters ABCA1 and ABCA7 as genetic risk factors for Alzheimer's disease (AD). A contribution of these transporters to amyloid-β (Aβ) production, degradation, and clearance has been discussed, however, their particular pathological roles in AD initiation and progression remain unclear. ABCA1 and ABCA7 are 'under-studied' ABC transporters which can barely be addressed by small-molecule modulators. Apart from their substrates cholesterol and phospholipids, only 14 modulators of ABCA1 are known, while ABCA7 modulators are unknown.

We developed a double-tracked polypharmacological approach for the rational discovery of novel ABCA1- and ABCA7-targeting agents. We generated a dataset comprising of all 113 known modulators of ABCA transporters[1] by data mining and curation, which were statistically analyzed for their substructural-features applying computer-aided pattern analysis (C@PA).[2] Qualified molecules were filtered applying a pharmacophore model generated on the basis of cryo-EM and homology structures of ABCA1 and ABCA7 as additional discriminator.

Using ABCA1- and ABCA7-expressing cells and fluorescence-labelled cholesterol and phospholipid tracers, several structurally distinctive hit molecules were identified which represent (i) optimal template molecules as *in vitro* tools to study ABCA1/ABCA7 biochemistry and physiology as well as (ii) transition molecules for the development of novel AD diagnostics and therapeutics to explore ABCA1 and ABCA7 as potential pharmacological AD drug targets of the future.

Acknowledgements
German Research Foundation (DFG, Germany; #504079349 PANABC, #466106904 #446812474, #263024513); Nasjonalforeningen (Norway; #16154), HelseSØ (Norway; #2019054, #2019055, #2022046); Barnekreftforeningen (Norway; #19008); EEA and Norway grants (Iceland, Liechtenstein, Norway, Czech Republic; TAČR TARIMAD #TO100078); Norges forskningsrådet (Norway; #295910 NAPI, #327571 PETABC).

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Polyphenolic compounds, antibacterial and antioxidant properties of flower and leaf extracts of *Tanacetum vulgare*

Renāte Šukele[1,2], Ingus Skadiņš[1,3], Inga Sīle[1,4], Rudīte Koka[1], Dace Bandere[1,3]

[1] Riga Stradins university Latvia
ingus.skadins@rsu.lv, rudite.koka@rsu.lv, dace.bandere@rsu.lv
[2] Red Cross Medical college of Riga Stradins university, Latvia
renate.sukele@rcmc.lv
[3] Baltic Biomaterials Centre of Excellence, Riga Technical University, Latvia
[4] Latvian Institute of Organic Synthesis, Riga, Latvia
inga.sile@farm.osi.lv

Tanacetum vulgare or Tansy is aromatic medicinal plant. Its aerial parts are rich in essential oil, polyphenolic compounds and phenolic acids.[1,2]Aim of this study was to assess antibacterial and antioxidant properties of ethanol and acetone extracts of *T. vulgare* flower (FE) and leaf (LE).

Aqueous ethanol and aqueous acetone (30, 50, 70%) extracts from flowers and leaves of *T. vulgare* growing in Riga district, Latvia were analysed. Antibacterial tests performed were agar disc diffusion test, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Six clinical isolates of bovine mastitis and two reference bacterial cultures were used. Antiradical activity was measured using the DPPH assay, total phenolic content (TPC) was tested using the Folin–Ciocalteu method and total flavonoid content (TFC) was determined using the $AlCl_3$ colorimetric method.

The MIC and MBC of FE were lower for *S. aureus* (range 3.4–6.4 mg/ml) than *E. coli* and *Strept. agalactiae* (53.9–107.8 mg/ml). None of the FE were effective against *Strep. uberis* and *Serratia liquefaciens*. The 70% ethanol of LE had effects against all bacteria except *E. coli* strains, and MIC and MBC ranged 7.8–125.9 mg/ml. The 50% acetone lyophilized LE and showed a better results than 50% ethanol FE, LE: TPC ranged 154–255 mg GAE/g of extract, TFC ranged 25–54 mg QE/g, and the IC_{50} value of DPPH radical scavenging activity was 147–194 µg/ml.

Our study showed that *T. vulgare* aqueous acetone and aqueous ethanol extracts of both flower and leaves have potential antibacterial and antioxidant properties.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement No 857287.

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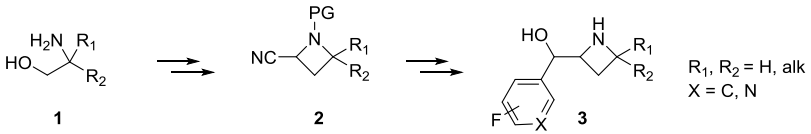
Synthesis of enantiopure (azetidine-2-yl)benzyl alcohols

Linda Supe[1], Ilga Mutule[1], Tore Bengtsson[2], Benjamin Pelcman[3]

[1] Latvian Institute of Organic sytnhtesis, Aizkraukles 21, Riga, Latvia
[2] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Svante Arrhenius väg 20C, 106 91 Stockholm, Sweden
[3] Atrogi AB, Tomtebodavägen 6, 171 65 Solna, Sweden
linda_supe@osi.lv

β-Amino alcohols are versatile chemicals used as scaffolds in medicinal chemistry and are key factors for the efficacy of numerous pharmaceutical products. Incorporation of structurally rigid azetidine ring into β-amino alcohols thus locking the conformation of active fragment may lead to increased potency and selectivity towards target receptor.

Our objective was development and synthesis of cyclic β-amino alcohols **3** with defined stereocenters (Scheme 1). Commercially available amino alcohols **1** were used as starting materials and converted to 2-cyanoazetidines **2**. Use of enantiopure starting amino alcohols (R_1 = H, R_2 = alk) allowed access to both *cis*- and *trans*-2,4-disubstituted azetidines with defined stereocenters. Cyano group was conveniently transformed to (hetero)arylamino alcohol as a mixture of *syn*- and *anti*-diastereomers, which after separation and deprotection gave desired (azetidine-2-yl) benzyl alcohols **3**.[1]



Scheme 1. General scheme for the synthesis of (azetidine-2-yl)benzyl alcohols **3**.

Acknowledgements
The research was funded by ERDF (Grant No. 1.1.1.2/VIAA/4/20/755)

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The *in vivo* interaction between the sigma-1 and GABA-B receptors reveals new opportunities for the development of novel anti-seizure drugs

Edijs Vavers[1,2], Gundega Stelfa[1,3], Baiba Svalbe[1], Anna Miteniece[1,4], Liga Zvejniece[1], Maija Dambrova[1,2]

[1] Latvian Institute of Organic Synthesis, Riga, Latvia
[2] Riga Stradins University, Riga, Latvia
[3] Latvia University of Life Sciences and Technologies, Jelgava, Latvia
[4] Imperial College London, London, UK
edijs.vavers@farm.osi.lv

During the last decade sigma-1 receptor (Sig1R) has been recognized as a valid target for the treatment of seizures. For example, Sig1R-related compounds are being studied in clinical trials for the treatment of Dravet, Lennox-Gastaut and Rett syndrome. Metabotropic GABA-B receptors are also known to play an important role in seizure modulation and have been studied as a possible target in absence seizures. Furthermore, *De Novo* mutations of GABA-B receptors have been shown to determine the phenotype in developmental and epileptic encephalopathies. Therefore, the aim of our study was to analyse possible interaction between sigma-1 and GABA-B receptors.

By using quantitative PCR, Western blotting and immunohistochemical analysis, a significantly decreased expression of the R2 subunit of the GABA-B receptors was revealed in the habenula of CD-1 background Sig1R knockout mice (provided by Laboratorios Dr Esteve, S.A.) brain samples. Compared to wild-type mice, the staining intensity of GABA-B R2 in the ventral part of the medial habenula was reduced by 70%. In addition, Sig1R knockout mice were more susceptible to pentylenetetrazol- and bicuculline-induced tonic seizures than their age-matched wild-type animals.

Our results demonstrate significant evidence of an *in vivo* interaction between the Sig1R and GABA-B receptors. The involvement of Sig1R in G-protein-coupled GABA-B receptor-related signalling mechanisms warrants further investigation and could be used to develop novel antiseizure drugs.

Acknowledgements
European Regional Development Fund Project No. 1.1.1.2/VIAA/2/18/376 "*Sigma chaperone protein as a novel drug target*" and Horizon 2020 Twinning Project No. 857394 FAT4BRAIN "*Networking for excellence in functional pharmacology to study the role of fatty acid metabolism in neurological disorders*".

Structurally simplified Diazonamide A analogs as anticancer agents

Viktorija Vitkovska[1], Mihail Kazak[1], Toms Kalnins[1], Edgars Suna[1]

[1] Latvian Institute of Organic Synthesis, Aizkraukles Str. 21, Riga, Latvia
viktorijav@osi.lv

Diazonamides are a structurally unique class of secondary metabolites first isolated by Fenical and coworkers from the colonial marine ascidian *Diazona angulata.*[1] The structurally complex Diazonamide A (**1**) was found to be highly cytotoxic anticancer agent (IC_{50} = 57 nM).[2] Studies conducted by Harran[3] revealed that DZ-2384 (**2**), a structurally simplified analog of **1**, is more potent (IC_{50} = 0.47 nM) and it lacks neurotoxicity at effective doses. Compound **2** binds to tubulin at vinca site disrupting microtubule dynamics. However, **2** still remains to be synthetically challenging and its preparation requires many steps with poor overall yield.

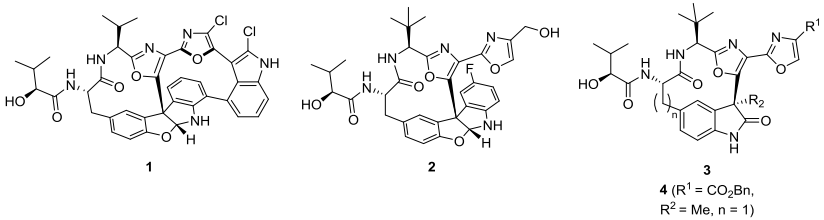

Figure 1. Structures of diazonamide A (**1**), DZ-2384 (**2**) and analogs **3** and **4**.

Herein we report the less complex synthesis of oxindole-containing macrocycles **3** using diastereoselective S_NAr-type macrocyclization as the key step. *In vitro* cytotoxicity measurements indicated that analog **4** exhibits nanomolar activity against relevant cancer cell lines making it more cytotoxic than diazonamide A (**1**).

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Structure-based peptide design targeting intrinsically disordered proteins: Novel histone H4 and H2A peptidic inhibitors

Kanin Wichapong[1], Carlos Silvestre-Roig[2], Quinte Braster[2], Ariane Schumski[2], Oliver Soehnlein[2,3,4], Gerry A.F. Nicolaes[1]

[1] Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands
[2] Institute for Cardiovascular Prevention (IPEK), LMU Munich Hospital, Munich, Germany
[3] Institute for Experimental Pathology (ExPat), Center for Molecular Biology of Inflammation, WWU Münster, Münster, Germany
[4] Department of Physiology and Pharmacology (FyFa),Karolinska Institute, Stockholm, Sweden
k.wichapong@maastrichtuniversity.nl

A growing body of research has demonstrated that targeting intrinsically disordered proteins (IDPs) and intrinsically disordered protein regions (IDPRs) is feasible and represents a new trending strategy in drug discovery. However, the number of inhibitors targeting IDPs/IDPRs is increasing slowly due to limitations of the methods that can be used to accelerate the discovery process. We have applied structure-based methods to successfully develop the first peptidic inhibitor (**HIPe** – **H**istone **I**nhibitory **Pe**ptide)[1] that targets histone H4 that are released from NETs (Neutrophil Extracellular Traps). HIPe binds stably to the disordered N-terminal tail of histone H4, thereby preventing histone H4-induced cell death. Recently, by utilisation of the same state-of-the-art approaches, we have developed a novel peptidic inhibitor (**CHIP** – **C**yclical **H**istone H2A **I**nterference **P**eptide)[2] that binds to NET-resident histone H2A, which results in a blockade of monocyte adhesion and consequently reduction in atheroprogression. Here, we present comprehensive details on the computational methods utilised to design and develop HIPe and CHIP[3]. We have exploited protein–protein complexes as starting structures for rational peptide design and then applied binding free energy methods to predict and prioritise binding strength of the designed peptides with histone H4 and H2A. By doing this way, we have modelled only around 20 peptides and from these were able to select 4–5 peptides, from a total of more than a trillion candidate peptides, for functional characterisation in different experiments. The developed computational protocols are generic and can be widely used to design and develop novel inhibitors for other disordered proteins.

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Temporal characterization of behavioral and neuropathological changes in an enhanced Huntington's disease mouse model: zQ175$^{\Delta neo}$

Jingyun Wu[1], **Jens Pahnke**[1,2,3]

[1] Department of Pathology, Section of Neuropathology, www.pahnkelab.eu, University of Oslo (UiO) and Oslo University Hospital (OUS), Oslo, Norway
[2] LIED, University of Lübeck, Lübeck, Germany
[3] Department of Pharmacology, Faculty of Medicine, University of Latvia, Rīga, Latvia
wu@jingyun.eu

Huntington's disease (HD) is an inherited and autosomal-dominant neurodegenerative disease characterized by the disordered control of voluntary movement, psychiatric disturbance, and cognitive impairment. We generated the zQ175$^{\Delta neo}$ HD mice from zQ175 mice to perform a temporal characterization of several behavioral and neuropathological features. The zQ175$^{\Delta neo}$ mice exhibited motor coordination dysfunctions and body weight loss at an early age of around 29 weeks. In addition, zQ175$^{\Delta neo}$ demonstrated muscular problems, anxiety-like behaviors, striatal atrophy, testicular atrophy and increased neuroinflammation after 36 weeks of age. However, microglia activation and whole brain atrophy only manifested at a late symptomatic stage of our experimental set-up. Overall, the zQ175$^{\Delta neo}$ is a reliable knock-in mouse model that showed significant HD-like phenotypes at a heterozygous state and only at late stage of mouse life span, which is more relevant to human HD. Removing the neo-cassette resulted in a more robust and long-term onset of HD symptoms, which enabled the zQ175$^{\Delta neo}$ mice to show severe and progressive phenotypes and provide a great value for preclinical studies.

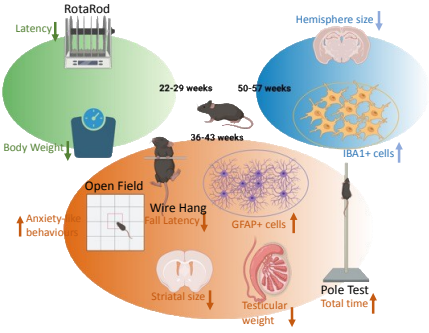

Figure 1. Graphic summary of main findings in zQ175$^{\Delta neo}$ characterization.

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Binding specificity and thermodynamics of fatty acids and acylcarnitines affinity to heart-type fatty acid binding protein

Diana Zelencova-Gopejenko[1], **Kristaps Jaudzems**[1]

[1] Latvian Institute of Organic Synthesis
zelencova@osi.lv

Fatty-acid binding proteins (FABPs) are well known as cytosolic transport proteins with the main function of binding and relocating highly hydrophobic long-chain fatty acids (LCFAs). Recent studies on energy metabolism emphasize LCFAs and LCFA-carnitine esters as important intermediates for cellular energy homeostasis. However, high toxicity of LCFA-carnitines makes their accumulation undesirable especially under ischemia/reperfusion condition.

In this work, we focused on investigating binding mechanism and possible substrate diversity of heart-type binding protein (FABP3) by means of nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC). Several approaches like formation of heterogeneous micelles with dimyristoyl-phosphatidylcholine (DMPC)[1] or addition of detergents (Triton X-100, Tween-20) were investigated in detail to increase solubility and prevent micellization of LCFA and corresponding carnitine esters. Additionally, 2D $^{1}H–^{15}N$ HSQC NMR spectroscopy was used to confirm specific binding of LCFA-carnitines in the active site of FABP3 (Figure 1).

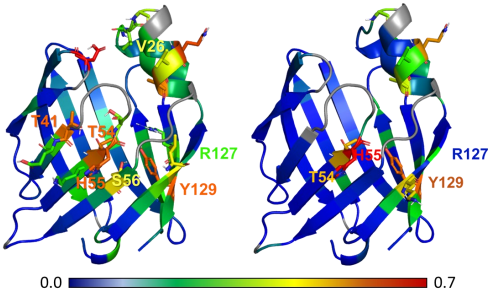

Figure 1. Chemical shift perturbation in the active site of FABP3 (PDB ID 1G5W) caused by oleate (C18:1 cis-Δ^9, left) and oleoylcarnitine (right) coloured according to the colour bar. Gray regions were not assigned.

Acknowledgements
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