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Achievements of the SPRINGBOARD project

May 2-3, 2024
Riga, Latvia

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the European Union's Horizon 2020 research and innovation
programme under grant agreement No 951883

02/05 THURSDAY	
9:00	**Registration**
9:45	**Prof. Raivis Žalubovskis** *Welcome*
10:00	**Carbonic anhydrase-targeted approach for photodynamic therapy** *Prof. Jean-Yves Winum*
10:30	**Coffee break**
11:00	**Towards the validation of the Carbonic Anhydrases as new antiinfectives** *Dr. Fabrizio Carta*
11:30	**Exploration of structure-activity relationships for antimicrobial peptides and peptoid/peptide hybrids** *Assoc. Prof. Henrik Franzyk*
12:00	**Lunch**
13:30	**PhD students/Post-Docs session** *(8 talks,15 min each)*
15:30	**Coffee break**
16:00	**Poster session** **Annual Springboard meeting**
from 18:00	**Dinner**

03/05 FRIDAY	
9:00	**Registration**
9:30	**Horizon Europe actualities** *Dr. Jānis Ancāns*
10:00	**Healthcare associated infections and antimicrobial resistance** *Dr. Madara Tirzite*
10:30	**Coffee break**
11:00	**Diazaborines: unveiling forgotten antibacterial potential by iterative screening approach** *Dr. Polina Ilina*
11:30	**Preclinical antibacterial drug discovery: exploring challenges in safety, exposure, and beyond** *Dr. Edgars Liepins*
12:00	**Lunch**
13:30	**Oxazolidinone-based carbonic anhydrase inhibitors: a promising strategy to discover antibiotics against gram-positive bacteria?** *Dr. Simone Giovannuzzi*
14:00	**Microbial natural products and their antibacterial activities** *Dr. Chin-Soon Phan*
14:30	**Human microbiome in health and disease: current research trends in Latvia** *Dr. Ilze Elbere*
15:00	**Closing remarks**
15:30	**Coffee break**

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Invited Talks

Human microbiome in health and disease: current research trends in Latvia

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The human microbiome plays a pivotal role in health and disease, influencing diverse physiological pathways and disease processes. In Latvia, recent advancements in microbiome research have begun to illuminate its significant implications in both maintaining health and contributing to disease pathogenesis. This presentation provides an insight of the cutting-edge research being conducted in Latvia nationally and at international level, focusing on the characterization and manipulation of the microbiome to enhance health and therapy outcomes as well as to mitigate disease progression.

Exploration of structure-activity relationships for antimicrobial peptides and peptoid/peptide hybrids.

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Recent studies of synthetic analogues of polymyxin B, tridecaptin A_1 and oncocin as well as of peptoid-peptide hybrid peptidomimetics will be presented. This includes a discussion of the influence of hydrophobicity, introduction of cationic and polar moieties as well as of end group modifications on antibacterial activity and hemolytic properties.

OXAZOLIDINONE-BASED CARBONIC ANHYDRASE INHIBITORS: A PROMISING STRATEGY TO DISCOVER ANTIBIOTICS AGAINST GRAM-POSITIVE BACTERIA?

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Over the past decade, bacterial carbonic anhydrases (CAs, EC 4.2.1.1) have emerged as promising targets for the development of novel antibiotic agents. Among the eight identified CA families (α, β, γ, δ, η, ζ, θ, and ι), only the α-, β-, γ-, and ι-classes have been identified in bacteria.[1-4] Specifically, α- and ι-CAs are periplasmic and exclusive to Gram-negative bacteria, while β- and γ-CAs are cytosolic and present in both Gram-positive and Gram-negative microorganisms.[1,2] Microbial CAs play crucial roles in various physiological processes, including pH regulation, virulence, growth, and acclimatization, making them valuable targets for designing anti-infective strategies.[1,4]

By applying a multi-target directed ligand (MTDL) approach, we developed oxazolidinone-based CA inhibitors to combine the inhibition of bacterial CAs with the antibiotic effects of oxazolidinones, such as Linezolid and Tedizolid, which disrupt bacterial protein synthesis in Gram-positive bacteria. A novel synthetic pathway was set up to incorporate (hetero)aryl sulfonamides into the oxazolidinone scaffold using various linkers and spacers. All multitargeting compounds were assayed as inhibitors against CAs expressed in Gram-positive bacteria by a Stopped-Flow kinetic assay. A subset of derivatives showed potent anti-enterococcal effects against various multidrug-resistant E. faecium and E. faecalis strains with several compounds significantly surpassing the efficacy of the lead Linezolid and CA inhibitor drugs (MIC values in the range 0.25 to >64 μg/mL).

References:

1. C.T. Supuran, C. Capasso (2020). *Expert Opin. Ther. Pat.* 30, 963-982.
2. C.T. Supuran, C. Capasso (2017). Metabolites. 7, 56.
3. N.S. Abutaleb, A. Elkashif, et al. (2021). Antimicrob. Agents Chemother. 65, e01715-e01720.
4. N.S. Abutaleb, A.E.M. Elhassanny, et al. (2021). *PeerJ.* 9, e11059.

Diazaborines: unveiling forgotten antibacterial potential through iterative screening approach

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This study investigates the potential of diazaborine compounds for antibacterial drug development. Since their initial discovery in 1960s, diazaborines have been for decades neglected. We revisited antibacterial potential of this group of boron-containing compounds, using screening in a range of clinically relevant bacterial pathogens followed by a panel of other bioassays including cytotoxicity, stability in human plasma, antibiofilm activity, synergy with colistin etc. As a result of this work, we identified diazaborines with optimized antibacterial performance against clinically relevant gram-negative pathogens like *E. coli*, *K. pneumoniae*, *A. baumannii*, and *S. typhimurium*.

Acknowledgements:
The Drug Discovery and Chemical Biology Network, supported by Biocenter Finland and University of Helsinki.

Preclinical antibacterial drug discovery: exploring challenges in safety, exposure, and beyond

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Delivering safer and more effective antibacterial medicines is becoming a progressively demanding task for scientists and the pharmaceutical industry. Traditionally, drug discovery programs were driven solely by antibacterial drug potency, regardless of their metabolic and safety properties. Drug discovery project teams synthesize a huge number of compounds that bind to the therapeutic target, but typically only a fraction of them have sufficient drug-like properties to become a pharmaceutical product. Safety issues of compounds may stop the project, or dramatically slow down the development process and increase the overall project costs. Drug-like properties, such as solubility, permeability, metabolic stability and transporter effects are of critical importance for the success of antibacterial treatment in preclinical and clinical studies. Within academia and small companies, the cost-effective testing and optimization process is essential. An efficient team using a low-throughput intelligent approach can effectively replace costly high-throughput ADME/TOX assays to advise and drive drug discovery projects. Early assessment of in vivo tolerability can provide valuable information about possible off-target effects of new compounds. Addressing safety and pharmacokinetic challenges requires a multidisciplinary approach, integrating pharmacological, medicinal chemistry, microbiology, and clinical expertise. Ultimately, teamwork is essential to overcome antibacterial drug discovery challenges for the development of safe and effective treatments for infections. The presentation will share practical cases from the antibacterial drug discovery projects at the Latvian Institute of Organic Synthesis. This presentation is supported by The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) projects APRINHA and MURYXIN.

Microbial Natural Products and Their Antibacterial Activities

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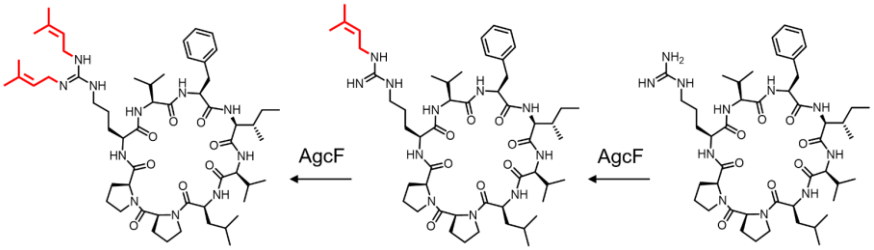
Body of the abstract

Chemical diversity and biological activity of natural products from cyanobacteria is continuing to be a subject of interest to researchers. This presentation focus on the chemistry and biology of cyanobacterial natural products, which led to the discovery of argicyclamides, a new class of cyanobactin from *Microcystis aeruginosa* NIES-88. We performed biochemical characterization on the new prenyltransferase, AgcF a key enzyme involved in enhancing antibiotic activity on the cyclic peptides, argicyclamides. This work disclosed bioactive natural products from cyanobacterium and characterized enzymes which expand the biocatalysis toolbox for prenylations.



References:

Phan, C.-S.; Matsuda, K.; Balloo, N.; Fujita, K.; Wakimoto, T.; Okino, T. Argicyclamides A-C unveil enzymatic basis for guanidine bis-prenylation. *J. Am. Chem. Soc*. **2021**, 143, 10083.

Healthcare associated infections and antimicrobial resistance

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Healthcare associated infections are emerging and are a serious and concerning threat. High number of healthcare associated infections (HAI) are caused by resistant strains of microorganisms (up to 70% organisms causing HAI are resistant to at least one antibiotic). Both European Centre of Disease control and prevention and local / national disease control centres have addressed this urgency issuing various recommendations, leading task forces and releasing statements about the urgency of the matter, related prevention activities, educational activities and further points of attention. HAI are larger burden than other infectious diseases and beyond – it is estimated that as from year 2050 deaths attributable to antimicrobial resistance every year will be circa 10 million. That will be significantly more than such other major cause of death as cancer. Finding the right balance when prescribing antibiotics, following indication sets, taking into account the capability and pharmacokinetic properties of each antibacterial agent is crucial just as finding new solutions in a world of high antibacterial resistance. Teamwork inbetween clinicists, epidemiologists, infectologists, biologists, microbiologists, pharmacologists and other specialities should be fostered in order to gain a network to successfully battle this emerging threat.

Carbonic anhydrase-targeted approach for photodynamic therapy

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Photodynamic Therapy (PDT) harnesses the power of reactive oxygen species (ROS) to combat cancer and microbial infections, making it a promising therapeutic approach. Combining carbonic anhydrase (CA) inhibitors with photosensitizers in targeted therapies could significantly enhance the efficacy of PDT systems. By addressing the limitations inherent in traditional PDT methods, such as cancer treatment and infection control, this innovative approach holds great potential for advancing medical treatment strategies.

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1- Clément S, Winum JY. Photodynamic therapy alone or in combination to counteract bacterial infections. *Expert Opin Ther Pat.* **2024**:1-14.
2- Merabti A, Richeter S, Supuran CT, Clement S, Winum JY. Are tumour-associated carbonic anhydrases genuine therapeutic targets for photodynamic therapy? *Expert Opin Ther Targets.* **2023**;27(9):817-826.
3- Merabti A, Roger M, Nguyen C, Nocentini A, Gerbier P, Richeter S, Gary-Bobo M, Supuran C-T, Clement S, Winum J-Y. Carbonic Anhydrase Inhibitors Featuring a Porphyrin Scaffold: Synthesis, Optical and Biological Properties. *Eur. J. Org. Chem.* **2022**; 21: e202101538.
4- Merabti A,Puchán Sánchez D, Nocentini A, Al LMA Nguyen C, Durand D, Hamon K, Ghanem T, Arnoux P, Josse P, Frochot C, Zalubovskis R, Richeter S, Gary-Bobo M, Supuran CT, Cabanetos C, Winum J-Y, Clément S. Thiochromenocarbazole imide-based photosensitizers decorated with carbonic anhydrase inhibitors for targeted treatment of hypoxic tumours. *Mater. Adv*. **2024**; advance article.

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Short talks of PhD students and PostDocs

Advantages and challenges associated with natural product antibacterial testing methods

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The growing threat of antimicrobial resistance demands the search for new active substances or more effective combinations of already known antimicrobials. Recently, the focus has shifted to the investigation of natural products. However, chemical and antibacterial testing of these types of materials comes with its own set of challenges. The goal of this study was to investigate the antibacterial potential of various natural products and evaluate the suitability and application of common antibacterial testing methods. Medicinal plant extracts (blueberry, bilberry, cranberry, lemon balm, purple coneflower) and honey samples were analyzed. The chemical profiles of said natural products were obtained using HPLC-UV and UHPLC-MS/MS analysis. Antibacterial activity was evaluated using well-diffusion, MIC/MBC methods, and antibiofilm tests. Overall, stronger antibacterial activity was observed towards Gram-positive than Gram-negative bacterial strains. The color of anthocyanin-rich plant extracts hinders precise evaluation of antibacterial activity. MIC/MBC analysis, in addition to qualitative analysis methods like well-diffusion, is necessary to draw accurate conclusions about the antibacterial potential of natural products.

Acknowledgements: BBCE – Baltic Biomaterials Centre of Excellence project (grant agreement No. 857287).

Development of a highly optimized engineered PETase enzyme for polyester degradation

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The fast and uncontrolled accumulation of plastic waste in the environment has long begun to impact on the natural ecosystems and to pose an existential threat to all forms of life on our planet. In this respect, enzymatic degradation of polymeric materials holds great promises as new and more efficient enzymes are constantly being developed. We aimed at applying a computer-aided enzyme engineering approach to improve the efficiency of enzymatic PET degradation.
Based on available crystal structures, we carried out molecular dynamics simulations to identify flexible regions of the enzyme and use this information to define enzyme variants with improved thermal stability, which validated our laboratory. We described an engineered Leaf-branch Compost Cutinase (LCC) that features enhanced PETase activity and thermal stability relative to the current gold standard (ICCG). Our LCC mutant shows a Tm > 96 °C and measurable activity beyond 6 days.

References:

Estiri, Hajar, et al. 'Tailoring FPOX Enzymes for Enhanced Stability and Expanded Substrate Recognition'. Scientific Reports, vol. 13, no. 1, Oct. 2023
Tournier, V. et al. An engineered PET depolymerase to break down and recycle plastic bottles. Nature 580, 216–219 (2020).

Mycothione reductase as a potential target in the fight against *Mycobacterium abscessus* infections

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Body of the abstract
While infections caused by Mycobacterium abscessus complex (MABC) are rising worldwide, the current treatment of these infections is far from ideal due to its numerous shortcomings thereby increasing the urge for novel drug targets. In this study, mycothione reductase (Mtr) was evaluated for its potential as a drug target for MABC infections since it is a key enzyme needed in the recycling of mycothiol, the main low-molecular-weight thiol protecting the bacteria against reactive oxygen species and other reactive intermediates. First, a MabΔmtr mutant strain was generated, lacking mtr expression. Next, the in vitro sensitivity of MabΔmtr to oxidative stress and antimycobacterial drugs was determined. Finally, we evaluated the intramacrophage survival and the virulence of MabΔmtr in Galleria mellonella larvae. MabΔmtr demonstrated a 39.5-fold reduction in IC90 when exposed to bedaquiline in vitro. Furthermore, the MabΔmtr mutant showed a decreased ability to proliferate inside macrophages and larvae, suggesting that Mtr plays an important role during MABC infection.

STRUCTURAL BASIS FOR INHIBITION OF THE SARS-COV-2 NSP16 BY SUBSTRATE-BASED DUAL SITE INHIBITORS

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Coronaviruses, including SARS-CoV-2, possess an mRNA 5' capping apparatus capable of mimicking the natural eukaryotic capping signature. The 2'O-methylation performed by nsp16-nsp10 is crucial for the escape of the viral RNA from innate immunity. Inhibition of this enzymatic activity has been proposed as a way to combat coronaviruses.
In our study, we employed X-ray crystallography to analyze the binding of the SAM analogues to the active site of nsp16-nsp10. We obtained thirteen novel 3D crystal structures of the nsp16-nsp10 complexes with SAM-derived inhibitors, demonstrated different conformations of the methionine substituting part of the molecules, and confirmed that simultaneous dual-site targeting of both SAM and RNA sites correlates with higher inhibitory potential.

Acknowledgements: This work was supported by VPP-COVID-2020/1-0014, VPP-EM-BIOMEDICĪNA-2022/1-0001 grants.

References:
Bobiļeva O, et al. ACS Med Chem Lett. 2021;12(7):1102-1107
Bobrovs R, et al. Pharmaceuticals (Basel). 2021;14(12):1243.

Photo-catalyzed Synthesis of Monofluoromethyl-containing Heteroarenes and their applications

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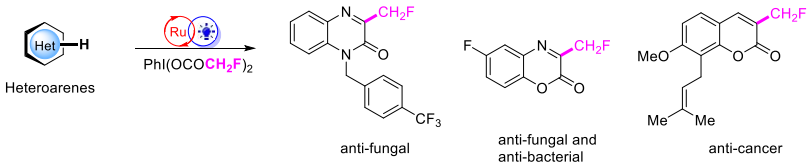
Abstract
Fluoroalkyl groups play a crucial role in pharmaceutical substances and agrochemicals, where their installation onto aryl and heterocyclic rings can often lead to improved physicochemical and biological properties. Here we developed a method for the synthesis of monofluoromethyl-containing heterocycles by using an iodine(III) reagent as a radical source of monofluoromethyl group via visible-light photoredox catalysis. Moreover, the monofluoromethylated heteroarenes exhibit promising antimicrobial and anticancer activities, indicating that this methodology might assist in drug discovery as a dependable tool for lead drug modification in the development of new small-molecule drugs.



Acknowledgements:
Postdoc Latvia ERDF project Nr. 1.1.1.2/VIAA/4/20/748.

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From Phenotype to Genotype: Investigating Antibiotic Tolerance in *Streptococcus pneumoniae*

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Persisters, a subset of bacteria tolerant to lethal antibiotic concentrations, are widespread among reference and clinical strains of *Streptococcus pneumoniae* (Geerts et. al, 2022).
Our ongoing research aims to characterize a large, diverse set (>600) of *S. pneumoniae* isolates both phenotypically and genotypically to further map the clinical landscape of pneumococcal persistence.
Through genome-wide association studies, genetic markers of persisters will be investigated, opening the road for future research on novel biomarkers and therapeutics.

Battling the biofilm in ventilator-associated pneumonia: Developing antimicrobial coatings on endotracheal tubing

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Ventilator-associated pneumonia (VAP) is one of the most common hospital-acquired infections and suffering from this disease results in a considerable increase of mortality rates at the intensive care unit. The presented work aims to develop an antimicrobial coating containing antimicrobial peptides (AMPs) on endotracheal tubing. Several coating strategies, such as polydopamine-assisted immobilization or hydrogel formation, were subject to quantitative antibacterial assays and visual validation with scanning electron microscopy.

Acknowledgements: FWO fellowship fundamental research (file number: 11I5523N)

Repurposing S1PR modulators as antibacterial agents

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Drug repurposing presents a promising strategy to complement traditional antibacterial drug discovery. In a previous study, fingolimod, an S1PR modulator for the treatment of multiple sclerosis, was identified as a hit antibacterial compound in a screening of FDA-approved drugs.[1] Following these findings, we screened a small library of other S1PR modulators against bacteria and identified etrasimod, a marketed drug for the treatment of ulcerative colitis, as a potent antibacterial agent against several Gram-positive bacteria, including drug-resistant strains (MIC = 5–10 μM).[2] Subsequent structure-activity relationship studies led to further identification of novel potent antibacterial compounds with improved biological properties.[3] Indole derivative **24f** showed the most potent activity against several Gram-positive bacteria (MIC = 2.5 μM). Furthermore, it showed bactericidal activity, synergy with gentamicin, low toxicity towards mammalian cells, and importantly, a low likelihood for rapid development of resistance in staphylococci.[3] Overall, this study highlights the potential of etrasimod and its derivatives as novel antibacterial compounds against Gram-positive bacteria.

References:

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2. Zore, M. et al. *Front. Microbiol.* **2022**, 13.
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Poster Presentations

N-((4-Sulfamoylphenyl)carbamothioyl) amides: Potential antibacterial agents

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Abstract

A panel of twelve structurally diverse *N*-((4-sulfamoylphenyl)carbamothioyl) amides were synthesized by selective acylation of easily available 4-thioureidobenzenesulfonamide with various aliphatic, benzylic, vinylic, and aromatic acid chlorides under mild conditions. The compounds were investigated as inhibitors of three bacterial β-CAs from Mycobacterium tuberculosis (MtCA1-MtCA3). The mycobacterial enzymes MtCA1 and MtCA2 were effectively inhibited by the targeted compounds. MtCA3 was, on the other hand, poorly inhibited by the sulfonamides reported here. The most sensitive mycobacterial enzyme to these inhibitors was MtCA2 in which 10 of the 12 evaluated compounds showed KIs (KI, the inhibitor constant) in the low nanomolar range. In order to unveil the relationship between the structural features and inhibition profiles, the binding mode of two selected compounds was investigated in silico in the active site of all three studied bacterial β-CAs.

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BENZOXAPHOSPHEPINE 2-OXIDES — A NOVEL CLASS OF CARBONIC ANHYDRASE INHIBITORS

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Carbonic anhydrases (CA, EC 4.2.1.1) are essential metalloenzymes found across all kingdoms of life and are already an established drug target for a range of diseases, including bacterial infections, malaria, cancer and glaucoma. Our research interests were aimed at development of novel and isoform-selective CA inhibitors, which can potentially serve as anti-tumour agents. Herein we present the facile synthesis of benzoxaphosphepine 2-oxides along with their evaluation of inhibitory activity towards tumour-associated CA isoforms IX and XII[1,2]. In addition to remarkable inhibition, this novel class of CA inhibitors displays excellent water solubility, making them attractive drug-like candidates for further studies.

Acknowledgements:
The work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 95188 within SPRINGBOARD project.

References:
1. Pustenko, A.; Balašova, A.; et al. *J. Enzyme Inhib. Med. Chem.* **2023**, 38, 216–224.
2. Balašova, A.; Pustenko, A.; et al. *J. Enzyme Inhib. Med. Chem.* **2023**, 38, 2249267.

PHOSPHOCOUMARIN DERIVATIVES AS CARBONIC ANHYDRASE INHIBITORS

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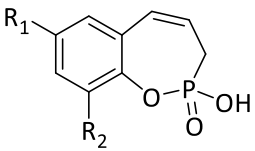
Carbonic anhydrases (CA) are metalloenzymes involved in vital physiological processes, such as pH regulation and CO_2 homeostasis.

In last two decades, CA have been identified as drug target. CA inhibitors can act as anticancer, antiglaucoma and, as shown in recent years, as antibacterial agents.

Previously in our research group, organophosphorus compounds **1** were synthesized, which have been identified to be isoform-selective and effective CA inhibitors [1]. Extending our research, we decided to develop potential CA inhibitors – 2-hydroxybenzo-1,2-oxaphosphinine 2-oxide **2**, which are considered phosphocoumarin.



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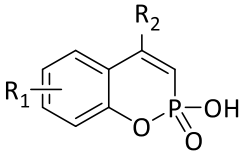

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Acknowledgements: This work has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 951883 within SPRINGBOARD project.

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1. Pustenko, A.; Balašova, A.; Nocentini, A.; Supuran, C.T.; Žalubovskis, R. *J. Enzyme Inhib. Med. Chem.* **2023**, *38*, 216–224.

Effects of expression and purification conditions on activity and stability of recombinant seryl-tRNA synthetase from *S. aureus*

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Body of the abstract
Aminoacyl-tRNA synthetases (aaRS) are enzymes catalysing the addition of an amino acid to a tRNA molecule containing the corresponding anticodon. Malfunctioning of any of these 20 enzymes results in a formation of incorrect protein sequence, thus disrupting functions of the cell and leading to death of unicellular organisms. This makes aaRS suitable antibacterial drug targets. Our current work is focusing on seryl-tRNA synthetases (SerRS).
Modern medicinal chemistry utilizes several biophysical methods to detect the interactions of antibacterial drug target with the potential inhibitors. Thus, obtaining a biologically active target enzyme *in vitro* is crucial for the initial ligand (or fragment) screening to succeed.
Production of recombinant biologically active SerRS from *S. aureus* (SaSerRS) in *E. coli* cells has been challenging. Current work describes approaches used for optimisation of protein expression and purification. Furthermore, initial results of SaSerRS stability testing are presented.

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Unraveling The Molecular Mechanisms of Cytotoxicity Induced By Physically Crosslinked Hyaluronic Acid/Poly-L-Lysine Hydrogel

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Creating versatile hydrogels with hyaluronic acid (HA) and ε-poly-L-lysine (PLL) for biomedicine shows potential but faces fibroblast toxicity challenges. Understanding its toxicity mechanisms is crucial for optimizing antibacterial properties and safe patient use. We produced HA/PLL hydrogels with varied polymer ratios, assessing fibroblast viability and metabolite profiles using CCK-8, LIVE/DEAD staining, and LC-MS metabolomics. Redox status and glutamine metabolism were explored via 13C tracing and lipid peroxidation was assessed through lipidomics. Co-cultures with iron, selenium, and cystine examined Fenton reactions and GPX4 activity. Confocal imaging revealed cytoskeletal damage. Physically crosslinked HA/PLL hydrogels increased cystine and reduced glutamine levels, correlating with ROS elevation and cytoskeletal degradation. Glutamine pathway inhibition, altered gene expressions like SLC7A11, reflecting a complex cytotoxicity process affecting redox balance and cytoskeletal structure.

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INACTIVATION OF BACTERIA AND RNA VIRUSES USING HIGH-INTENSITY UV-A LIGHT

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LED-based disinfection is emerging as a sustainable and cost-efficient substitute for mercury lamps. UV-A light primarily inactivates pathogens by generating reactive oxygen species, which cause oxidative damage to pathogens. This approach is generally effective against bacteria and enveloped viruses, but non-enveloped viruses are typically more resistant. In this study, we investigated the disinfection capabilities of high-intensity UV-A LED lamps (~460 mW/cm², 365–375 nm). We hypothesized that intense UV-A light might cause direct photochemical damage to pathogen nucleic acids, enhancing disinfection. The inactivation of the bacterium *Escherichia coli*, the non-enveloped RNA bacteriophage MS2, and the enveloped RNA virus Semliki Forest Virus (SFV) were studied, along with the inactivation of self-replicating RNA from SFV. The results showed a 4 log10 reduction of *E. coli* and SFV, as well as improved efficiency against MS2. Moreover, direct irradiation of RNA led to a significant reduction in viral gene expression in transfected cells.

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Exploration of Novel 1,2-Dihydropyridine Derivatives: Potential Antimicrobial Agents and Innovative Delivery Platforms

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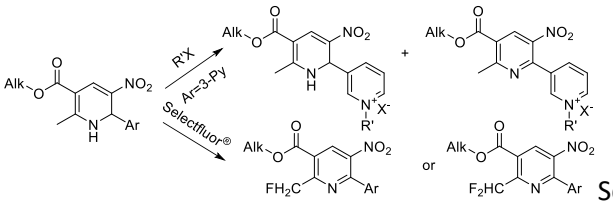
Introduction of fluorine or additional lipophilic alkyl substituents into heterocycles may modulate their properties and enhance biological activity.[1] Synthesis of 1,2-DHP and Py derivatives with fluorine atoms or lipophilic moieties; characterization of formed nanoparticles; studies of antibacterial activities and toxicity; evaluation of the structure-activity relationships were performed. Synthesis of compounds was performed using reported methods.[2] Modifications of 1,2-DHP were performed in two ways (Scheme 1). Fluorination leads to the fluoro/difluoromethyl substituted derivatives. Quaternization of pyridine moiety with alkyl halides forms cationic compounds with additional alkyl moieties.



Scheme 1.

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BENZOXABORININES AS ALTERNATIVE CHEMOTYPE FOR CARBONIC ANHYDRASE INHIBITION

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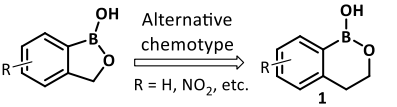
The disregulation, and abnormal enzymatic activity of different types of metalloenzymes carbonic anhydrases (CAs) are usually related to pathological conditions such as cancer, psoriasis, fungal infections etc. The incorporation of boron into CA inhibitors has proven to be a valuable approach for the modulation of the ligand ability to recognize the target, thus influencing the selectivity towards different human CA (hCA) isoforms. A number of boron-containing compounds are currently approved in clinics; moreover, benzoxaboroles, five-members boron-heterocycles, are able to interact with hCA and have shown a prominent activity against pathogenic CA. In order to promote new interactions within the active site, increase the selectivity and stability in physiological conditions, herein we present the synthesis and hCA enzymatic activity evaluation of benzoxaborinines **1** [2].



Benzoxaborole Benzoxaborinine

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Serum metabolite changes in a fracture-related infection model

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Fracture-related infection (FRI) is a major and hard-to-treat complication in orthopedic surgery. We are the first to analyze serum metabolites, using an FRI sheep model to quantify 41 metabolites over a 12-week time course using LC-MS-based targeted metabolomics. During the initial infection, we observed significant changes in only 3 metabolites: A decrease in the redox metabolite cysteine and the energy intermediate octanoylcarnitine, and an increase in the collagen breakdown product hydroxyproline.
Importantly, cysteine exhibited only a slow recovery over time. We were able to develop a classification model for FRI using the ratios (Hydroxyproline/Glycine, Creatinine/Serotonin), exhibiting a 90.9% predictive power in our samples.
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Structure-Activity Relationship Studies of Amphiphilic Transfection Agents with Antimicrobial properties

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Cationic lipids are one of most versatile tools for the delivery of genetic material and therapeutic molecules. Our previous studies highlighted 1,4-dihydropyridine (1,4-DHP) amphiphiles as potential transfection agents, exhibiting multifunctional properties [1].
In this work, our focus is on role of the linker between hydrophilic and lipophilic parts. Six-membered 1,4-DHP was replaced with five-membered heterocycles. For better comparison, 'opened analogues' were synthesized as well. Structures containing heterocyclic linkers demonstrated *p*DNA transfection activity, whereas the 'opened analogues' did not. Three amphiphiles showed both transfection and antimicrobial activity at same range of concentration and can be considered for further development. More results are discussed in poster.
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Insights into Production of Recombinant Stabilized Plasmepsin V in *E. Coli* and insect cells

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Despite significant improvements in its treatment, malaria still remains a life-threatening infectious disease caused by *Plasmodium* parasites. The development of resistance to existing antimalarial drugs emphasizes the urgent need in novel effective therapeutic strategies. Plasmepsins (PMs), aspartic proteases of *Plasmodium*, have been selected as promising targets for inhibitor design. PM V is the most structurally distinct enzyme from the plasmepsin family. It exhibits minimal similarity to human aspartic proteases, as well. Thus, PM V is an excellent candidate for the development of highly selective antimalarial drugs.
Nevertheless, preparation of soluble and catalytically active PM V remains the challenging task. In this work, we generalized current knowledge on PM V production in both bacterial and baculovirus expression systems to optimize expression condition and develop an assay for large scale PM V production necessary for further fragment screening and targeted covalent inhibitor binding studies.

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