Edited by

Michal Jurášek, Radmila Řápková, Zina Pecková, Pavel Drašar

Corresponding authors are fully responsible for the content of their communications; the editors in some cases corrected the format of articles that were not submitted in accordance with the CCSSS journal rules.

ENCAPSULATION AND BIOLOGICAL STUDIES OF BIOACTIVE RUTHENIUM(III) COMPLEXES IN CD-MOF-1

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Ruthenium-DMSO complexes are promising among ruthenium-based anticancer drugs due to their good selectivity for solid tumor metastases and minimal host toxicity. Nevertheless, given the poor stability, low water solubility, and some toxicity associated with the unspecific delivery of these metallodrugs, research has focused on the encapsulation of ruthenium complexes into host systems[1]. With the rapid advancement of material chemistry, much effort has been directed to the formation of innovative platforms for controlled and smart drug release systems with the goal of maximizing therapeutic efficacy while minimizing side effects. One of such systems are Metal-Organic Frameworks (MOFs), composed of organic ligands and metal ions/clusters linked together by coordinative bonds[2]. Cyclodextrins (CDs) are hydrophilic cyclic oligosaccharides having truncated cone-shaped molecules. The outside hydrophilic surface is decorated by a number of hydroxyl groups, whereas the inner hydrophobic cavities are lined by glycosidic oxygens and C-H units. The γ-CD (8 glucose unit) has been utilized in green synthesis of biocompatible and non-toxic MOFs using alkali and alkaline earth metal ions[3]. In this research the model complex (MC) composed of ruthenium(III) metal center coordinated with 4-methylpyridine and DMSO as axial ligands and four chlorides in equatorial plane was synthesized, studied for its stability, and used for adsorption studies into CD-MOF-1 (composed of potassium hydroxide and γ-CD). Herein, we present the first pharmaceutical formulation of potential metallodrug inside the CD-MOF-1 and it's *in vitro* viability studies on spheroids of human hepatoblastoma cell line HepG2 (Fig. 1).

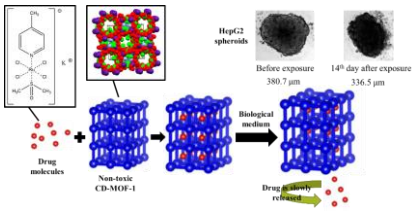

Fig. 1. **Graphical abstract.**

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FROM MAMMARY GLAND TO NIPPLE: AN ATTRACTIVE STORY OF FSP1+ MESENCHYMAL CELLS

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The mammary gland has an essential role in the survival and health of mammals, where the produced milk stands as a unique source of nourishment for the progeny. While mammary gland produces the milk itself, the nipple represents the crucial point of milk delivery.

The nipple is a specialized type of epidermis, which is histologically and functionally distinct from the abdominal skin of an animal. It is formed around the lactiferous duct, possesses deep epidermal ridges and it is hairless[1]. The nipple is not just a channel delivering the milk to the progeny, but also a sensitive organ that receives and transduces sensory signals and triggers hormonal responses crucial for sustained milk production.

The development of both, mammary gland and nipple, is tightly connected and depends on instructive signals from the mammary mesenchyme, more specifically, fibroblasts[2]. Yet, the developmental origin, heterogeneity and plasticity of these fibroblasts remains to be determined.

In this study, we use genetically engineered mouse model *Fsp1-Cre*, which allows us to target cells expressing *Fsp1* (*fibroblast specific protein 1*) to describe a role of these cells in development and function of the mammary gland, and the nipple. FSP1 is a marker of mesenchymal cells, mostly considered to be a marker specific for fibroblasts[3].

Here, by *in vivo* lineage tracing approach, we confirmed a mesenchymal nature of FSP1+ cells. We found that FSP1+ mesenchymal cells give rise to a heterogeneous stromal cell population, including fibroblasts and immune cells. Remarkably, the cell populations derived from FSP1+ progenitor are different in mammary and nipple stroma.

Furthermore, by cell-specific ablation study, we observed a severe lactation defect in the mice with depleted FSP1+ cells. Even with preserved lactating capacity, the dams fail to feed the offspring resulting in early mortality of the whole litter due to dramatic nipple underdevelopment.

Taken together, we described FSP1+ mesenchymal cells and their progeny as a heterogeneous stromal cell population which is crucial for nipple development and mammary gland function.

This work was funded by project MUNI/IGA/1311/2021, MUNI/G/1775/2020 and MUNI/A/1301/2022, GAČR 23-04974S, MŠMT ERC-CZ LL2323 FIBROFORCE and Brno city municipality (Brno Ph.D. Talent Scholarship). We acknowledge the core facility CELLIM supported by MEYS CR (LM2023050 Czech-BioImaging).

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POTENTIAL ANTITUMOR EFFECTS OF TRIBUTYLTIN PROPIONATE AND TRIBUTYLTIN SALICYLATE IN BREAST CANCER-DERIVED CELL LINES

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Breast cancer is the most common form of cancer diagnosed in women. Among non-platinum chemotherapeutics based on metal complexes, triorganotin compounds are potential treatment candidates due to their antiproliferative activity and apoptosis-inducing abilities[1]. Several cytotoxic mechanisms are involved in the antitumor effect of organotin compounds, which include modulating the energy metabolism of mitochondria, inhibiting the synthesis of macromolecules and reducing DNA synthesis, as well as increasing the concentration of calcium ions in the cytosol by affecting the cell membrane.

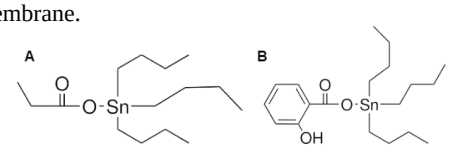

Fig. 1. **Structural formulas of tributyltin propionate (A) and tributyltin salicylate (B).**

The aim of this study was to test the *in vitro* cytotoxic and antitumor effect of two selected triorganotin compounds (Fig. 1): tributyltin propionate (TBT-PROP) and tributyltin salicylate (TBT-SAL) on cell lines derived from breast cancer MCF-7, MDA-MB-231 and MDA-MB-436. The effect of selected compounds on the proliferation of treated cell lines and the effect on the membrane integrity was studied. The cytotoxic effect was demonstrated for both tested compounds (MTT and LDH-cytotoxicity assay). The IC50 ranged from 0.11 to 0.17 μM for TBT-PROP and 0.18 to 0.3 μM for TBT-SAL. Both substances slowed cell migration in a time-dependent manner (Wound healing assay), induced cell apoptosis (RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay) and associated increased activity of caspases, which undergo a cascade of catalytic activation in the early stages of apoptosis (Caspase-3/7-Glo® Assay). In the case of the MCF-7 cell line, there was a decrease in the expression of the apoptosis-suppressing Bcl-2 and the proliferation marker PCNA and an increase in the pro-apoptotic BAX (real-time PCR and Western blot).

This work was supported by grants APVV-20-0314, VEGA 02/0116/21 and VEGA 2/0042/24.

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PHOTOCHEMISTRY AND PHOTOPHYSICS OF NICKEL(II)–BIPYRIDINE ARYL HALIDE COMPLEXES

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Ni(II)–bipyridine aryl halide complexes serve as pivotal photoredox catalysts in organic synthesis, yet specific details regarding their photoactivation remain unresolved[1]. Previous studies have indicated that direct excitation induces homolytic cleavage of the Ni(II)–C(aryl) bond, resulting in the generation of an aryl radical and a Ni(I) species, which is essential component in the proposed Ni(I)/Ni(III) catalytic cycle[2].

Our investigation employs a combination of spectroscopic and computational analyses on diverse Ni(II)-bipyridine photoredox complexes to elucidate the mechanism behind this excited-state Ni(II)–C(aryl) bond homolysis[3,4]. We identify structural and electronic factors influencing Ni(I) formation, demonstrating the ability to finely tune ground- and excited-state properties through the modification of bipyridine ligand substituents. Systematic Hammett analysis, supported by quantum chemical calculations, reveals a direct correlation between reactivity and electronic structure.

Moreover, we introduce a novel class of structurally constrained Ni(II)–bipyridine aryl halide complexes that exhibit a remarkable photochemical stability. Through kinetic analyses and ultrafast transient absorption spectroscopy, we attribute this stability to geometric restrictions, establishing a reversible Ni(II)–C(aryl) ⇄ [Ni(I)…C(aryl)•] equilibrium. Intriguingly, the introduction of an electrophile during photoirradiation shifts the preference for the forward aryl radical photodissociation reaction, establishing the parent Ni(II) complex as a stable source of a reactive Ni(I) intermediate. The insights presented here lay the foundation for designing precisely tailored photoactive catalysts for organic synthesis.

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DEVELOPING SPECIALIZED MOUNTING SYSTEM FOR LONGITUDINAL LIVE CELL IMAGING WITH MULTIVIEW SPIM

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Tissues are formed and maintained by strictly regulated interactions between various cell types. Each cell undertakes a specific fate, such as proliferation, migration, death or survival. These decisions are regulated by numerous signaling molecules that assume different states over time. The investigations of these processes highly rely on the fixed time-point collections of pooled cells. Genetic engineering and light microscopy advancements allow single-cell tracking and analysis through extended time periods with high spatiotemporal resolution.

Multiview selective plane illumination microscopy (SPIM) features illumination and detection objectives on a horizontal plane of the microscope[1]. It utilizes a planar laser light sheet to illuminate the whole sample plane, which increases acquisition speed and reduces photobleaching and phototoxicity. The observed sample is suspended from vertical support inside the imaging chamber, which enables image acquisition from multiple sides. SPIM is, therefore, especially suitable for large samples and live cell longitudinal imaging.

Here, we utilized primary organoids derived from transgenic mouse strain EKAREV-NLS[2] to observe processes and developmental dynamics of mammary gland epithelium. We tested and developed several sample holders and mounting techniques for organoid imaging in multiview SPIM (Fig. 1), including commercially available glass capillaries with different mounting media and in-house-made holders.

We found that the organoids require proper matrix support without tight spatial restrictions to maintain viability and to resume proper morphology and behaviour. Further, efficient support and rigidness are needed to reduce imaging artefacts and unwanted sample displacement. Lastly, we identified two holder setups that incorporate the abovementioned characteristics while allowing proper nutrient exchange and maintaining organoid viability.

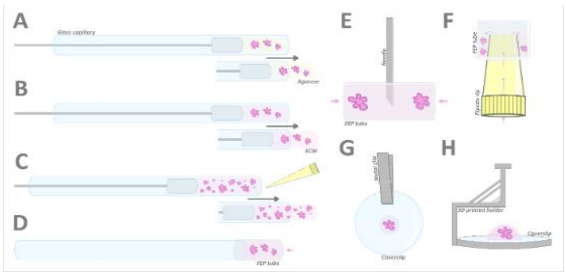

Fig. 1. **The schematics of organoid mounting approaches for multiview SPIM.**

This project was funded by MUNI/G/1775/2020, MUNI/A/1301/2022 and GAČR GA23-04974S.

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ON THE WAY TO CHIRAL SHAPE PERSISTENT π-ELECTRON MACROCYCLES

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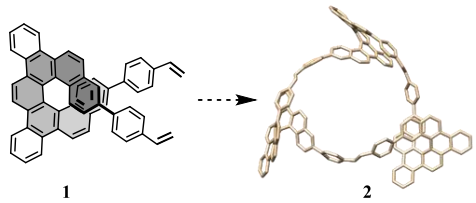
Fully aromatic helicenes are attractive building blocks for the construction of inherently chiral π-conjugated macrocyclic hydrocarbons[1]. These still rare molecular architectures are expected to exhibit interesting (chir)optical properties, self-assembly, charge/spin transport or fascinating Möbius topology. In our group, a macrocycle containing three conformationally unstable dibenzo[5]helicene units has already been prepared in the form of a mixture of stereomers[2].

In order to prepare enantio- and diastereomerically pure macrocycles, it is necessary to synthesize the appropriate conformationally stable helicene building blocks. Here, we report the synthesis of the conformationally stable bis(4-vinylphenyl) derivative of dibenzo[7]helicene **1** (Scheme 1), which was prepared from dichloro dibenzo[7]helicene by Suzuki reaction with the respective boronic acid. Macrocyclization to connect three helicene units via metathesis reaction is now in progress. The optimized structure of macrocycle **2** shows an interesting globular geometry and Möbius topology.



Scheme 1. **Bis(4-vinylphenyl) derivative of dibenzo[7]helicene 1 as a proposed precursor of macrocycle 2 composed of three helicene units.**

This work was supported by Czech Science Foundation (Reg. No. 20-23566S), MSMT (Grant INTER-ACTION, Reg. No. LUAUS24212), and IOCB CAS (RVO: 61388963).

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NANOPARTICLE COUNTING FOR THEIR ABSOLUTE QUANTIFICATION AND CHEMICAL CHARACTERIZATION

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In recent days, nanomaterials have become a new generation of materials with many applications in the life and material sciences including e.g. bioimaging or drug delivery[1]. Their use is governed by their physical-chemical properties including surface chemistry, which affects the potential for conjugation to ligands and biomolecules. Therefore, the chemical characterization of nanoparticle surfaces is critical for understanding and controlling their behavior. Herein, the quantification of carboxyl groups on a variety of nanoparticle types is presented[2].

For this purpose, the quantification of nanoparticles and the quantification of the carboxyl groups have to be carried out. The concentration of nanoparticles can be estimated by several analytical methods[3] with the use of ensemble techniques such as gravimetry or absolute single-nanoparticle counting approaches. Compared to other methods, the counting approaches determine the concentration of nanoparticles without the need for calibration standards, which are often unavailable, especially for new nanomaterials. The counting approaches are based either on counting individual nanoparticles focused through a channel (e.g. resistive pulse sensing or sp-ICP-MS) or on microscopy imaging. Despite two basic imaging techniques available – TEM and optical microscopy – the absolute quantification by single nanoparticle counting remains a technical challenge.

Recently, we reported a new absolute method for nanoparticle counting after their immobilization in anisotropically collapsed gels, which is followed by optical microscopy and nanoparticle counting[4]. The method exhibits excellent properties, such as limit of detection and good accuracy, and it was successfully used for extremely sensitive counting even in a multiplexed detection setting. This study shows its application for the chemical characterization of nanoparticles – a variety of nanoparticle types are quantified by counting, and the number of carboxyl groups per single nanoparticle is estimated by acid-base titrimetry.

The authors acknowledge the grant 24-11183S (Czech Science Foundation), and support from UIACH (RVO: 68081715).

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MYRISTOYL SWITCH AND ITS IMPLICATIONS IN THE LATE PHASE OF M-PMV LIFE CYCLE *IN VIVO*

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Retroviral life cycle has two phases. The early phase includes infection of the host cell, reverse transcription and integration of the transcribed viral DNA into the host genome. The late phase begins with the production of retroviral polyprotein precursors that eventually form an immature viral particle that buds through the cytoplasmic membrane. The particle then undergoes maturation, which causes radical morphological changes following proteolytic cleavage of the polyprotein precursors. Upon maturation, the viral particles become infectious[1]. Assembly of Mason-Pfizer monkey virus (M-PMV) immature particles takes place in cytoplasm of the host cell and they are transported towards the cytoplasmic membrane where budding occurs[2]. The myristoyl moiety of matrix protein (MA), the N-terminal domain of structural polyprotein Gag, is an important late-phase regulatory element as it can be either sequestered in the protein core or expelled and thus available for plasma membrane interaction. This change of myristoyl position (myr switch) also alters the conformation of the first protease cleavage site of the Gag which then becomes accessible for cleavage by viral protease *in vitro* (data currently under review for publication). To study this phenomenon *in vivo*, we introduced point mutations preventing or facilitating myr switch (myr IN or myr OUT

variants) into the vector encoding M-PMV genome. Similarly, mutations blocking proteolytic cleavage of MA from the rest of the Gag were introduced. HEK 293T cells were transfected and viral particles were harvested. Our data show that myr OUT variants undergo proteasomal degradation and therefore their effect on virus maturation could not be confirmed. On the other hand, myr IN variant shows delayed maturation, consistent with our *in vitro* results. Additionally, blocked MA cleavage also delays the maturation process. These data support the *in vitro* findings of the involvement of the myristoyl switch in the regulation of the Gag proteolytic cleavage during M-PMV maturation.

This work was supported by the project National Institute of Virology and Bacteriology (EXCELES, ID Project No. LX22NPO5103).

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NEW PHOTOACTIVABLE RUTHENIUM COMPOUND TRIGGERS ONCOSIS IN CANCER CELLS BY DISRUPTING CELLULAR MEMBRANES

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Poor selectivity of anti-cancer therapy often hinders successful treatment outcomes. Therefore, strategies that can confine the cytostatic effect to cancerous tissue while preserving surrounding tissues are in high demand. Photodynamic therapy (PDT) addresses this challenge by combining systemic administration of a drug with targeted tumor irradiation, aiming to eliminate cancer cells selectively[1].

Photosensitizers, the drugs used in PDT, are known to generate reactive oxygen species upon irradiation. Reactive oxygen species exhibit a very short half-life, so they damage biomolecules in their close vicinity[2]. Consequently, the subcellular localization and affinity for biomolecules play a crucial role in the activity and mechanism of action of the photosensitizers. Metallodrugs emerge as promising candidates for PDT due to their highly tunable photophysical properties[3].

The investigated new photoactivatable ruthenium(II) octahedral complexes based on benzimidazole (synthesized and characterized in the laboratory of Prof. José Ruiz from Universidad de Murcia, Spain) exhibited promising anti-cancer activity in a panel of cancer cell lines, notably demonstrating a more than tenfold increase in activity upon irradiation with green light. The representative compound **Ru1** accumulated in membrane-rich compartments, including the cytoplasmic membrane, mitochondria, and endoplasmic reticulum. Upon irradiation, all these compartments were damaged in treated cells. Based on *in vitro* experiments, we deduced that **Ru1** has the capability to disrupt phospholipid membranes directly. Additionally, differential scanning calorimetry of living cells also indicated denaturation of cytoplasmic/membrane proteins. In summary, the compound appears to effectively impair numerous intracellular membrane systems, ultimately leading to a cell death termed oncosis (see Fig. 1).

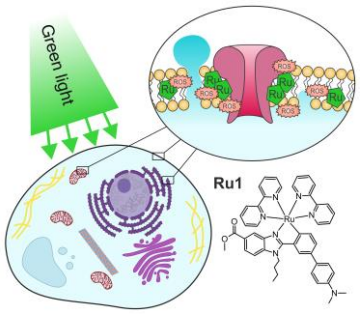

Fig. 1. **Graphical representation of proposed mechanism of action of the Ru1 compound in cancer cells.**

This work was supported by the Czech Science Foundation (Grant 23-06316S).

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DEVELOPMENT TOWARDS THERAPY OF MACULAR DEGENERATION OF RETINA BY DERIVATIVES OF HUMAN PLURIPOTENT STEM CELLS

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Regenerative medicine in the field of ophthalmology was revolutionized in the 1990s through stem cell-based therapies focusing on treating corneal diseases[1]. However, there still exist numerous incurable diseases associated with the perceptive part itself, the retina. The pathogenesis of the multiple forms of retinopathies is often associated with the

dysfunction and loss of retinal cell types, including retinal pigment epithelium (RPE).

Our objective is, thus, to establish for the first time in the Czech Republic the current Good Manufacturing Practise (cGMP) compliant methodological and regulatory grounds for producing a key component of the Advanced Therapy Medicinal Product – human pluripotent stem cell-derived RPE cells[2]. By doing so, we aim to contribute to the advancement of regenerative medicine, particularly in the treatment of retinal diseases.

In our ongoing research, we have determined the optimal conditions for differentiation of hPSC into RPE cells, utilizing established protocols as a foundational reference. We have started with the establishment of the morphological, molecular, and functional criteria to assess the efficacy of the differentiation process, alongside a comprehensive evaluation of the genetic stability to ensure their safety. Our next steps involve the crucial transfer of this methodology to cGMP conditions, laying the groundwork for the translation of our findings into the realm of clinical applications.

This project is supported by Ministry of Health of the Czech Republic (NU22-08-00629) and Grant Agency of Masaryk University (MUNI/A/1598/2023).

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UNVEILING THE IMPACT OF SARS-CoV-2 RdRp MUTATIONS: INSIGHTS INTO PROTEIN STABILITY, ENZYMATIC ACTIVITY, AND DRUG RESPONSE

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Since the SARS-CoV-2 outbreak, missense mutations have repeatedly been identified in all three subunits of the viral RNA-dependent RNA polymerase (RdRp). Despite RdRp being a primary target for antiviral drugs[1], experimental studies of these mutations and the resulting amino acid substitutions have thus far been limited.

To address this, we performed a multiscale analysis of amino acid substitutions in all three subunits of RdRp: nsp7, nsp8, and nsp12[2]. The representative substitutions were selected based on their frequency of occurrence and their potential impact on the RdRp complex. Nano-Differential Scanning Fluorimetry (NanoDSF) and Microscale Thermophoresis (MST) were employed to examine the impact on protein stability and the RdRp complex assembly. Intriguingly, a single substitution in nsp8 increased its melting temperature by 13 °C. In contrast, specific substitutions in either nsp7 or nsp8 resulted in a reduced binding affinity to the main enzymatic unit, nsp12, during the assembly of the RdRp complex. The overall effect on RNA polymerase activity was evaluated utilizing a fluorometric RNA extension assay. As anticipated, most substitutions affecting the polymerase activity correlated with changes in protein or complex stability. Notably, a 50% increase in enzymatic activity was observed with the combination of the nsp8 A21V and nsp12 P323L variants. Moreover, the activity evaluation revealed that some of the investigated substitutions in the RdRp subunits alter the response to Remdesivir treatment, significantly decreasing the efficacy of the drug.

To our knowledge, our results are the first to demonstrate the impact of mutations in all three RdRp subunits and thus provide a basis for understanding novel RdRp variants and their potential relevance for drug development.

This study was supported by the project National Institute of Virology and Bacteriology (EXCELES, ID Project No. LX22NPO5103) – Funded by the EU – Next Generation EU.

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SYNTHESIS OF DIAZAELICENES BY USING DOUBLE SCHMIDT REARRANGEMENT

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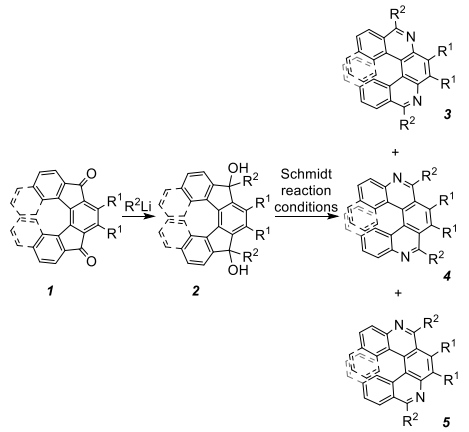
Helicenes are non-planar polycyclic aromatics composed of *ortho*-fused benzene rings[1]. Introducing heteroatoms, such as nitrogen, gives access to azahelicene derivatives, which can remarkably tune optical, electronic, and supramolecular properties[2]. The synthesis of azahelicene derivatives is still challenging and to this day, there are only a handful of methods for synthesizing diazahelicenes.

In this work, our attention was focused on developing a methodology for transforming helical indeno[2,1-*c*]fluorene diones[4,5] ***1*** to diazahelicenes using Schmidt rearrangement[3], exploring the regioselectivity, and expanding the methodology for the synthesis of highly enantioenriched diaza[7]helicenes. Using double Schmidt rearrangement on tertiary diols ***2*** in acidic conditions with sodium azide we were able to obtain, and fully characterize, all three possible regioisomeric

diazahelicenes ***3***-***5***. The highest selectivity for symmetrical product ***4*** with an isolated yield of up to 68% was observed using a strong acid with pK_a below -2 (Scheme 1). The reaction was carried out with several different substrates bearing electron-withdrawing and electron-donating groups. The stepwise nitrogen insertion allowed us to understand the observed regioselectivity to some extent.



Scheme 1. **Transformation of diketones to diazahelicenes.**

This work was supported by Czech Science Foundation (Grant No. 21-29124S).

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PROLONGED METHIONINE DEPRIVATION LEADS TO THE UPREGULATION OF GROWTH SIGNALING IN MALIGNANT MELANOMA

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Methionine is an essential amino acid used in several intracellular processes. It is necessary for translation initiation, nucleotide and polyamine synthesis, redox balance, and synthesis of S-adenosylmethionine (SAM) substrate for methylation reactions. The level of methionine in the cell is sensed by a specific sensor which affects the mammalian target of rapamycin complex 1 (mTORC1), the main regulator of cell metabolism. Compared to leucine or arginine, which are recognized by their sensors Sestrin2 and CASTOR1, methionine is sensed indirectly through its metabolite SAM which binds to its sensor SAMTOR. The interaction of SAM and SAMTOR enables mTORC1 activation and the promotion of cell survival and proliferation[1–3].

Due to its important role in cell growth, mTORC1 pathway is commonly hyperactivated in different types of cancer, including malignant melanoma, the most aggressive type of skin cancer. Its progression usually stems from hyperactivating mutations in growth signaling pathways like ERK and mTORC1 that lead to acceleration of cell division and tumor growth. Despite the existence of new therapeutics that specifically target growth signaling pathways, resistance commonly occurs within a few months. Therefore, new approaches are necessary to find suitable treatment for patients with malignant melanoma.

We tested *BRAF*^{V600E} mutant melanoma cells for their dependence on methionine. They were treated with media without methionine, and although we saw a decrease in global translation in the cell, we did not see the inhibition of the mTORC1 pathway. In contrast, we observed the activation of p70S6K which is a direct target of mTORC1. Even more interestingly, when we examined connected signaling pathways ERK and AMPK, they were upregulated as well. These pathways usually act against each other. ERK pathway promotes cell growth and AMPK inhibits catabolic processes when activated.

These surprising results indicate that *BRAF*^{V600E} mutant melanoma cells are affected by methionine deprivation, and additionally implies that there might be some kind of backup mechanism that boosts all pathways to keep cells alive as long as possible, until there are better nutrient conditions.

This work is supported by the Czech Science Foundation (GA22-30397S), the EU – Next Generation EU – the project National Institute for Cancer Research (EXCELES, Project No. LX22NPO5102), and Brno city municipality (Brno Ph.D. Talent Scholarship).

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PIEZOELECTRICALLY-DRIVEN SYNTHESIS OF PHENANTHRENES UNDER MECHANOCHEMICAL CONDITIONS

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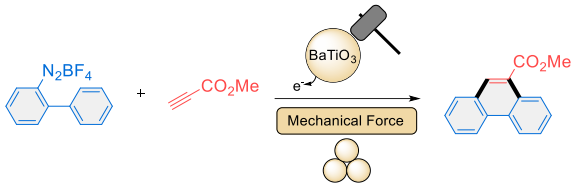
The phenanthrene building block is often used as a precursor for material and medicinal chemistry. Preparation

is provided primarily by the radical cyclization reaction of biphenyldiazonium salts with alkynes[1]. The radical species required for this process are obtained by single-electron redox transformations, typically achieved through photoredox catalysis or electrochemistry[2]. Recently, mechanochemically driven piezoelectric redox catalysis has been discovered significantly simplifying the reaction setup and its efficiency[3]. The study aimed to integrate piezoelectric redox catalysis into phenanthrene synthesis *via* mechanochemical conditions and optimize the proposed radical cyclization conditions. Targeted parameters for optimization included solvent screening and loading (liquid-assisted grinding agent, LAG), the amount of piezoelectrically active catalyst, substrate concentration and time. To our surprise, the key to improving the reaction yield was purifying commercial piezoelectrically active BaTiO3 from inorganic impurities originating from its synthesis. This also led to a significant reduction in reaction time from 60 minutes to 5 minutes, a dramatic improvement over examples in the literature. To our delight, optimized conditions resulted in an 80% isolated yield within 5 minutes of reaction time under discovered mechanochemical conditions.



Scheme 1. **Reaction scheme for preparation of phenanthrenes.**

Funded by the EU (ERC, CAPELE, 101078608). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the EU or the ERC. Neither the EU nor the granting authority can be held responsible for them.

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POTENTIAL OF SPIDER SILK PROTEIN-DNA BIOCONJUGATES FOR BINDING BIOLOGICALS

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Most of the proteins are able to undergo a self-assembly, an autonomous process, based on non-covalent interactions between partially unfolded protein molecules, which requires no additional energy sources[1]. As a result, higher order fibrillar nanostructures are formed. Together with the possibility of chemical modification of proteins with functional ligands such as DNA oligonucleotides there is a great potential for processing new bio-based nanomaterials with supplementary functions[2].

The objective of this study was to chemically modify recombinant spider silk protein, eADF4(C16)[2], with exceptional characteristics, for instance biocompatibility, biodegradability, high mechanical strength and toughness, by binding of functional DNA oligonucleotides TBA15 and TBA29 known for their specific binding to thrombin enzyme[3]. The synthesis of such bioconjugates was performed by "click" reaction after modifying the N-terminus of eADF4(C16) with azide group and 5' end of DNA aptamers with 5'-di-benzocyclooctyne[4]. Methods like MALDI-TOF, HPLC and native gel electrophoresis were used to verify these reactions.

Variety of biochemical/biophysical spectroscopy techniques and atomic force microscopy have been utilized to investigate the self-assembly process of prepared TBAx-eADF4(C16). Obtained experimental data revealed that conjugates preserve their ability to self-assembly into fibrils and this process could be controlled by experimental conditions, i.e. by the concentration of phosphate ions, K^+/Na^+ ions, temperature. Addition of biologically relevant agent thrombin, which binds to TBA15 and TBA29 moiety of conjugates into solution and extensive characterization of the interactions between known components may result in formation of hierarchically organized structures that may possess potential applications, for example, as the drug delivery systems or biosensors.

This work was supported by the grants VEGA 2/0034/22, SK-DAAD 2024-2025 and Preveda OZ.

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THE EFFECT OF THE MYC ONCOPROTEIN ON SENSITIVITY TO HSP90 INHIBITION

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Hsp90 inhibitors such as Luminespib or Pimitespib have recently entered clinical trials with acceptable results in progression free and overall survival[1–3]. The aim of this study is to better understand the mechanisms of Hsp90 inhibition and try specifying factors that could increase effectivity of therapy.

We decided to test whether increased protein synthesis via induced MYC expression would result in an increased sensitivity to Hsp90 inhibition. The MYC oncoprotein is a transcription factor that binds specific sequences called E-boxes, that serve as promoters to a variety of genes of which many influence the rate of rRNA transcription, and therefore regulate ribosome activity. MYC overexpression is a frequent marker of tumorigenesis facilitating increased protein synthesis and cell proliferation.

The experiments were conducted on RPE-1 non-tumor cell lines possessing inducible expression of MYC. We have observed that cells with elevated MYC expression are significantly more sensitive to cellular stress induced by Hsp90 inhibition. Conversely, cells treated with inhibitors of protein synthesis exhibit higher tolerance to proteotoxic stress.

The findings suggest that novel therapeutics targeting Hsp90, such as Pimitespib, should be primarily used in tumors exhibiting heightened MYC expression and preferably not combined with conventionally prescribed inhibitors of protein synthesis, such as mTOR inhibitors, to ensure maximum efficiency of therapy.

This work was supported by the project National Institute for Cancer Research (EXCELES, ID Project No. LX22NPO5102) – funded by the EU – Next Generation EU, by Ministry of Health Development of Research Organisation, MH CZ - DRO (MMCI, 00209805) and by the Czech Science Foundation (22-17102S).

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KILL ME WHEN YOU CAN ELSE CATCH ME IF YOU CAN: LESSONS FROM CAR-T CELL MEDIATED CD19 ESCAPE IN B CELL MALIGNANCIES

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Chimeric antigen receptor T (CAR-T) cells are genetically engineered T lymphocytes widely being investigated for their ability to treat certain malignancies, especially leukaemia and lymphomas. They target, recognise and kill the malignant cells specifically and effectively. Despite their precisive mode of action, CAR-T cells still possess certain limitations, often leading to a relapsed disease state. Target antigen escape and downregulation of antigen is a prominent mechanism in relapsed patients treated with CAR-T cells. Reports have shown target antigen escape may be due to mutations in the CD19 gene[1], alternative splicing, glycosylation[2], trogocytosis and hypermethylation of promoter[3].

Our mouse model demonstrated a certain fraction of recurring tumours that had lost CD19 expression. To investigate it further *in vitro*, co-cultures using CAR-Jurkat cells or primary CAR-T cells showed target antigen loss across many tested malignant B-cell lines. We didn't find any mutations in the CD19 exons, nor trogocytosis as the mechanism. To observe the effect of killing dynamics, we used primary CD4+CAR-T, CD8+CAR-T, Jurkat-CAR and two NK lines KHYG-CAR (less cytotoxic) and NK92-CAR (highly cytotoxic). The cells with high cytotoxic effect were able to efficiently kill target cells while the cells with less cytotoxicity were unable to kill the cells as they mediate downregulation of CD19 antigen. This may be due to the importance of timing in CAR-T cell-mediated killing. We found prolonged interaction between CAR-T cell and CD19 leads to CD19 antigen loss as CAR-T cells failed to kill them within this timeframe.

This work has received funding from the Czech Science Foundation, project no. 22-35273S and MUNI/A/1558/2023.

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NOVEL SPECIALISED METABOLITE WITH 4-ALKYL-L-PROLINE MOIETY ISOLATED FROM MARINE ACTINOBACTERIA

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The marine environment is a rich source of actinobacteria, which are significant producers of specialised metabolites with a wide range of bioactivities and applications[1,2]. 4-Alkyl-L-proline derivatives (APDs) represent unusual moiety that are incorporated into structurally and functionally diverse groups of complex natural products[3]. APDs are structurally similar to the proteinogenic amino acid L-proline, however, they are biosynthesised in a specialised metabolic pathway from L-tyrosine. APDs are included in the lincosamide antibiotic lincomycin[4], the large group of

antitumor agents pyrrolobenzodiazepines (PBDs)[5], the signalling molecule hormaomycin[6] and last, but not least, lucentamycins with antitumor activity[7].

The advent of high-throughput sequencing technologies is opening opportunities for the discovery of new bioactive compounds. Genome mining uses these advances to explore and characterise the genetic potential of microorganisms[8,9]. Specifically, based on the sequencing data of the genome, we find the homologue genes for the biosynthesis of APD from the marine actinobacteria genus *Salinispora*. With the help of bioinformatics tools, we identified the *apd1*, *apd2*, *apd3*, *apd4* and *apd6* genes that correspond to the APD moiety from the structure of hormaomycin. In addition, the novel APD compound is a non-ribosomal peptide that is structurally different from the well-known lincosamides, PBDs, hormaomycin or lucentamycins, thus expanding this group of APD compounds. So far, preliminary bioassay shows antibacterial activity against Gram-positive *Kocuria rhizophila*.

The work was supported by the Lumina Quaeruntur Program No. LQ200202002 from the CAS and by the SEA-Europe Joint Funding Scheme 4th Joint Call for Proposals (Innovation) in the Thematic Area of Infectious Diseases (including Antimicrobial Resistance [AMR]) Project No. SEAEUROPEJFS19IN-080.

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AUTOMATIC BENCH TOP ELECTROCHEMICAL STATION FOR DEVELOPMENT AND ANALYTICAL APPLICATION OF DOPAMINE AND PARACETAMOL SENSORS

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The proposed contribution deals with possibilities of automation in the field of performing an analytical process, expedite the development of the new analytical methods and high-performing preparation of modified sensor/biosensors by implementing automatic benchtop electrochemical station. Gold nanoparticles (AuNPs) represent promising material for electroanalytical chemistry especially for biosensors development and sensors surface modification. The benefit of AuNPs comes from excellent conductivity, high surface to volume ratio and favourable catalytic properties[1].

In situ preparation of AuNPs modified screen-printed carbon electrode (AuNPs-SPCE) was accomplished by electrodeposition technique and individual deposition parameters such as concentration of deposition solution ($HAuCl_4$), deposition time t_{DEP} and applied deposition potential E_{DEP} were systematically examined in detail on 1 mM hexaammineruthenium (III) chloride and 50 μM dopamine (DOP). The surface morphology of modified SPCE sensor at different modification conditions was investigated by SEM. Analytical performance of AuNPs-SPCE sensors was assessed by construction of calibration curves of DOP and paracetamol (PAR) as a model/target analytes. SPCE and AuNPs-SPCE at modification condition providing the best sensitivity was successfully applied to determine DOP and PAR in real sample of tap water using "spike-recovery" accession. Automatic analyser helps to yield exceptional reproducibility of the modification of SPCE by AuNPs in comparison, when SPCE was manually modified with AuNPs by highly qualified lab analyst. Automatic benchtop electrochemical station can significantly decrease handling cost while saving time and using environmentally friendly materials what highly meets the requirements of the green analytical chemistry[2].

The authors thank the VEGA project No. 1/0036/24 and the IUPAC project No. 2023-010-2-500.

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NAPTHOYL-BENZOTHIAZOLE BASED HYDRAZONE PHOTOSWITCHES

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In recent years, there has been a growing scientific interest in advanced molecular materials capable of modulating their physicochemical properties in response to external stimuli. Among the most captivating representatives

are molecular photoswitches, which undergo light-induced reversible structural changes. This property endows them with considerable potential for applications across a wide range of technological domains, including optoelectronics, solar energy storage, imaging photo-pharmacology, and other biological aplications[1,2].

Triarylhydrazones[3,4] represent a novel compelling class of photochromic compounds offering intriguing features, including good addressability, high molar absorptivity, extraordinary thermal stability, and particularly effective light-absorption above 365 nm, contrary to many diarylhydrazone-based photoswitches[5,6]. However, some of them suffer from low quantum yields of the light induced *Z*→*E* isomerization due to the formation of a strong hydrogen bond in the excited state.

We have developed[7] a novel subclass of hydrazone photoswitches based on naphthoyl-benzothiazole with preserved attractive absorption characteristics, high thermal stability, but also particularly enhanced efficiency of *Z*→*E* photoisomerization (Fig. 1).

This design presents a promising advancement in the field of hydrazone-based molecular photoswitches, opening up new possibilities for diverse applications in photo-pharmacology and high-tech fields.

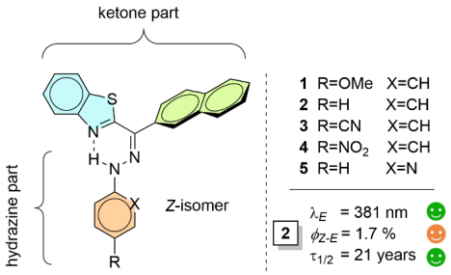

Fig. 1. **Molecular structure of napthoyl-benzothiazole based photochromic triarylhydrazones.**

This work was funded by Slovak Research and Development Agency (APVV-20-0098).

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SYNTHESIS AND EVALUATION OF NEW ZWITTERIONIC CHIRAL STATIONARY PHASES FOR SEPARATION OF POLAR SUBSTANCES

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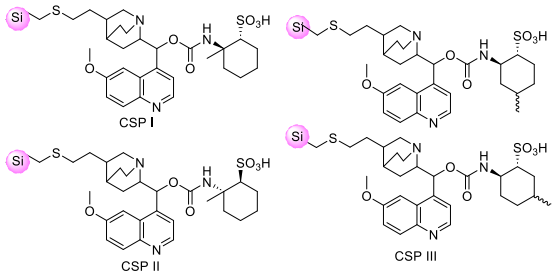
Chiral separation of racemic mixtures on chiral stationary phases (CSPs) is recognized as the most common first-choice approach to obtain pure enantiomers. Nowadays, there is a broad variety of commercially available CSPs, which are routinely used in research laboratories and industry, and allow for resolution of almost any racemic mixture of choice[1]. The developed CSPs are mostly optimized to have the broadest possible scope of application for compounds possessing various structures and chemical properties. Specific CSPs are usually required for the chiral separation of charged or chargeable compounds. To address this need, chiral ion exchangers, which can separate a broad variety of such substances, have been developed[2].

In this work, we have focused on novel zwitterionic CSPs, which are well suited for chiral separation of acidic, basic and zwitterionic analytes. We synthesized a series of selectors, which were immobilized onto 3-mercaptopropyl-modified silica by means of radical reaction. The target chiral stationary phases (Scheme 1) were slurry packed into chromatographic columns and evaluated in different mobile phases using LC-UV and LC-MS systems. We have optimized the chromatographic conditions (mobile phase composition, buffer type and concentration, flow rate, etc.). We show that the composition of mobile phase play a crucial role in separation mechanism by influencing which structural unit preferentially interacts with analytes.



Scheme 1. **Structure of new synthetised ZWIX selectors.**

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USE OF MACHINE LEARNING FOR DETECTION OF GUT MICROBIOME BIOMARKERS OF PATIENTS WITH AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a neurological and developmental disorder that impacts behavior and communication. ASD is heritable, caused mainly by genetics. However, environmental factors are also highly relevant. There is a high prevalence of gastrointestinal disorders among ASD patients, suggesting that gut microbiota may be a potential trigger in ASD pathogenesis[1]. The gut-brain axis communication is also mediated through microbiota-derived signaling molecules. Alterations in the gut microbiome or its dysbiosis may alter the production of bacterial metabolites, such as short-chain fatty acids, indoles, and polysaccharides, which were observed to be modified in autistic patients. Dysbiosis of gut microbiota is frequently observed in ASD patients, but thus far, no reliable diagnostic biomarkers are available[2]. To search for such biomarkers, a machine learning approach was used.

Gut microbiome samples were obtained from young female ASD patients. Extracted DNA was sequenced with the use of the NGS. Quality control of data and taxonomical classification was performed with the qiime2 pipeline.
Obtained gut microbiome data were subjected to a machine learning algorithm. For the ASD, 16 bacterial genera were identified as potential biomarkers. The biomarkers with the highest impact included bacterial genera *Agathobacter*, *Faecalibacterium*, *Alistipes*, and *Bacteroides*. For the pilot analysis, a test dataset of 9 samples, including 6 patients and 3 controls, was chosen. The area under the curve of the receiver operating characteristic reached the value of 0.92 with an accuracy of 0.89. Except for one, all the samples were classified correctly.

This work was supported by the project APVV-20-0114 funded by the Slovak Research and Development Agency.

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SHEDDING LIGHT ON THE SECRETS OF NANOLUC, ITS MECHANISM, AND ALLOSTERIC BEHAVIOUR

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NanoLuc luciferase is a tiny enzyme with an exceptionally bright bioluminescence. Thanks to these desirable properties, NanoLuc has been widely used in biotechnology and biomedicine since being designed in 2012 from the luciferase of a deep-sea shrimp *Oplophorus gracilirostris*[1]. Unfortunately, the mechanism of NanoLuc's light-emitting reaction has not been solved. This knowledge is, however, vital for designing the next generation of bioluminescent systems. Therefore, we applied multiple lab- and computer-based methods to study NanoLuc's catalysis, including crystallography, kinetic measurements, molecular docking, and molecular dynamics simulations with enhanced sampling.

One of the advantages of NanoLuc is its small size of 171 amino acid residues compared to luciferases from sea pansy *Renilla reniformis* (311 residues) and firefly *Photinus pyralis* (550 residues). We confirmed that NanoLuc is indeed monomeric in solution; however, it is packed as a crystallographic homotetramer in some crystals. Moreover, we identified two distinct binding sites of the substrate molecule: the catalytic site, which is buried in the core of NanoLuc in its monomeric form, and an allosteric binding pocket shaped on the oligomerization interface of NanoLuc crystals[2]. Importantly, we have demonstrated that modifying the allosteric site can enhance the bioluminescent reaction in the active site.

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DETERMINATION OF KRATOM ALKALOIDS CONTENT IN URINE BY CAPILLARY ZONE ELECTROPHORESIS HYPHENATED WITH MASS SPECTROMETRY DETECTION

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Kratom is an herbal drug belonging to the group of new psychoactive substances. Its psychoactive effect is mediated by the presence of two indol alkaloids – mitragynine (MIT) and 7-hydroxymitragynine (7-OH-MIT). These alkaloids exhibit dose-dependent effect. The psychostimulant effect of kratom appears at doses to 5 g of dried plant, while doses above 5–10 g are responsible for opioid-like effect[1].

Liquid chromatography (LC) is considered as the standard analytical method for kratom alkaloids determination in biological matrices. In the proposed work, we developed an alternative capillary zone electrophoretic (CZE) method characterised by many advantages over conventionally used LC approach, such as low environmental load (due to minimal consumption of organic reagents), high selectivity, sensitivity and separation efficiency, or cost-effectiveness. Moreover, the in-capillary preconcentration approach based on dynamic pH junction was introduced to provide enhancement of separation effectivity.

The background electrolyte composed of 100 mM formic acid (pH 2.39) was chosen as the optimal separation environment. The proposed CZE-MS/MS method successfully passed the complex validation protocol according to FDA validation guidelines for bioanalytical methods. Parameters such as linearity ($r^2 > 0.99$), precision, accuracy or short-term stability met the defined criteria (%RSD and %RE values where within ± 15 %). Limit of detection for MIT and 7-OH-MIT in urine samples was 0.5 ng/mL and 2 ng/mL, respectively.

The developed method was finally used for MIT and 7-OH-MIT content determination in model urine samples. Obtained results clearly demonstrated the ability of the method to be implemented in routine toxicology use.

This work was supported by the project VEGA 1/0514/22 and was carried out in the Toxicological and Antidoping Center at the Faculty of Pharmacy, Comenius University in Bratislava.

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MASS SPECTROMETRY-BASED SIDEROPHORE SCREENING OF EMERGING FUNGAL PATHOGENS

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Fungal siderophores are virulence factors biosynthesized to scavenge iron in iron-limited settings and biomarkers whose detection overcomes current limitations in clinical mycology[1]. *Scedosporium apiospermum* (*Sa*) and *Lomentospora prolificans* (*Lp*) have been considered by WHO medium-level fungal threats to public health. However, their invasive infections can result in a mortality rate of up to 90%[2]. So far, the detection of *Sa* siderophores is limited to N^{α}-methyl-coprogen B (*N*-CopB) and dimerumic acid (DMA) in sputum samples from cystic fibrosis patients[3]. There are no reports on *Lp* siderophores. This work provides new insights on the metabolome of *Sa* and *Lp*.

The study began by comparing sensitivities through analysis of coprogen (Cop) standard using LC-MS-ESI+ and MALDI+. Following this, *Sa* and *Lp* clinical strains were challenged by cultivation under iron restriction. Supernatants were collected at eight time points (0–96 hours) and analyzed using LC-MS-ESI+. Biomass was harvested at the 96th hour and the collected data underwent screening with CycloBranch.

LC-MS-ESI+ yielded 11-fold better detection limit for Cop (LOD 0.9 and LOQ 2.7 ng/mL) than MALDI+ (LOD 9.8 and LOQ 29.6 ng/mL). Both strains biosynthesized the same spectrum of siderophores: coprogen B (Cop B), *N*-CopB, dimethyl-coprogen (DM-Cop), and DMA, with *N*-CopB being secreted earliest at the 19th hour. Upon normalization of *N*-CopB concentration to dry biomass, *Lp* was found to be a 100-times stronger coprogen producer.

The metabolome of *Sa* and *Lp* was updated by identifying potential biomarkers (Cop B, *N*-CopB, DM-Cop, DMA) for mass spectrometry-based diagnosis of invasive scedosporiosis or lomentosporiosis. Although MALDI+ has an 11-fold worse LOD and LOQ, it can still be used in clinical mycology as a time-saving ionization technique compared to LC-MS-ESI+.

Support: MSMT (CZ.02.01.01/00/22_008/0004597), Petr Hubáček, Radim Dobiáš, the IGA of Palacký University (IGA_PrF_2023_027).

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UNFOLDED PROTEIN RESPONSE IN MITOCHONDRIA AND ENDOPLASMIC RETICULUM INDUCED BY 3-NITROPROPIONIC ACID OF SH-SY5Y CELLS

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All major neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and prion-related diseases are also known as misfolded protein diseases, and show similarities concerning protein aggregation and neuronal loss or dysfunction[1]. Accumulation of misfolded protein in mitochondria or ER leads to the activation of organelle-specific unfolded protein response (UPR), which cells use to restore homeostasis. Prolonged stress, when cells are not able to restore homeostasis, can lead to cell death[2]. It is known that mitochondria and ER have very close contact sites, which they use for communication and for the exchange of Ca^{2+} ions, proteins, and lipids, which it's called mitochondrial-associated endoplasmic reticulum membranes (MAMs). Therefore, dysfunction of ER is associated with mitochondrial dysfunction and vice versa[3].

For experimental studies, we use neuroblastoma cell line SH-SY5Y, frequently used in neurobiology as an *in vitro* model for the study of neurotoxicity in dopaminergic neurons. We treated our cells with 3-nitropropionic acid (3-NPA), which is an irreversible inhibitor of complex II of the respiratory chain. The relative viability of the cells was determined by MTT assay. We observed that the viability of the cells treated with 1 mM 3-NPA for 72 hours were significantly decreased. The expression of specific proteins was analysed using *western blot* analysis of the cell extracts prepared from the cells treated with 3-NPA at concentrations of 0.2 and 1 mM for 24 hours. We observed down-regulation of both, mitochondrial chaperon HSP60 that represents an important protein of mtUPR and voltage-dependent anion channel 1 (VDAC1), which is part of MAMs. GRP78 which is crucial in the first step of ER specific UPR was also down-regulated.

Based on our findings we suggest, that the cells died through mitophagy and the structure of ER was disrupted, but the activation of UPR was not observed. However, because the exact mechanism of the impact of 3-NPA on ER and mitochondria is not known, it should be further investigated.

Supported by VEGA 1/0183/23.

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***MONASCUS* SPP. EXTRACTS AS PHOTOANTIMICROBIALS EFFICIENT AGAINST BACTERIA**

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The fungus *Monascus* is a pigment- and pharmaceutical-producing genus well known in Asian countries. The main secondary metabolites are *Monascus* pigments (MPs) which are used as food colorants, but are currently studied also for their beneficial biological properties; such as antimicrobial, antioxidant, anti-inflammatory and anticancer[1].

The antimicrobial activity of MPs against various microorganisms has been reported[2]. Yellow MPs exhibit selective antimicrobial activity against G^+ bacteria[3,4], though their effect against *B. subtilis* is lower than that of orange MPs[3]. Orange MPs have displayed antimicrobial activity against *S. aureus*[5,6], *E. coli*[7] and *B. subtilis*[3]. Red MPs and their derivatives are effective against G^+ and G^- bacteria, as well as against some filamentous fungi[5,8–10].

However, the potential use of MPs as photosensitizers (PSs) in antimicrobial photodynamic therapy (aPDT) has not been tested yet. PSs are synthetic or natural compounds, that can be activated by visible light at an appropriate wavelength, and, in the presence of oxygen, induce the release of reactive oxygen species (ROSs) that cause oxidative stress, leading to the death of eukaryotic or prokaryotic cells[11].

We have screened the effects of eight different extracts from the liquid- and solid-state fermentation of *Monascus purpureus* DBM 4360 and *Monascus* sp. DBM 4361 against model G^+ and G^- bacteria. It was found that all extracts showed varying antimicrobial activity against *B. subtilis* in dark which was further increased after irradiation. *E. coli* was tolerant to the extracts' exposure in the dark but sensitivity to almost all extracts occurred after irradiation. The *Monascus* sp. DBM 4361 extracts seemed to be the best potential candidate for aPDT against Gram-positive bacteria, being efficient at low doses.

This work was supported by the grant of Specific university research – grant A2_FPBT_2022_016.

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JULIA-KNOCIENSKI-LIKE CONNECTIVE C-C AND C=C BOND-FORMING REACTION AND ITS APPLICATION

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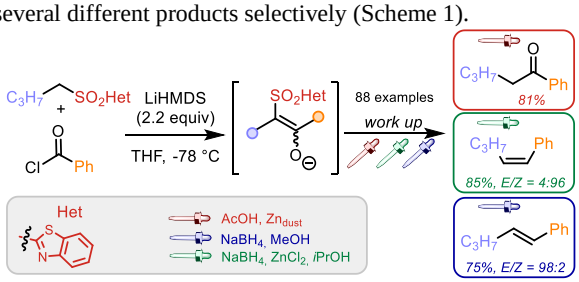
Carbon-carbon bond-forming reactions are the keystone methods in organic chemistry. Not surprisingly, many such methods have been developed over the past few centuries, but only a few of them allow the formation of various products (from stereochemical or functional group presence viewpoint) from the same set of the starting materials only by varying the conditions of the reaction work-up. The typical example of such reaction is Peterson olefination, which allows the selective formation of (*E*) or (*Z*)-olefins with respect of the selected work-up procedure (acidic vs. basic)[1]. The aim of our project was to extend the Julia-Kocienski olefination protocol to (**a**) previously unexplored substrates (use of acyl halides as electrophiles), (**b**) use of reaction work-up protocols to allow selective formation of (*E*) or (*Z*) olefins and (**c**) introduce the product functional group diversity to the coupling (to form other products than olefins). In short, a set of starting materials should produce by altering the reaction work-up protocol several different products selectively (Scheme 1).



Scheme 1. **Pseudo-Julia-Kocienski-like connective C-C and C=C bond-forming reaction[2].**

In this contribution, the scope and limitations of the developed method, the proposed reaction mechanisms, and the application of the developed methods in the context of natural product synthesis (fatty and nitro-fatty acid synthesis) will be discussed[2].

This work was supported by the IGA of Palacky University (IGA_PrF_2023_031 and IGA_PrF_2023_20) and Grant Agency of The Czech Republic (no. 23-06051S).

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SMALL DRUG-LIKE MOLECULES TARGETING RNA STRUCTURES

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Most of the currently available market drugs are designed to target proteins, but around 80% of disease-related proteins are considered undruggable; therefore, selecting another suitable candidate is crucial for treating many systemic and cancer-related pathologies. Around 75 % of the human genome is transcribed into RNA, while only a small fraction (~3%) of it translates to protein[1]. Hence, RNA can be exploited as a potential therapeutic target. Specifically, the 3' untranslated regions (3'UTRs) of RNAs which play a crucial role in *mRNA* stability and gene expression regulation, have distinct secondary and tertiary structures that interact with proteins within cells. In this study, the goal is to identify small drug-like molecules that can target the 3'UTRs of several non-druggable oncogenes and non-oncogene addiction genes, such as *MYC*, *KRAS*, *HSF1*, *CDK12*, *NRF2*, etc.

A library of specialized heterocyclic compounds representing drug-like small molecules was screened against 120 nucleotide fragments of the *MYC mRNA* 3'UTR using a high-throughput fluorescence-based anisotropy assay (FA)[2]. Selected RNA fragments were subsequently screened using another biophysical technique called Surface Plasmon Resonance (SPR) to confirm the binders. Our screening approach leverages the conformational changes in RNA fragments upon small molecule binding[3]. Further characterization of these interactions will enable us to create a library of RNA motif-small molecule interaction pairs that can be utilized to target other *mRNAs*. These interaction pairs will aid in identifying lead compounds capable of modulating *mRNAs* and *mRNA*-protein complexes within cells[4]. Disrupting RNA-protein interactions with small molecules can result in the downregulation of disease-related protein expression, with potential therapeutic implications.

We successfully established the FA as a high-throughput method for screening of small molecules against the RNA fragments. We found significant binders that affected specific RNA fragments by extending or compacting the RNA structure. Through RNA structure webserver (Mathews labs) we looked at the predictive secondary structure of RNA fragments and found that majority of significant binders either interact with RNA having multivalent stem-loops in a particular fashion or with RNA having higher number of internal loops. By looking at the functional screening results of the same 344 compounds against *MYC mRNA* 3'UTR expressed cells using Dual-Luciferace assay, we found four hits that showed upregulation. Upon comparing these results with FA readout, we found these four hits interacted with the *MYC* fragments having multivalent stem-loop structures only and thus compacted the RNA structure.

We conclude that multivalent stem-loop structures have propensity to form highly significant tertiary structures and can act as pockets for small molecule binding. This can ultimately cause changes in the gene regulation of cancer-related genes and therefore can be used as a drug target.

The project is supported by Czech Science Foundation Grant (GACR: GA22-20110S). Sincere thanks to Dr. Lumír Krejčí, Dr. Josef Houser, CEITEC core facility, NCBR Masaryk University and my lab colleagues.

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APPLICATION OF 5-SUBSTITUTED *N*-FLUOROALKYLATED 1,2,3-TRIAZOLES IN DENITROGENATIVE REACTIONS

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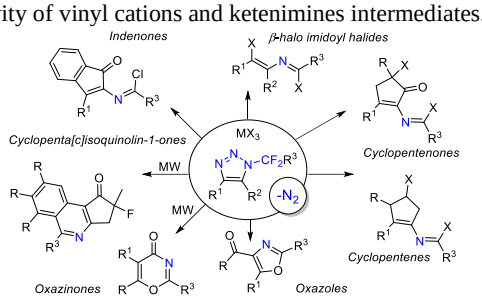
N-electron-acceptor-substituted 1,2,3-triazoles are excellent substrates for denitrogenative reactions[1]. 5-Substituted *N*-fluoroalkyl-1,2,3-triazoles, available by the click reaction of copper acetylides and fluorinated azides, react with Lewis acids (AlX_3 or $BF_3{\cdot}OEt_2$) to vinyl cation species and ultimately to *N*-haloalkenyl imidoyl halides[2] or multi-substituted cyclopentenes[3]. Moreover, it also is possible to generate ketenimine intermediates under thermal microwave assisted conditions[4]. In this report, novel strategies leading to multi-substituted cyclopentenones, indenones, oxazoles, oxazinones and cyclopenta[c]isoquinolinones will be presented. These processes rely on the generation and reactivity of vinyl cations and ketenimines intermediates.



Scheme 1. **Utilization of 5-substituted *N*-fluoroalkyl-1,2,3-triazoles in denitrogenative transformations initiated by Lewis acids or microwave conditions leading to various heterocyclic or *N*-alkenyl products.**

This work was financially supported by the Czech Science Foundation (23-04659S).

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NEW METAL-CATALYZED METHOD FOR STRUCTURAL MODIFICATION AT POSITION 10 ON THE CYTOCHALASAN CORE

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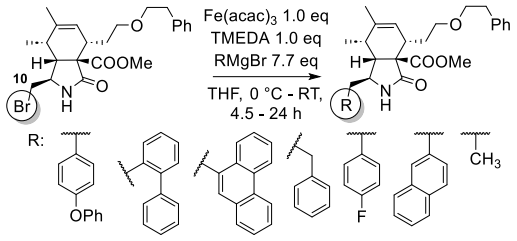
Cytochalasans are natural fungal metabolites that exhibit various biological activities, including cytotoxic and antimetastatic effects. Over 400 of them have been isolated, indicating significant structural variability on a six-membered ring of a bicyclic lactam, on a macrocycle or at position 10 originating from an amino acid[1,2].

Only a limited number of cytochalasans with the structural variation at position 10 have been isolated or prepared. Therefore, sufficient SAR studies have not been carried out so far. Furthermore, there is no universal method for synthesising them in addition to the chemoenzymatic way[3].

The new synthetic approach to obtain cytochalasan analogues with structural modification at position 10, where aryl and benzyl groups are located, is presented (Scheme 1). The cross-coupling method was first studied on a pyrrolidinone-type model compound, followed by the

preparation of suitable starting material with cytochalasan core. The method was then tested and optimised using previously prepared starting material (see above) and a library of seven new analogues was synthesised. The target compounds were characterized and their biological activities were assessed.



Scheme 1. **Novel cross-coupling method to obtain cytochalasan analogues with structural modification at position 10.**

This work was supported by the Czech Science Foundation (project nr. GM23-05336M), a grant of Specific university research, and institutional support of the Dagmar Procházková fund.

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SOLID-PHASE SYNTHESIS OF HYPERMODIFIED HYDROPHOBIC OLIGONUCLEOTIDES

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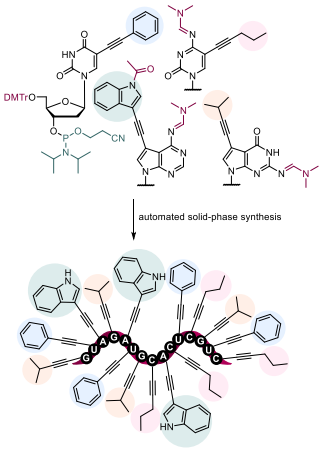
For targeting undruggable lipophilic proteins by oligonucleotide aptamers, the incorporation of hydrophobic moiety to the oligonucleotide backbone was previously shown to enhance the aptamer affinity[1]. Therefore, hypermodified oligonucleotides, with every nucleobase displaying hydrophobic modification, could increase the binding efficiency of the aptamers targeting hydrophobic proteins. Previously, such hypermodified oligonucleotides were synthesized only enzymatically[2], but to fully explore their potential for the selection of aptamers, it is essential to prepare them in large scales using the automated solid-phase synthesis of oligonucleotides. Therefore, we designed and synthesized a set of 2'-deoxyribonucleoside 3'-phosphoramidites derived from 5-phenylethynyluracil, 5-(pentyn-1-yl)cytosine, 7-(indol-3-yl)ethynyl-7-deazaadenine and 7-isopropyl-ethynyl-7-deazaguanine[3]. These nucleoside phosphoramidites were then successfully applied in the solid-phase synthesis of oligonucleotides synthesizing sequences containing one or several modifications, eventually synthesizing fully modified sequences and additionally investigating their hybridization.



Scheme 1. **Four differently modified nucleoside phopshoramidites were synthesized and used for solid-phase synthesis of hyper-modified hydrophobic oligonucleotides.**

This work was supported by the Czech Science Foundation (20-00885X).

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CREATING A CUSTOMIZED MICROENVIRONMENT FOR CELL AND ORGANOID DIFFERENTIATION USING MICROFLUIDICS

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Advances in material science and engineering have paved the way for what has come to be known as microphysiological systems, or organs-on-chips. In these systems, *in vitro* cell cultivation takes place in a tailor-made microfluidic device. This allows for the recapitulation of specific physiological processes or conditions seen *in vivo* in ways that could not have been possible in conventional cell culture vessels. Examples of these include the processes of vascularization[1], signalling gradient formation[2], or the

crosstalk of various tissue analogues[3]. In this work, microfluidic devices are used to better recapitulate the microenvironmental niche for cell differentiation.

The cells microenvironment is of interest because the mechanical and biochemical cues that make up this microenvironment influence many critical functions of the cell, such as metabolism or differentiation[4]. This can be seen when mesenchymal stem cells are directed towards neurogenesis, myogenesis, or osteogenesis based on the stiffness of the surrounding extracellular matrix matching the stiffness of brain, muscle, or bone tissue respectively[4,5].

In this study, a microfluidic device was designed to mimic various elements of developmental lung tissue. While the device is intended for lung differentiation, it is readily adaptable for other tissue types. To this end, a first device was made for the formation of cell spheroids. Next, an additional design was made for the cultivation of cell constructs embedded in a hydrogel. Subsequent efforts focused on the inclusion of additional features found *in vivo*, most notably biochemical gradients and a vascular element. It is hypothesized that the environment created in our device will allow for lung organoid differentiation in a more physiologically relevant, *in vivo*-like fashion.

This work was funded by Brno city municipality (Brno Ph.D. Talent Scholarship).

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UNCOVERING MECHANISMS OF ALZHEIMER'S DISEASE DEVELOPMENT THROUGH IPSC-DERIVED CEREBRAL ORGANOIDS

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Alzheimer's disease (AD) is a neurodegenerative disorder primarily characterized by the presence of amyloid plaques, neurofibrillary tangles, and loss of neuronal connections in the brain[1]. Nowadays, AD holds its position as the most prevalent cause of dementia in middle-aged and elderly individuals, and with the gradual extension of human life, it has become a significant health problem worldwide. Despite extensive efforts to explore various therapeutic approaches, AD remains an incurable disease. Consequently, scientists have turned their attention to enhancing *in vitro* models that more accurately replicate the pathophysiology observed in AD patients, aiming to achieve a deeper understanding of the disease's biology. Currently, induced pluripotent stem cells (iPSCs) are widely utilized for investigating human neurodevelopment and diseases. In the context of AD, iPSCs have been used to generate three-dimensional cerebral organoids (COs), effectively mimicking AD pathology *in vitro*[2]. These *in vitro* models not only replicate AD pathology but also stimulate the early stages of brain development. As a result, they could play a crucial role in revealing the initiation steps and molecular mechanisms underlying this disease. These, however, remain to be described.

In our laboratory, we recently generated iPSC lines from patients with the familial form of AD. Our AD cell lines carry mutations in *PSEN1*(A246E) and *PSEN2*(N141I) genes and are sex- and age-matched to non-demented controls (NDC)[3]. These cell lines were subsequently used to generate COs. Our previous data revealed that 60-days-old organoids not only manifested AD-like pathology but also had altered development[4]. Moreover, single-cell sequencing analysis suggested their premature differentiation. To explore the initial stages of Alzheimer's disease, we now examined the expression of genes associated with brain development and the activity of pathways crucial for neurodevelopment. Our findings thus far show that AD-COs undergo distinct developmental patterns. Specifically, AD-COs show a preference for the differentiation into prosencephalon, problems in proper dorsal-ventral development, and likely altered key signaling pathways, including Notch and Wnt. Further examination of these differences in the early stages of development will be the key pillar for understanding the initiation mechanisms of disease.

Funding for this research was provided by the Czech Science Foundation GA24-12028S; NU21-08-00373; NPO-Neuro-D LX22NPO5107; MUNI/A/1301/2022; ADDIT-CE 101087124 and CETOCOEN 857560, financed by EU.

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AMINO ACID PREFERENCES OF POST-PROLINE CLEAVING ENDOPEPTIDASES REVISITED

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Classical as well structural proteomic workflows rely strongly on protein digestion. In hydrogen/deuterium exchange (HDX) it provides spatial resolution, in fast photochemical oxidation or cross-linking it enables precise localization of modifications and in general proteomics it helps to overcome issues with ionization of entire proteins in complex mixtures. Even though each workflow has its own "golden standard" protease, the search for new enzymes is a never-ending quest. Recently, proline specific enzymes such as neprosin or *Aspergillus niger* (*An*PEP, ProAlanase) were introduced as interesting alternatives for protein digestion due to their complementary selectivity targeting mostly proline and alanine.

Our study on *An*PEP utilization in HDX setup however revealed additional unexpected selectivity towards cysteine residues. We evaluated research grade *An*PEP, its industrial alternative (Clarity-Ferm), both acid serine proteases, and neprosin, glutamic protease, and confirmed the post-Cys selectivity in all these enzymes. We also showed that modified (alkylated, oxidized) Cys or Cys involved in disulfide bond is not targeted. In addition, we showed that and the research grade *An*PEP can be efficiently immobilized and used in online microreactors either alone or in combination with other enzymes such as pepsin or nepenthesin which is crucial for successful digestion of prion proteins and enables their structural analysis. Finally, to facilitate data processing of MS-based analyses of protease specificities, we developed a new software tool named DigDig. It streamlines comparison and parametrization of protein digestions, enables extraction of cleavage preferences, and works well even with complex mixtures like human serum.

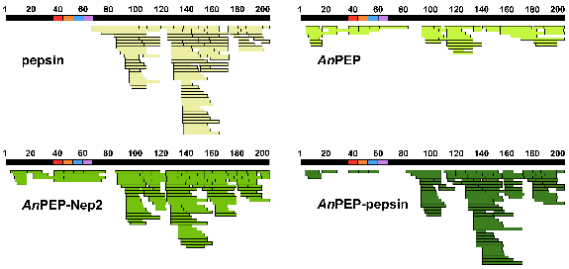

Fig. 1. **Utilization of *An*PEP for cleavage of prion protein leads to better sequence coverage and spatial resolution in the HDX-MS analysis.**

Financial support from NPO-NEURO-EXCELLES – LX22NPO5107, Horizon2020 EPIC-XS (82383) and Czech Science Foundation (22-27695S) is gratefully acknowledged.

MADIS: GENOMIC ANALYSIS TOOL FOR THE REVELATION OF MULTIPLE ALLELES WITHIN A SINGLE GENE

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Understanding crop diversification through evolution and domestication is crucial for crop breeding. Traditional breeding methods are time-consuming and inefficient; to accelerate and enhance the precision of the breeding process, identifying the concrete candidate genes and causative mutations (CM) is essential. Lately, genome-wide association study (GWAS) has been widely used to identify genomic loci underlying important traits. Numerous post-GWAS analyses were developed to narrow down the associated genomic regions, however, in many cases, they are still unable to identify candidate genes or CMs. Historical natural selection and breeding-related artificial selection alter allele frequencies in genes that control phenotypes. This raises the likelihood that numerous alleles with independent CM would be present in a single gene, which presents issues for GWAS employing large datasets and samples from isolated regions.

Up until now, there has been no association method addressing the issue of multiple alleles in a single gene. Therefore, we developed a tool that computes a score for a combination of variant positions in a single candidate gene and based on the highest score, identifies the best number and combination of CMs. The tool is available as a Python package on GitHub and as a web-based Multiple Alleles discovery (MADis) tool specifically designed for a curated panel of 1066 soybean resequenced accessions[1].

We tested and validated the algorithm and presented the utilization of MADis in the *L1* gene associated with black pod color in soybean[2]. We then successfully identified a candidate gene for the soybean pod color *L2* locus and predicted the existence of multiple alleles that potentially cause loss of pod pigmentation in soybeans. In this work, we demonstrated the use of genomic analysis in the exploration of the natural and artificial selection of multiple alleles. The MADis tool can be applied to other species to aid in the discovery of genes under selection for improved breeding.

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BIFUNCTIONAL REAGENTS FOR SELECTIVE BIOCONJUGATION

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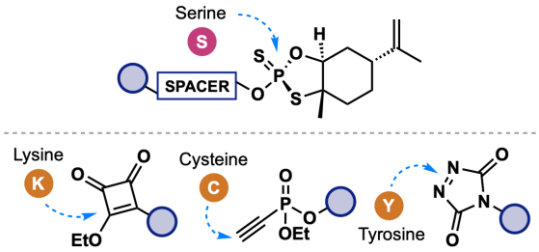
Protein modification and bioconjugation methods have rapidly emerged as an invaluable tool in chemical biology, enabling numerous applications such as development of novel therapeutics and studies on protein functions[1,2]. However, most of the currently available protein modification and bioconjugation technologies suffer from low selectivity and poor yields, require protein prefunctionalization, and proceed under rather harsh reaction conditions[3]. Therefore, superior technologies are required to overcome these drawbacks and enable selective and high-yielding modifications of native proteins under mild conditions.

In this project, we designed and prepared a series of novel and modular bifunctional bioconjugation reagents which selectively react with two natural amino acid residues (Ser, Cys, Lys, Tyr, among others) under mild and biocompatible reaction conditions. In the future, these novel bifunctional bioconjugation reagents will be applied to the synthesis of antibody-drug conjugates, protein labelling and peptide stapling, thereby addressing current challenges in chemistry and biology.



Scheme 1. **Structure and reactivity of novel bifunctional bioconjugation reagents.**

This work was supported by The Czech Science Foundation (reg. No. 23-05752S).

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IMPROVED GEOGRAPHICAL ORIGIN ASSESSMENT OF BOTRYTIZED WINES: A FRESH METHODOLOGY UTILIZING GC×GC-HRTOF-MS TECHNIQUE AND TILE-BASED *F*-RATIO APPROACH

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Combining high-resolution time-of-flight mass spectrometers (HRTOF-MS) with comprehensive two-dimensional gas chromatography (GC×GC) is a highly precise method for analysing wine samples[1,2]. Detecting statistically significant differences in chromatograms from a sample series can be challenging, but specialized chemometric and statistical tools enable precise identification of composition and concentration variances, even in trace compounds[3].

This research was conducted with the objective of emphasizing geographical variations by examining the volatile organic compounds (VOC) presence in botrytized wines from Slovakia, Hungary, France, and Austria. Following the extraction of volatile organic compounds using solid-phase microextraction, GC×GC-HRTOF-MS technique was employed to separate and analyse the extracts. The resulting chromatograms were analysed using a tile-based *Fisher*-ratio approach, involving the partitioning of two-dimensional chromatograms into tiles of equal dimensions which were then compared across all samples and sample classes[4]. Initially, 70 VOC were identified to play a significant role in classifying samples. However, it was demonstrated that selecting just 10 compounds with the highest *F*-ratio enabled the classification of samples[1,5]. Among these, the following 10 compounds were found to significantly contribute to the observed cluster patterns in the principal component analysis (PCA) results (see Fig. 1): methyl-octadecanoate, 2-cyanophenyl-β-phenylpropionate, α-ionone, *n*-octanoic acid, 1,2-dihydro-1,1,6-trimethyl-naphthalene, methyl-hexadecanoate, ethyl-pentadecanoate, ethyl-decanoate, and γ-nonalactone.

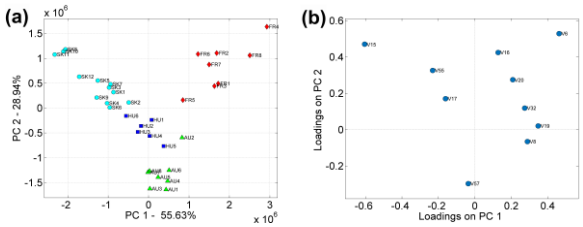

Fig. 1. **PCA results—Scores (a) and loadings (b) plots for the 10 highest statistically significant features. Austrian (green), Hungarian (blue), Slovak (cyan), and French botrytized wine samples (red).**

This project has received funding from Slovak Research and Development Agency APVV-21-0211, Scientific Grant Agency Ministry of Education, Science, Research and Sports of the Slovak Republic VEGA 1/0298/23, the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska Curie grant agreement No 945478, the STU Grant Scheme for Support of Excellent Creative Teams of Young Researchers SOLUTION, and CNPq fellowship (313381/2021-6) and National Institute of Science and Technology in Molecular Sciences (INCT-CiMol) Project 406804/2022-2/CNPq 58/2022.

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2,6,9-TRISUBSTITUTED DERIVATIVES OF PURINE WITH NEUROPROTECTIVE EFFECT

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Parkinson's disease (PD), with a predicted prevalence of 12 million people worldwide by 2040, is a chronic degenerative disease of the central nervous system and the second most common type of neurodegenerative disorder. It is characterized by the selective loss of dopaminergic neurons in substantia nigra pars compacta, which leads to a non-motor and motor symptoms[1]. As the disease progresses, symptoms such as bradykinesia, tremor, rigidity, sleep deprivation, and cognitive and behavioural problems develop[2]. The pathophysiology of PD is complex and involves multiple mechanism, such as mitochondrial dysfunction, oxidative stress, protein misfolding and neuroinflammation. However, the exact cause is not yet fully understood. Existing treatments primarily focus on managing symptoms, utilizing dopaminergic therapy (mainly the well know precursor of dopamine, L-DOPA), dopaminergic agonists such as pramipexole and rotigotine, dopamine metabolism inhibitors rasagiline and selegiline, also rivastigmine or other cholinesterases inhibitors, and similar agents. However, many of these medications are associated with pronounced adverse effects, and long-term administration often renders the treatment ineffectual. To investigate potential neuroprotective effect, we conducted test on the human neuroblastoma cell line SH-SY5Y, exploring 2,6,9-trisubstituted derivatives of purine in a 3-nitropropionic acid-induced model of mitochondrial damage. Several of these compounds demonstrated promising neuroprotective effects. Consequently, selected derivatives were evaluated for their cytotoxic, neuroprotective and pro-neurogenesis effect in cerebral organoids as *in vitro* model of human brain. Our current efforts are aimed at clarifying the underlying mechanism responsible for their neuroprotective actions.

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EFFECT OF THE PRESENCE OF G-QUADRUPLEX PRONE DNA SEQUENCES ON TRANSCRIPTION INDUCED BY CANCER-RELEVANT P53 TUMOR SUPPRESSOR MUTANTS

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The p53 protein plays an indispensable role in cells as a guardian of the genome and tumor suppressor. Loss or mutation of the *TP53* gene leads to reduced production of wild-type protein or formation of its mutant forms. Reduced function of P53 or complete loss of the gene may contribute to genomic instability and promote tumorigenesis, proliferation of tumor cells, and generation of resistance to commonly used medical treatments[1]. Similarly, G-quadruplex motifs (G4s) forming at the wrong time or place in the genome may also contribute to genomic instability. G4s are secondary non-canonical DNA (or RNA) structures. The presence of G4s has been localized in important regions of the genome, such as telomeres and promoters of oncogenes and the genomes of many viruses and pathogens. The involvement of G4s in the regulation of gene expression and its localization in important regions of the genome make G4s a promising target in cancer therapy[2].

In this study, the effect of G4 prone DNA sequences on the transactivation properties of cancer-relevant P53 partial loss-of-function mutants in the yeast one-hybrid expression system (Y1H) was investigated. Seven isogenic *Saccharomyces cerevisiae* yLFM strains containing the identified KSHV-derived G4 prone DNA sequences placed downstream of the p53 responsive element of the *BBC3* gene (*PUMA*) were used. The activity of the mutant P53s and P53 wild-type proteins was monitored under an inducible promoter to obtain moderate or high levels of protein expression. G4

prone DNA sequences were themselves biophysically characterized by ThT assay and CD spectroscopy.

The results of functional assays in Y1H showed that basal reporter transcription in the absence of specific transcription factors is stimulated by the presence of G4, correlating proportionally with the propensity to form G4 structures. In association with the higher constitutive activity of the yeast minimal promoter containing G4, the transcriptional activity of mutant P53 proteins (A161T, R213L, N235S, V272L, R282W, R283C, R337C, R337H, and G360V) was investigated. Our results demonstrated that the G4 forming sequences presence increase the transactivation ability of partial function P53 family proteins. These observations are pointing to the importance of DNA structural characteristics for accurate classification of P53 protein functionality in the context of the wide variety of TP53 germline and somatic mutations.

This work was supported by funds from the Faculty of Chemistry BUT (FCH-S-23-8303) and the Institute of Biophysics of the CAS.

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IMMUNOSURVEILLANCE IN HER2+ BREAST CANCER – ROLE OF ENDOPLASMIC RETICULUM STRESS

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HER2+ breast cancer (BC) is one of the most aggressive types of BC, which accounts for about 15–20 % of BC cases[1]. It is considered to be an immunogenic type of cancer based on the high levels of tumour-infiltrating T-lymphocytes (TILs)[2]. The interaction of immune (IC) and cancer cells (CC) occurs within the complex cellular network of tumour immune microenvironment (TIME). Various factors secreted in TIME cause immunosuppressive and chronic inflammatory environment with hypoxia, low pH and nutrient deficiency. These factors can cause Endoplasmic Reticulum Stress (ERS). The main cellular response to ERS is activation of signalling pathways known as the Unfolded Protein Response (UPR). In CC this leads to decreased expression of MHC class I molecules, increased angiogenesis, hypoxia survival or resistance to therapies, whereas IC lack antigen presentation, exhibit suppressor phenotype, and can initiate apoptosis[3]. The alleviation of ERS could therefore enhance anticancer immunity to BC.

The aim of this project is to study the role of ERS in the interaction of IC and CC, and the response to targeted therapy in *in vitro* preclinical models. To study the response of IC in stressful environment, we modulate ERS in co-cultures of CC with peripheral blood mononuclear cells (PBMC) using chemical chaperone TUDCA and inhibitor of N-glycosylation Tunicamycin. Then, by analysing cellular viability and motility (MTT, scratch wound assay), gene expression (RT-qPCR), protein profiles (western blotting, immunofluorescence staining), we study various ERS markers (BiP, CHOP, PERK etc.) and immune tumour profile (FoxP3, PD-1 etc.). We correlate these results with analysis of clinical samples from patients with HER2+ BC treated with conventional immunotherapy.

The alleviation of ERS led to decreased ERS markers expression in IC (PBMC). It also increased the viability of CC when cultured by themselves, whereas in co-culture with PBMC, their viability, as well as motility, decreased. These results suggest enhanced effectiveness of anticancer immune reaction. We relate these results to the molecular background in patient samples, where we observed a positive correlation between good clinical outcomes, higher TILs levels in TIME and elevated levels of ERS markers in CC. These data could lead to discovering new treatment targets and improved prognosis for patients with HER2+ BC.

This project is supported by Grand Agency of Masaryk University, Student Research support programme – Excellent diploma thesis, project number MUNI/C/0142/2023

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LASER ABLATION SYNTHESIS OF TELLURIUM NANOPARTICLES FOR MALDI MS APPLICATIONS

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This study showcases the potential of pulsed laser ablation synthesis (PLA) in producing tellurium nanoparticles (Te NPs) for matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) applications. To prevent unwanted aggregation of uncoated Te NPs and avoid the need

for additional modifiers, an experimental laboratory setup for PLA synthesis of fresh Te NPs was designed. It was discovered that performing PLA in acetone was the optimal method for preparing Te NPs. An alternative is to use commercially available laser ablation devices for laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) to perform PLA in a helium atmosphere. However, this method is less effective and leads to the formation of undesired larger particles[1,2].

Laser ablation synthesis proved to be a viable method for producing new Te NPs for MALDI-MS applications. Uncoated Te NPs tend to aggregate, forming self-assembled nano chains that eventually sediment into a dark, spongy mass at the bottom of the container after several tens of minutes. Several organic solvents (ethanol and acetone) were used for the synthesis. The water was not used due to the dissolving of atmospheric oxygen. Dissolved oxygen readily reacts with pure metal NPs. The example of synthesized NPs is possible to see in Fig. 1 (ref.[1,2]).

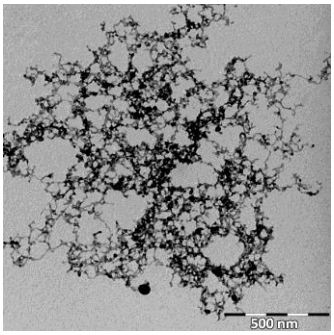

Fig. 1. **TEM picture of synthesized NPs.**

The Te NPs were utilized in a pilot study with the MALDI MS technique, revealing several observations that opened up new application of Te NPs in the field of MS. A significant effect was observed when Te NPs were used in a MALDI-MS analysis of the α-cyclodextrin (α CD) and cucurbit[7]uril (CB7) macrocycles. Addition of Te NPs to MALDI matrix resulted in the formation of no analytes matrix adducts. Te NPs caused several changes in the obtained mass spectra when used in MALDI-MS of intact cells. The scope of this work does not include the interpretation of the observed effects, which will be the subject of further research. Additionally, we observed a positive effect of Te NPs on the crystallization of the MALDI-MS matrix[3].

This work was supported by project no. GA22-07635S, no. MUNI/A/1598/2023 and no. MUNI/A/1575/2023.

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SYNTHESIS OF NOVEL HETEROCYCLIC AZA-β-LACTAMS DERIVED FROM 1,3-DIAZETIDIN-2-ONE

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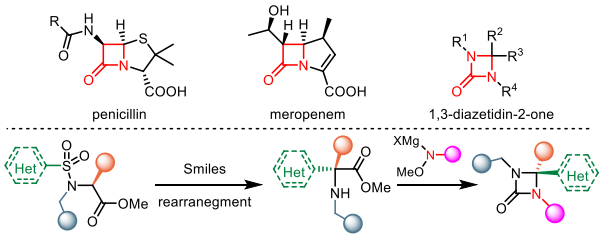
The most well-known and widely used antibiotics are probably β-lactam-containing drugs such as penicillin or meropenem.[1] The increasing resistance of microorganisms to this type of antibiotics recently led to substantial structural modifications of β-lactam with aim to ensure its efficacy. In recent years, our group has become interested in the synthesis of 1,3-diazetidin-2-one (aza-β-lactams) synthesis. According to various (*in silico)* studies, such a structural motif should be able to act as an inhibitor of proteins that cause penicillin resistance in bacteria, and in some cases, it could also act as a reversible inhibitor of serine β-lactamases.[2]

In this contribution, we wish to disclose a new approach for the aza-β-lactam derivative synthesis that is based on a sequence of two intramolecular rearrangements that allows a short and efficient transformation of optically pure *N,N*-di-substituted sulfonamides[3] to targeted derivatives. In this contribution we discuss not only the scope and limitations of the method but also the investigation of the reaction mechanism and biological activity of prepared compounds.



Scheme 1. **β-lactams and unprecedented synthesis of 1,3-diazetidin-2-one.**

This work was supported by the European Regional Development Fund-Project "Centre for Experimental Plant Biology" (no. CZ.02.1.01/0.0/0.0/16_019/0000738) and by the IGA of Palacky University (IGA_PrF_2023_20).

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SYNTHESIS OF FLUOROALKYLATED ISOQUINOLINES AND FUSED PYRIDINES FROM *N*-FLUOROALKYL-1,2,3-TRIAZOLES

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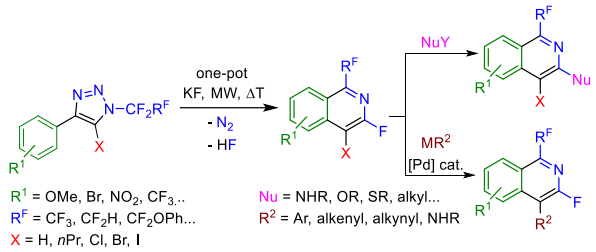
Isoquinoline core is present in a great number of naturally occurring alkaloids, which in many cases possess interesting pharmaceutical properties. Consequently, there is a demand to introduce the isoquinoline moiety even to synthetically prepared drugs to improve their bioactivity. The implementation of fluorine atom or fluoroalkyl groups into a lead molecule is another widely used strategy to enhance pharmacologically relevant properties. Yet, the procedures for the preparation of 1-fluoroalkylated isoquinolines remain underdeveloped and they are often very substrate specific, low yielding, or require expensive, non-selective and atom non-economical fluoroalkylation methods and/or transition metal catalysis[1,2].

In the subsequent work to our published methodology leading towards reactive *N*-fluoroalkylketenimines[3], upon further heating of these species we observed the formation of 1-fluoroalkyl-3-fluoroisoquinolines, which can be further modified in position 3 and/or 4 of the ring. After extensive optimization we found potassium fluoride to be a suitable additive to enhance the process and we were able to develop effiecient diverse metal-free one-pot procedure for the preparation of these isoquinolines and fused pyridines from starting aryl or heteroaryl substituted *N*-fluoroalkyl-1,2,3-triazoles (Scheme 1). The reaction scope, limitations, further reactivity, and reaction mechanism will be discussed.



Scheme 1. **Synthesis of 1-fluoroalkylisoquinolines from triazoles.**

This work was financially supported by Czech Science Foundation – grant No 23-04659S.

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IDENTIFICATION AND CHARACTERIZATION OF NEW SMALL-MOLECULE MODULATORS OF p53 TUMOR SUPPRESSOR ISOFORMS EXPRESSION

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The tumor suppressor p53, encoded by the *TP53* gene, is an essential regulator of a cellular stress response. Within the human transcriptome and proteome, p53 can be found in many isoforms differing in function, transcriptional activity, interactions with other cellular proteins, and sub-cellular localization. These variants can originate in alternative promoters of the *TP53* gene, p53 transcript alternative splicing, or the use of alternative translation initiation sites. The p53 isoform expression is often tissue-specific and could be used as a prognostic marker or in targeted cancer therapy.

We identified a series of small-molecule compounds which modulate p53 isoform expression in cancer cells. The compounds are structurally very similar, yet they have a considerably different impact on the expression of alternatively spliced p53 isoforms. Using siRNA-mediated gene knock-down, western blot analysis, and RT-qPCR, we ascertained that these modulators dramatically change the ratio between the p53α and p53β isoforms. Besides the potent increase in p53β formation, there is also a modest upregulation of p53γ expression. Our data also indicate the impact of these small-molecule modulators on the nonsense-mediated mRNA decay pathway.

Since p53β expression was associated with better prognosis in breast cancer[1], melanoma[2], renal carcinoma[3] or acute myeloid leukemia[4], the small-molecule modulators could be beneficial in the therapy of these diseases.

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PUSHING THE LIMITS: ENZYMATIC SYNTHESIS OF HEAVILY MODIFIED DNA BEARING FOUR DIFFERENT ANIONIC SUBSTITUENTS AT ALL FOUR NUCLEOBASES

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Base-modified DNA find a number of applications in different fields of biotechnology and chemical biology. In most of them, DNA contain one or two modified nucleotides which bring an additional function, while the other nucleotides are non-modified. So far, there have been only several reports on fully-modified DNA where each nucleotide bears a different modification (further referred to as hypermodified DNA). Lipophilic hypermodified DNA have recently been studied in our group[1]. In this work we report[2] on the synthesis of four new base-modified dNTPs bearing anionic groups and their further studies as building blocks in enzymatic synthesis of hypermodified 'superanionic' DNA (Fig. 1). Biophysical studies suggest that the presence of anionic modifications in one strand decreases the stability of duplexes while still preserving B-DNA conformation, but DNA hypermodified in both strands adopts a different secondary structure.

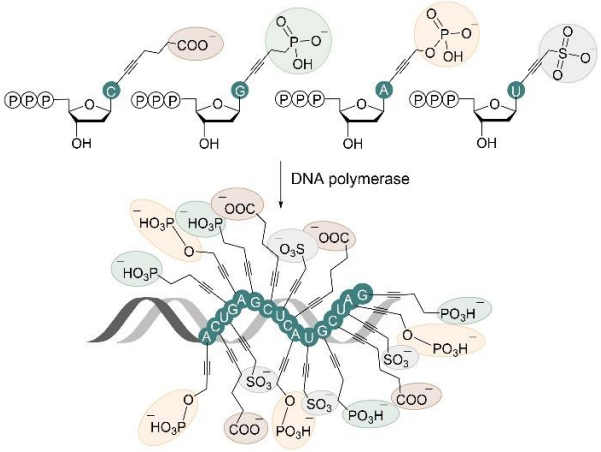

Fig. 1. **Enzymatic synthesis of 'superanionic' DNA.**

This work was supported by Czech Science Foundation(grant No 20-00885X).

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FLUORINE-INDUCED STRENGTHENING OF CH-PI INTERACTION AS AN IMPORTANT DRIVING FORCE BEHIND THE RECOGNITION OF FLUORINATED DISACCHARIDES BY WHEAT GERM AGGLUTININE

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Wheat germ agglutinin (WGA) is a carbohydrate-binding protein (lectin) abundant in the seeds of Triticum vulgare. This lectin exhibits antifungal, antibacterial, and cytotoxic properties with potential application in chemotherapy, targeted drug delivery, and antibiotic-resistant bacteria elimination[1]. Such functions are dependent on WGA ability to recognise *N*-acetylglucosamine, a key constituent of chitin polysaccharide.

In this work, we report a systematic study of WGA recognition properties using two series of mono-fluorinated glycomimetics based on *N*,*N*′-diacetyl chitobiose (CB) and *N*,*N*′-diacetyl lactosamine (LN). In these series, 6′-fluoro-CB was identified as ca. one order of magnitude stronger binder than CB. To rationalize such observations, the recognition of 6′-fluoro-CB by WGA was investigated by ^{1}H STD, ^{1}H-^{1}H tr-NOESY, and ^{1}H-^{19}F tr-HOESY NMR. These techniques revealed that fluorine-induced polarization of 6′$_{proR}$ geminal proton strengthens its CH..pi interaction with aromatic Phe109 residue (Fig. 1), demonstrating the potential of specific deoxyfluorination in the development of novel glycomimetics[2].

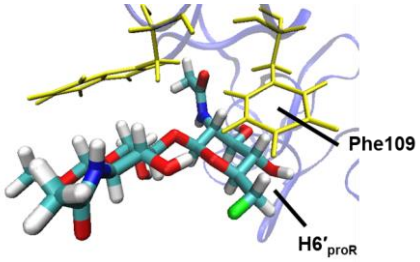

Fig. 1. **Supramolecular complex of methyl *N*, *N*-diacetyl 6′-deoxy-6′-fluoro-chitobioside with WGA.**

Support from MEYS (INTER COST, no. LTC20052) and GAČR (23-05146S) is gratefully acknowledged.

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VIBRATIONAL OPTICAL ACTIVITY STUDY OF POLY-GLUTAMIC ACID CONFORMATIONS

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Disordered and aggregated proteins are difficult to study due to conformational heterogeneity. Thus, development of methods capable to describe irregular species in solutions is desirable.

Vibrational optical activity (VOA) is a promising spectroscopic technique, which allows a unique insight into structure and dynamics of chiral molecules in solution. It comprises vibrational circular dichroism (VCD) and Raman optical activity (ROA) which measure circularly polarized absorption and scattering, respectively. Previously, it was shown that VOA is sensitive to variations in protein conformation and provides rich and characteristic spectra[1]. However, interpretation of these spectra is not trivial and requires time consuming quantum-chemical calculations. To make them more accessible, we employed a peptide with repeating sequence such as poly-glutamic acid (PGA). It is a common model of amyloid fibrils and conformational transitions, which take place upon side chain protonation.

In this work, we studied PGA in forms of disordered coil, helix and β-sheet aggregates (fibrils) by VOA spectroscopies. The availability of both L- and D-enantiomers of PGA was particularly useful for obtaining "mirrored spectra" (Fig. 1), allowing to avoid artifacts in the experiment[2].

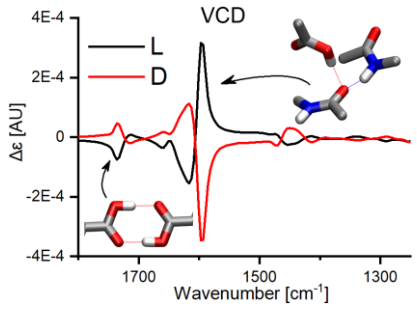

Fig. 1. **Experimental VCD spectra of PGA fibrils prepared form L- and D-enantiomers in D_2O. Insertions show molecular arrangements responsible for specific signals.**

The experiments were performed in H_2O and D_2O, which simplified band assignment of exchangeable chemical groups. For spectra interpretation, we simulated the geometry by molecular dynamics and calculated VOA spectra on the basis of density functional theory.

The spectra of PGA in the disordered conformation could be well reproduced with poly-proline II model[3], with a reduced flexibility of the backbone. An explicit hydration was found to be crucial for more accurate simulation of the amide bond vibrations.

For the helical conformation, we developed models based on α-helix and 3_{10}-helix, and found that the experimental spectrum was best reproduced by the α-helix. This allowed us to determine spectral regions suitable for distinguishing these two conformations, difficult do distinguish by other methods.

The most challenging part was to interpret spectra of amyloid fibrils, structure of which is not understood yet. We determined that chiral signals of protonated carboxyl groups originate from hydrogen bonded network between β-sheet layers. We could also reproduce unusually shifted amide vibration[4], as a result of a bifurcated hydrogen bonds of the amide groups. These findings show that with our methodology VOA can significantly contribute to the understanding of the PGA structure, including amyloid aggregates.

The work was supported by the Czech grant agency, 24-10558S.

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FUNCTIONAL IMPACT OF ANKRD26 VARIANT c.-140C>G IN INHERITED THROMBOCYTOPENIA

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Inherited thrombocytopenias, marked by reduced platelet count, represent a more prevalent group of disorders than previously thought. Some of these conditions are associated with a germline predisposition to hematologic malignancies. Inherited thrombocytopenia type 2 (THC2) is caused by pathogenic germline variants in a critical region (c.-140C to c.-113A) of the 5' untranslated region (5'UTR) of the *ANKRD26* gene. The presence of the pathogenic variant leads to persistent overexpression of *ANKRD26* due to failure of regulation from transcription factors, resulting in defects in platelet formation and subsequent thrombocytopenia[1]. The c.-140C>G variant is reported in 6.2 % of the non-Finnish European population, according to dbSNP. The clinical interpretation of this variant is currently conflicting; recent ClinVar data suggest a likely benign or benign effect, contrary to earlier reports of its causality for THC2.

In our study, we performed a functional analysis of the c.-140C>G variant together with clinical tests. Individuals with c.-140C>G variant and wild-type individuals were selected based on whole genome sequencing. We determined the *ANKRD26* expression in platelets from selected individuals using real-time polymerase chain reaction. The results showed no significant difference in the *ANKRD26* expression between individuals with the c.-140C>G variant and the control group. Additionally, platelet aggregation tests were performed, revealing no consistent alteration in platelet function depending on the presence of the c.-140C>G variant. Moreover, all individuals with the c.-140C>G variant had normal platelet counts and normal bleeding scores. The results of this study present the first functional evidence of the benign nature of the c.-140C>G variant in 5'UTR of the *ANKRD26* gene. This highlights a significant impact on the prognosis of individuals diagnosed with this variant, especially in terms of the risk of developing malignancies.

This work was supported by MH CZ - DRO (FNBr, 65269705) and Masaryk University under Grant MUNI/A/1558/2023.

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ENVIRONMENTAL FORCES SHAPING BACTERIAL GENOMES

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Although originally thought to evolve clonally, most bacteria exchange DNA and bacterial genome evolution and speciation are shaped by multiple forces, including environmental pressure acting over time[1].

In our study, we analysed the genomes of two potentially new species of *Streptomyces* spp. isolated from heavily contaminated soil from a former lead-silver-zinc heap in Tarnowskie Góry, Poland. *Streptomyces* spp. are bacteria primarily found in the soil environment, including areas contaminated with heavy metals[2]. While heavy metals occur naturally in trace amounts and pose minimal threat to bacterial life, in higher concentrations can cause several damages to biological processes, culminating in bacterial death[3]. It has been confirmed that streptomycetes have the ability to tolerate heavy metals through a spectrum of specialized adaptation mechanisms, such as efflux pumps, superoxide dismutases, oxidation/reduction of toxic metal ions into a less harmful forms etc. Highly active secondary metabolism of *Streptomyces* spp. indicates that evolutionary adaptations allow them to thrive in soil habitats, particularly under the extreme environmental conditions[4] as well.

Whole genome sequencing of both P9 and P17 isolates was performed, and the taxonomic position of both strains was established by genome-based phylogenetic analysis, aligning them with the closest *Streptomyces* spp. strains in the TYGS database. P9 isolate was found to belong to the *S. lateritius* clade (Average nucleotide identity – ANI value – 88.36) and P17 was assigned to the *S. cupreus* clade (ANI value – 92.56). These findings show that despite inhabiting the same environment, the P9 and P17 isolates are phylogenetically distant (ANI value – 85.76).

The genome annotation using the Rapid Annotation using Subsystem Technology (RAST) confirmed the metabolic differences between P9 and P17 isolates. The significantly higher number of coding genes in 'Metabolism of Aromatic Compounds', 'Carbohydrates' and 'Fatty Acids, Lipids, and Isoprenoids' subsystems was detected in the P17 isolate compared to P9.

Using the antiSMASH server, some major classes of biosynthetic gene clusters (BGCs) in both strains were identified. While both strains exhibit the production of common *Actinomycetes* compounds, such as ectoine or desferrioxamine B[5,6], 15 of additional characterized BGCs in P9 do not share homology with 22 unique BGCs in P17 (Fig. 1). Additionally, we detected 5 BGCs in the P9 genome that did not match any reference gene clusters in the MIBiG database and 9 BGCs in the P17 genome with the unknown function, indicating the potential for the production of novel compounds.

Based on our results, we can assume that P9 and P17 are two different *Streptomyces* species differing in phylogeny, metabolism and with the potential to synthesize various types of BGCs. Intriguingly, despite these differences, we observed almost the same set of heavy metal resistance (HMR) genes within their genomes using the RAST annotation server, Bakta server and BacMet software specialized to heavy metals, suggesting a shared adaptive response to heavy metals including copper, zinc, mercury, arsenic, cadmium, tellurium and nickel. Such metabolic traits are frequently plasmid encoded in *Streptomyces* spp.[7], but putative megaplasmid (> 115kb) was detected in the P17 isolate only and all mechanisms associated with adaptations to increased heavy metals content are probably encoded chromosomally.

Presented results suggest the environmental influence on the evolution of phylogenetically different species of microorganisms isolated from the same extreme conditions.

Fig. 1. **Venn diagram showing shared and unique BGCs (biosynthetic gene clusters) and HMR (heavy metal resistance) genes.**

This project was supported by VEGA Grant Agency (grant no. 1/0779/21).

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EXPLORING THE IMPACT OF PALMITOYLATED PrRP31 AND LIRAGLUTIDE IN MOUSE MODEL OF OBESITY AND NAFLD

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Obesity and type 2 diabetes are key factors leading to non-alcoholic fatty liver disease (NAFLD), characterized by fat accumulation in the liver[1]. Currently, there is no known cure for NAFLD, however anorexigenic peptides like glucagon-like peptide 1 (GLP-1), its long-acting analogue liraglutide (LIRA), and prolactin-releasing peptide (PrRP) show promise for both obesity and NAFLD treatment[2]. Lipidization of peptides allows them to act centrally after peripheral administration, which have been shown in recent studies for palmitoylated PrRP31 (palm[11]-PrRP31)[3]. This study aims to investigate effects of palm[11]-PrRP31 and LIRA on a mouse model of obesity and NAFLD.

Mice with monosodium glutamate (MSG)-induced obesity, fed a high fat, high fructose and high cholesterol diet (FFC) or standard diet (STD) were used, while NMRI mice on either FFC or STD diet served as controls. MSG mice on FFC diet exhibited notable increases in body weight and liver weight, as well as elevated levels of leptin and CRP in plasma. Liver histology revealed a significant increase in the percentage of fat droplets and collagen in FFC diet mice, suggesting signs of fibrosis. After a period of seven months, a one-month intervention with palm[11]-PrRP31 and LIRA followed. Although body weight did not change significantly, there was a slight decrease in leptin level and a significant decrease in CRP level. Most importantly, a significant decrease in the percentage of fat droplets in the liver was observed after the treatment and the percentage of collagen in the liver was notably lower in the treated mice.

The findings suggest that anorexigenic peptides may have potential for treating NAFLD. However, further studies are required to determine the mechanism of action for its potential application.

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THIENO[3,2-*b*]PYRIDINE: AN ATTRACTIVE SCAFFOLD FOR HIGHLY SELECTIVE INHIBITORS OF UNDEREXPLORED PROTEIN KINASES

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Recently, we identified the thieno[3,2-*b*]pyridine scaffold as a versatile core for the design of new potent and highly selective protein kinase inhibitors. Protein kinases are key regulators of numerous biological processes, and their aberrant activity can cause various diseases, in particular cancer[1,2]. Kinome-wide screening of selected thieno[3,2-*b*]pyridine derivatives showed outstanding selectivity towards the kinase Haspin[3,4]. Complementary *in vitro* and *in vivo* studies with specific compounds demonstrated their potential use as high quality chemical biological probes for the study of this chromosomal protein. Apart from Haspin, other underexplored kinases such as CDKLs and TAF1L were found to be effectively inhibited by some analogs in our library. The syntheses of these thieno[3,2-*b*]pyridine-based inhibitors relied mainly on the use of regioselective consecutive transition metal-catalyzed

cross-coupling reactions. In the case of certain compounds where this methodology could not be applied, we adopted alternative approaches., in particular direct assembly of the thieno[3,2-*b*]pyridine core by various cyclization reactions. Overall, we prepared and tested >170 compounds, which allowed for thorough study of the structure-activity relationship (SAR) around the thieno[3,2-*b*]pyridine pharmacophore. The series of inhibitors is covered by our recently filed patent application[5] and the manuscript is in preparation.

European Structural and Investment Funds, Operational Programme Research, Development and Education – „Preclinical Progression of New Organic Compounds with Targeted Biological Activity" (Preclinprogress), the project CZ-OPENSCREEN: National Infrastructure for Chemical Biology (LM2023052), and Bader Philanthropies.

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ORTHO-QUINONE METHIDE BASED PHOTOCONJUGATION AND PHOTORELEASE REACTIONS

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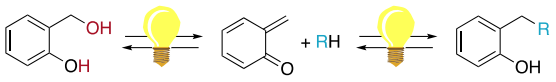
Photoclick chemistry represents a class of photo-activated click reactions generally characterised by precise spatial and temporal control, high selectivity, and quantitative yields[1,2]. Its importance resides in a remarkable selectivity that enables reactions to take place in highly complex environments. The photo-inducible precursors can be conjugated with various moieties of biological or synthetic significance.

A characteristic feature of numerous photoclick reactions is the formation of a stable adduct unsusceptible to reversible cleavage. Such bond-forming conjugations have indeed found their applications in biological research, most notably in photoaffinitive labelling[3]. However, the design of a reversible system allowing for dynamic control of photoclick reactions is an appealing research extension and the chemistry of *ortho*-quinone methides (*o*-QM)[4] suits such demand. The *o*-QM adduct generated by a nucleophilic attack is prone to further photo-mediated cleavage, making the reaction reversible (Scheme 1). The regenerated substance is repeatedly reactive towards water or other nucleophiles, the concentration of which could be modulated. The system thus exemplifies a "catch" and "release" strategy directed by light.

The utility of *o*-QMs in the catch and release strategy has been recently proposed by reversible photoderivatisation of cysteine residues[5]. We have expanded the reactivity landscape of *o*-QMs and characterised their behaviour towards N- S- and C-nucleophiles. Understanding the principles guiding the *o*-QM reactivity will enable us to apply this elegant behaviour in reversible photoaffinity labelling.



RH = BuSH, PhSH, CH_2CN_2, $C_4H_4N_2O_3$, KCN, $BuNH_2$, C_4H_9N, $PhNH_2$,

Scheme 1. **Generation and nucleophilic reactions of *ortho*-quinone methides.**

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TRANSCRIPTIONAL PROFILING OF BRAIN ORGANOID MODELS OF GAUCHER DISEASE

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Gaucher disease (GD) is a rare lysosomal storage disorder caused by mutations in the *GBA1* gene coding for glucocerebrosidase enzyme[1]. Reduced activity of this enzyme leads to accumulation of its substrate glucosylceramide, which is a component of glycosphingolipids in cell membranes. GD is characterized by diverse symptoms affecting liver, spleen, and bones. Some variants involve also neurological symptoms and have been associated with an increased risk of Parkinson's disease and other neurodegenerative disorders.

The neuropathology in GD is manifested by inflammation, activation of microglia, and impaired mitochondrial function, with region-specific effects on the brain[2]. Disbalance of growth and cell death-related processes has been proposed as a potential cause of neurodegeneration and impairment of neuronal functions[3]. However, the

understanding of the impact of *GBA1* dysfunction on early brain development has been limited.

Here, we prepared brain organoids derived from human iPSCs (induced pluripotent stem cells) by two different protocols, resulting in subpallial and neocortical organoids. We harvested the organoids after 75 and 150 days in culture and conducted single-cell RNA sequencing to identify cell populations and gene expression changes associated with GD and compare differentiation trajectories between GD and controls, as well as between the cultivation protocols.

Investigating the early neurodevelopmental consequences of GD-causing mutations may open new perspectives for the future research of GD, ultimately leading to improved early diagnosis and therapy.

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NOVEL JUVENILE HORMONE RECEPTOR AGONISTS DISRUPT INSECT DEVELOPMENT IN SPECIES-SPECIFIC MANNER

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Currently used insecticides remain poorly selective and undesirably kill non-target species, contributing to the global decline in insect abundance and species diversity[1]. Many insecticides exploit the natural signaling pathway of juvenile hormone (JH) to block insect metamorphosis. JH binds to Methoprene-tolerant protein (Met) that subsequently forms heterodimer with Taiman (Tai), creating the JH receptor complex (JHR)[2]. The JHR is a transcription factor activating *Krüppel-homolog 1* gene (*Kr-h1*) that inhibits metamorphosis[3]. During the final juvenile stage, disappearance of JH and downregulation of *Kr-h1* expression allows insects to metamorphose[4]. Thus, exposure to a JHR agonist at this time prevents adult development. Synthetic JH mimics that presently serve to control pests and disease vectors affect a broad spectrum of insects. Therefore, to obtain more selective means for insect control, we aim to develop compounds targeting only selected insect taxa. To that end, we screened a chemical library of 90 thousand molecules for activators of JHRs from 7 target species representing evolutionary distant groups. In high-throughput screening, we employed a two-hybrid assay probing the ability of tested molecules to induce dimerization of the JHR subunits Met and Tai, measured as luciferase expression. Selected compounds were subject to follow-up assays testing the ability to bind Met *in vitro* and block metamorphosis by inducing *Kr-h1* expression *in vivo*. Metamorphosis was either arrested without producing viable adults or the treated animal formed a supernumerary juvenile stage incapable of reproduction. Importantly, we found compounds that disrupted development of target species without affecting the beneficial honeybee. Together, our data demonstrate that novel, species-selective compounds for insect research and control can be developed.

Supported by grant 20-05151X (CSF) and project LM2018130 (MEYS) to the National Infrastructure for Chemical Biology CZ-OPENSCREEN.

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APPLICATION OF LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY FOR METABOLIC STUDIES OF METHOXPHENIDINE

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New psychoactive substances (NPSs), also known as designer drugs or legal highs, are psychotropic substances that are not controlled by international drug control agreements[1]. One of the NPSs classes is dissociative anesthetics, less common but dangerous group, which contain methoxphenidine (2-MeO-Diphenidine, MXP), initially patented as a potential therapy for neurotoxic damage in 1989[2]. MXP is an *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist sought by users for its hallucinogenic effects, which are accompanied by disconnection of thought, identity and memory[3]. Despite being available on the black market for at least a decade, there is still a lack of information on its pharmacological and toxicological properties. The significance of studying MXP metabolism is in identification of its metabolites, which can serve as biomarkers to confirm the use of this psychoactive substance.

Therefore, the method using high-performance liquid chromatography and high-resolution tandem mass spectrometry (UHPLC/HRMS) was developed for the identification and characterization of MXP phase I and phase

II metabolites. Biotransformation experiments were performed using three models: Wistar rats (urine), *Cunninghamella elegans* mycelia and human liver microsomes. Due to the heterogeneity and complexity of each matrix, proper sample preparation and isolation of analytes from the biological samples was necessary. Liquid-liquid extraction (LLE) was used for this purpose. The raw data was processed using FreeStyle for visualization and qualitative analysis, while Compound Discoverer was used to identify and determine the structure of metabolites present in real samples. In addition, the project involved the synthesis of reference standards for the identified metabolites. These standards were analysed under the same conditions as the original samples. The ultimate confirmation of the metabolite structure was achieved by comparing the fragmentation spectra and retention times of the synthesised metabolites with those of the proposed metabolites in the real samples.

This study presents the chemical structures of phase I and phase II MXP metabolites in all three media, where several differences were observed.

This work was part of the research project 'New psychoactive substances: forensic toxicology research center' supported by the Ministry of Interior of the Czech Republic from the OPSEC Program (grant No. VK01010212).

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A PLATFORM FOR THE SYNTHESIS OF OXIDATION PRODUCTS OF BILIRUBIN

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The yellow pigment bilirubin is recognized as the primary product of heme catabolism[1]. It is also a potent antioxidant and potential signaling molecule, with multiple biological functions speculated[2,3]. Elevated levels of bilirubin in the bloodstream (hyperbilirubinemia), particularly during the neonatal period, result in neurotoxicity and contribute to some cases of neonatal morbidity and mortality[4]. Despite the global application of phototherapy and numerous scientific investigations, many unanswered questions about the biology of bilirubin remain. Bilirubin produces diverse compounds through (photo)oxidation, especially during oxidative stress[5–7]. These oxidation products remain largely unexplored. A major challenge has been to obtain sufficient quantities of the oxidation products in high purity. Our primary aim was to establish a unified synthetic platform to produce all major categories of oxidation products derived from bilirubin. We designed and synthesized functionalized ready-to-couple monocyclic building blocks with high efficiency[8]. Utilizing metal-catalyzed cross-coupling chemistry, we combined these building blocks in a modular fashion to assemble structurally diverse bilirubin oxidation products. These are now available for future *in vitro* and *in vivo* investigations.

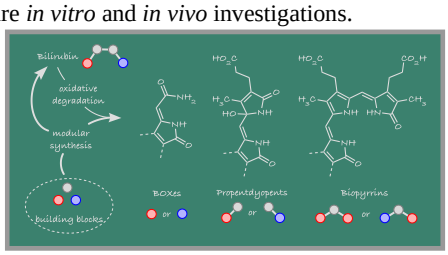

Fig. 1. **Graphical abstract.**

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REPURPOSING A COMPLEX III INHIBITOR: A TARGETED THERAPY FOR SDH-DEFICIENT CANCERS

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Succinate dehydrogenase (SDH), also known as mitochondrial respiratory complex II, is at the crossroads of the electron transport chain (ETC) and the citric acid cycle. Impairment of SDH function is associated with several types of cancer, including paraganglioma and pheochromocytoma and renal cell carcinoma. Current treatment options for these tumor types are inadequate, therefore development of new therapeutic strategies is crucial. Recent studies have unveiled

a unique vulnerability associated with SDH dysfunction: the impeded ability of cells to utilize fumarate as an alternative final electron acceptor when oxygen reduction in the ETC is compromised[1]. Inhibition of complex III or complex IV, thereby obstructing oxygen reduction, results in the suppression of electron flow and halts *de novo* pyrimidine synthesis - a process vital for tumor propagation[2]. Capitalizing on this metabolic vulnerability, we investigated the impact of atovaquone, a complex III inhibitor used in the prevention and treatment of malaria, on SDH-deficient cancer cells. Remarkably, atovaquone selectively inhibited the proliferation of SDH-deficient cells, while their SDH-proficient counterparts exhibited resistance to this treatment. Our findings underscore the pivotal role of *de novo* pyrimidine synthesis in the susceptibility of tumor cells to atovaquone. We propose a potential repurposing of atovaquone - an established antimicrobial drug with a proven safety profile in humans - for the targeted treatment of SDH-deficient tumors. Our results point to further exploration of atovaquone as a promising therapeutic strategy, offering new hope for patients with SDH-associated cancers.

This project is supported by Czech Science Foundation grant 23-05303S.

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QUANTITATION OF INTACT PROTEINS IN BIOLOGICAL FLUIDS USING CZE-MS WITH OFF-LINE μSPE SAMPLE PRETREATMENT

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Protein analysis in biological samples is one of the key areas of biomedical research. To miniaturize the entire analytical process and lower its environmental impact, attention is currently being paid to the development of new "greener" approaches and techniques aimed at the targeted determination of intact proteins, in contrast to the traditionally used approaches for protein analysis. In this field, capillary electrophoresis is becoming more popular and meets the criteria for greener techniques. When combined with mass spectrometry (MS), it can compete with established chromatographic techniques in terms of performance and meets the requirements to become a routine part of practice. However, when it comes to the analysis of biological matrices, its reliable application requires a comprehensive optimization of the separation and detection conditions in addition to the implementation of effective preconcentration techniques and pretreatment procedures[1].

In this work, we focused on the development of an on-line hyphenated capillary zone electrophoresis-mass spectrometry method (CZE-MS) employing off-line microelution solid-phase (μSPE) extraction as a sample pretreatment step for the quantitation of multiple intact proteins (<20 kDa) in various biological fluids (human serum, plasma, urine, and saliva). 19- to 127-fold increase in signal intensity was achieved by employing transient isotachophoresis (tITP) as an in-capillary preconcentration method. Off-line μSPE with various eluate treatment procedures was evaluated to ensure the compatibility of the sample with the selected in-capillary preconcentration, separation, and detection process. Achieved extraction recoveries of spiked proteins were in the range of 76–100% for urine, 12–54% for serum, 21–106% for plasma, and 25-98% for saliva. The optimum method was validated across different biological matrices, offering good linearity, accuracy, and precision, and making it suitable for proteomic studies in different biological samples.

This work was supported by the projects VEGA 1/0483/20, VEGA 1/0514/22, UK/3177/2024, and FaF/11/2023 and carried out in the Toxicological and Antidoping Center at the Faculty of Pharmacy Comenius University Bratislava.

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ENANTIOSEPARATION AND QUANTIFICATION OF METHOXPHENIDINE AND ITS METABOLITE IN WISTAR RAT MATRIX DETERMINED BY SUPERCRITICAL FLUID CHROMATOGRAPHY

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Methoxphenidine (MXP) belongs among dissociative anaesthetics and is widely abused for its psychotropic effects[1]. On the other hand, dissociative anaesthetics (particularly ketamine) have potential as next generation antidepressants, especially, for treatment of patients with treatment-refractory depression. Moreover, the effect usually varies for the *R*- or *S*-enantiomer thereby putting drug stereochemistry into the spotlight[1,2]. Analytical methods for the simultaneous determination of the enantiomers of MXP and its metabolite normethoxphenidine - norMXP are a prerequisite for future clinical investigations and a deeper understanding of the individual role of isomers.[2]

Therefore, our aim was to develop a method of chiral separation of MXP and norMXP using supercritical fluid chromatography coupled to mass spectrometry (SFC-MS), and apply it in an *in vivo* pharmacological study using an animal model.

The enantioseparations were carried out using chiral polysaccharide column and mobile phase composed of CO_2, ethanol and ammonia in gradient mode. Consequently, we have elaborated an extraction method for MXP and norMXP from Wistar rat serum and brain tissue. Resolution of enantiomers was determined as 2.3 for MXP and 1.8 for norMXP. Concentration range was 25–3000 ng mL^{-1} in serum and 60–7500 ng mL^{-1} for brain samples.

In conclusion, we determined the concentration of individual enantiomers of the MXP and norMXP in the extracted samples by newly developed and validated enantioselective SFC-MS method. Collected data will be discussed and correlated with data form a behavioural study performed at the National Institute of Mental Health.

This work was a part of the research project supported by the Ministry of Interior of the Czech Republic (grant No. VK01010212).

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FOXO TRANSCRIPTION FACTORS IN PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Survival and proliferation of chronic lymphocytic leukemia (CLL) cells is vitally dependent on B cell receptor (BCR) signaling. BCR loss in mouse models leads to B cell apoptosis. However, this can be fully compensated by constitutive activity of downstream PI3K/Akt signaling[1,2]. In CLL, the use of BCR signaling inhibitors such as ibrutinib became a standard of care and leads to a high degree of clinical responses, but that is often followed by relapse and resistance development. We have shown that in CLL, activity of transcription factor FoxO1 provides non-genetic mechanism of adaptation to BCR inhibitory therapy, interestingly by restoring activity of its own regulator, the Akt kinase[3]. In this study we aim to understand the precise molecular mechanisms responsible for this phenomenon.

We focused on revealing role of FoxO1 in transcriptional regulation of more BCR-related genes and its consequences for function of this signaling. We performed Cut&Run analysis, an efficient ChIP-seq alternative, of ibrutinib treated CLL-derived cell line MEC-1 and revealed increased binding of this transcription factor to promoters of several members of BCR pathway. Based on these results, we validated whether expression of 4 potential FoxO1 targets and intensity of BCR signaling is influenced by FoxO1 inhibitor (AS1842856, 0.5 μM, 48 hrs) in MEC-1 as well as primary CLL cells. The importance of FoxO1 for CLL cell survival is underscored by observed ~30% decrease in cell viability upon FoxO1 inhibitor treatment. Next, we also generated a FoxO1-overexpressing MEC-1 cell line, which we compared to our already established FoxO1-knockout MEC-1 cell line confirming the FoxO1-dependent regulation of several selected proteins.

Taken together, we demonstrated that FoxO1 influences the BCR signaling by regulating a number of genes involved in this pathway, with consequences for adaptation/resistance to BCR inhibitors. These observations have potential therapeutic implications for combinational targeted therapy.

This work was supported by: the Ministry of Health of the Czech Republic, grant no. NU23-08-00448, NU22-03-00117; ERC (grant agreement no.802644); the National Institute for Cancer Research (EXCELES, project no. LX22NPO5102), funded by the EU –Next Generation EU; MH CZ-DRO (FNBr, 65269705), and MUNI/A/1558/2023.

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UTILISING CRISPR/CAS9 TECHNOLOGY TO GENERATE ISOGENIC CELL LINE MODELS FOR THE STUDY OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is characterized by genetic heterogeneity and a variety of somatic mutations, the most frequent of which targeting *ATM*, *TP53*, *NOTCH1*, *MYD88* and *SF3B1* genes[1]. A thorough exploration of these mutations could shed light on the disease etiology, or even lead to the discovery of potential novel drug targets. However, CLL cells extracted from patients do not proliferate *ex vivo* and thus preclude many lengthy experiments.

We have therefore used CRISPR/Cas9 technology in HG3 and MEC1 cells, both well-established CLL-derived cell lines[2,3], to generate monoclonal isogenic cell lines carrying disruptive mutations in *ATM* or *TP53*. We also used CRISPR/Cas9-based homology directed repair to obtain HG3 cells with the most recurrent mutations of *NOTCH1* (P2514fs), *SF3B1* (K700E) and *MYD88* (L265P).

The generated knockout cell lines show a complete loss of ATM or p53 protein. The *TP53*^{KO} cells also show abrogation of downstream signaling pathways, documented by impaired activation of p21 on both mRNA and protein level. The *ATM*^{KO} cells have defective phosphorylation of downstream effector KAP1. The obtained heterozygous *SF3B1*^{KI} cell line shows defects in splicing, as evidenced by promoted aberrant splicing of *DVL2*. All generated *NOTCH1*^{KI} cell clones have a truncated NOTCH1 protein caused by the P2514fs mutation. Further functional validations of these cell lines are still ongoing.

Some of the established knockout and knock-in cell lines have been used by our research group in experiments such as drug screening with an FDA-approved drug library and genome-wide CRISPR/Cas9 dropout screening to identify genes whose deletion is selectively lethal to the introduced mutations. The knockout cell lines have also been used to generate CLL mouse models utilised for exploring CAR T-cell performance.

This research was partly financed by the grant MUNI/A/1558/2023 and supported by the project National Institute for Cancer Research (EXCELES, ID Project No. LX22NPO5102) - Funded by the EU – Next Generation EU, as well as by the Martina Roeselová Memorial Fellowship granted by the IOCB Tech Foundation.

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THE CONTEST OF NANOPARTICLES: SEARCHING FOR THE MOST EFFECTIVE TOPICAL DELIVERY OF CORTICOSTEROIDS

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Owing to their complicated pathophysiology, the treatment of skin diseases necessitates a complex approach. Conventional treatment using topical corticosteroids often results in low effectiveness and the incidence of local or even systemic side effects[1,2]. Nanoformulation of potent anti-inflammatory drugs has been selected as an optimal strategy for enhanced topical delivery of corticosteroids. In order to assess the efficiency of various nanoformulations, we formulated hydrocortisone (HC) into three different systems: lipid nanocapsules (LNC), polymeric nanoparticles (PNP), and ethosomes (ETZ). The systems were characterized using dynamic light scattering for their particle size and uniformity and the morphology of nanoparticles was observed by transmission electron microscopy. The nanosystems were tested using *ex vivo* full thickness porcine and human skin for the delivery of HC. The skin penetration was observed by confocal microscopy of fluorescently labelled nanosystems. ETZ were proposed as the most effective delivery system for both transdermal and dermal drug targeting but were also found to have a profound effect on the skin barrier with limited restoration. LNC and PNP were found to have significant effects in the dermal delivery of the actives with only minimal transdermal penetration.

This work was supported by project reg. number: CZ.02.01.01/00/22_008/0004607: New technology for translation research in pharmaceutical sciences/NETPHARM, co-founded by EU.

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PORINS AS A MEANS TO ENHANCE THE BIOTECHNOLOGICAL POTENTIAL OF BACTERIA

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Today's industries rely on non-renewable resources to produce fuels, chemicals, pharmaceuticals, or food additives. Biotechnologies offer more environmentally friendly solutions. Due to its high natural tolerance to toxic chemicals and stress, *Pseudomonas putida* is an established workhorse in modern microbial biotechnologies[1]. While the physiology and metabolism of this popular bacterial model have been extensively studied, one step has been largely overlooked – substrate uptake, particularly the role of outer membrane porins.

In this study, we thoroughly mapped the porin complement of *P. putida* and investigated its role in the transport of biotechnologically relevant substrates. Three paralogs of the glucose-specific porin OprB were inactivated in the wild-type *P. putida* and in a mutant strain engineered for

biotechnology using the CRISPR-derived base editor[2]. We then performed a detailed analysis of the resulting mutants growing on glucose, gluconate, or xylose. This included evaluation of growth parameters (growth rate, length of lag phase, maximum cell density) as well as whole genome sequencing and metabolomics.

We show that OprB porins in *P. putida* are not as specific as previously thought and that they enable uptake of both sugar and non-sugar substrates. Furthermore, we demonstrated that porin activity and regulation are closely linked to downstream metabolism. This new knowledge will be used to improve the growth and production properties of biotechnologically relevant bacterial hosts.

This work was funded by Czech Science Foundation Project 22-12505S granted to P.D.

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PITFALLS OF LIPOPROTEIN ANALYSIS: FROM SAMPLE PREPARATION TO DATA PROCESSING

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Lipidation - the covalent attachment of a lipid moiety to a protein structure - is a posttranslational modification firstly described by Folch and Lees in 1951 (ref.[1]). Since then, lipidation and lipidated proteins have been the subject of numerous studies. They have been found in all forms of life including eukaryotes, where they primarily regulate vesicle transport and cellular signaling[2]. However, lipopeptides exhibit specific physicochemical properties, which causes challenges during standard qualitative and quantitative proteomic analysis.

When analyzing lipopeptides in a complex protein-rich sample such as plasma or serum, it is essential to significantly reduce the sample matrix, commonly achieved through immunoprecipitation or extraction techniques. Despite the fact that the analysis of lipopeptides in a complex protein-rich sample such as plasma or serum shares many similarities with the widely utilized standard proteomic LC-MS approach, it has its specificities crucial for successful detection of lipid modification.

The aim of this project is to find optimal conditions for the analysis of lipopeptides prepared by enzymatic digestion of complex protein samples. The project focus on sample preparation, optimization of LC-MS conditions, and software data evaluation. This novel approach significantly increases the probability of detecting hydrophobic, low-abundant peptide modifications by fatty acids in proteomic analysis.

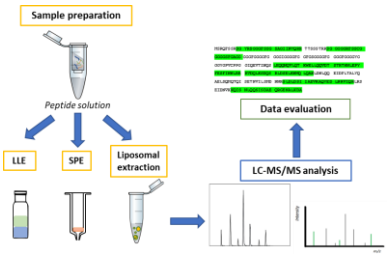

Fig. 1. **Workflow of lipopeptide analysis.**

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NOVEL INSIGHTS IN THE ELECTROCHEMICAL OXIDATION OF PHARMACEUTICALS

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Every drug undergoes degradation studies, which are part of the approval process. In these studies, pharmaceuticals are exposed to various environmental extremes. One of them is oxidation, traditionally carried out using hydrogen peroxide[1]. Electrochemical oxidation can represent a novel and significantly more effective method for degradation of various pharmaceutical substances[2]. In this study, to investigate the oxidative stability of the model active ingredient abacavir, a new electrochemical method was developed, involving the use of two different electrode materials: platinum and boron dopped diamond (BDD)[3]. While platinum represents a classical electrode material, BDD represents a new promising electrode material that allows oxidation by hydroxyl radicals. The degradation results were compared with traditional oxidation using hydrogen peroxide. Degradation up to 20% of abacavir loss, which is a requirement for pharmaceutical stability studies[4], occurred in only a few minutes using platinum and BDD anodes compared to the hours required for hydrogen peroxide oxidation. Electrochemical degradation also led to the formation of the same main degradation products as that for chemical. The measurements further showed that the electrochemical oxidation at both types of electrodes is strongly pH-dependent, and the pH of the electrolyte also influences the composition of the products, which are formed in different proportions. In particular, the advantage of electrochemical oxidation lies in the ability to adjust the oxidation strength by choosing the electrode material, the appropriate potential, and the pH value. Furthermore, electrochemical oxidation does not introduce an

external reagent into the system under study, which is a limitation in the case of chemical oxidation.

This research was funded by the Czech Science Foundation, grant number 23-05688S.

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TPM2.3 IN OSTEOSARCOMA GROWTH AND METASTASIS

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Osteosarcoma is the most common malignant tumour of bone tissue, which often forms pulmonary metastases. It most frequently occurs in children and adolescents, and the second most affected group consists of individuals over sixty years of age.

Tropomyosins belong to the family of actin-binding proteins (ABPs). They modulate access of other ABPs and myosins to the actin filaments thus controlling the stability/dynamics of microfilament network. Human genome contains four genes encoding tropomyosin (Tpm) proteins: TPM1, TPM2, TPM3 and TPM4. The TPM2 gene consists of eleven exons, two of them are alternatively spliced to create four isoforms[1]. Previous studies showed that the isoform Tpm2.1 affects the progression of colorectal and breast cancers[2,3].

The aim of this study is to elucidate the role of Tpm2.3, another highly expressed Tpm isoform, in osteosarcoma. We derived 143B and SAOS-2 LM5 osteosarcoma cells with elevated expression of Tpm2.3. Subsequently, we examined the impact of Tpm2.3 overexpression on growth, migration, chemosensitivity and metastasis of these and control cells. Elevated expression of Tpm2.3 resulted in decrease of 143B and SAOS-2 LM5 cell proliferation *in vitro* and *in vivo*. Furthermore, formation of spontaneous lung metastasis in immunodeficient mice was also reduced in Tpm2.3-overexpressing cells.

These results indicate that Tpm2.3 can be considered as potential tumour/metastasis suppressor in osteosarcoma.

This work is a joint research project supported by the Czech Science Foundation, grant no. 23-06303K and National Science Centre, Poland, grant WEAVE-UNISONO project number 2022/04/Y/NZ5/00064

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VISUALIZATION OF THE SORLA PROTEIN MUTATIONS IN THE ALZHEIMER'S DISEASE DEVELOPMENT

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Alzheimer's disease (AD) is a gradually advancing neurodegenerative disease. With over 55 million people affected worldwide, rising by 10 million annually, AD poses a significant health burden. The primary symptoms of AD involve progressive memory decline and are characterized by two main pathological hallmarks: the buildup of amyloid-β plaques and Tau neurofibrillary tangles formation[1]. The majority of the AD cases are sporadic and likely caused by the interaction between the genetic and environmental factors. Only a small percentage of cases belong to the familial form of AD, attributed to mutations in three genes: *APP*, *PSEN1* and *PSEN2*. Recently, the fourth gene - *SORL1* has been associated with the onset of AD[2].

SORLA protein (encoded by the *SORL1* gene) functions as an intracellular sorting receptor managing the sorting and trafficking of intracellular cargo between endosomes and the trans-Golgi network. Specifically, the SORLA protein plays a crucial role in the transport and recycling of the amyloid precursor protein (APP) which directly regulates the processing of the amyloid-β[3]. Despite individuals with pathological SORLA mutations show a higher incidence of AD, the precise molecular mechanisms underlying SORLA-dependent AD development remain uncertain. Therefore, more thorough research of this protein and its mutations on a human-relevant model is necessary.

To model the AD phenotype in this project, we used induced pluripotent stem cells (iPSCs) carrying selected pathological mutations in the *SORL1* gene. Using advanced microscopy techniques and imaging approaches on 2D

induced neurons and 3D cerebral organoids we confirmed the presence of key pathological hallmarks of AD: accumulated APP and swollen endosomes. Our results now confirm and visualize how pathogenic mutations of the SORLA protein affect AD development.

This research was supported by the project AZV No. NU22J-08-00075 and SORLA-FIX No. 8F20009, NPO No. LX22NPO5107 and CETOCOEN No. 857560, financed by EuropeanUnion. We also acknowledge the core facility CELLIM supported by MEYS CR (LM2023050 Czech-BioImaging).

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PEPTIDE HYDROGEN-DEFICIENT RADICAL ANIONS FORMATION IN SOFT X RAY ATMOSPHERIC PRESSURE PHOTOIONIZATION MASS SPECTROMETRY

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Bottom-up proteomics is a mass spectrometry technique extensively used in protein research. The most common workflow consists of protein digestion, creating subsets of peptides, which are then analyzed by HPLC ESI MS/MS. This approach is powerful; however, some limitations have been identified.

Among others, sufficient ionization of some peptides and adequate localization of the posttranslational modifications (PTMs) remains challenging. The ionization of peptides unfavorably detected in ESI may be enabled by a fundamentally different type of ion source, namely atmospheric pressure photoionization (APPI)[1]. A typical APPI relies on VUV photon emission, which initiates the detachment of thermal electrons from LC solvents and atmospheric gasses, enabling a complex ionization process. Recently, a novel in-house-built soft X ray APPI has been developed[2]. The soft X ray photon energy is about 500 times higher than the energy of VUV photons and allows the displacement of even inner-shell electrons. Consequently, soft X-rays may provide a more effective ionization of analytes than VUV APPI.

This work aims to determine whether newly introduced soft X ray APPI may be beneficial in proteomic analysis. A fundamental study of 22 peptides having four to nine amino acids was performed. The standards were selected to represent peptides generated by tryptic, chymotryptic, or Glu C protein digestion. Modifications such as phosphorylation, lipoylation, and amidation have also been considered. The utility of peptide analysis in soft X ray APPI was evaluated based on a comparison with commercially available VUV APPI and ESI. Exclusively, the soft X ray APPI generated hydrogen-deficient peptide radical anions, allowing improved peptide structural analysis and the successful localization of PTMs.

This work was supported by the Czech Science Foundation (Project No. 20-09126S).

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EXPLORING THE IMPLICATIONS OF HEME INTERACTION WITH THE PROTEINS FROM THE P53 FAMILY

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The p53 protein is a prominent transcription factor that plays an essential role in the suppression of tumorigenesis. In response to cellular stress or DNA damage, p53 binds to the promoters of genes involved in apoptosis, cell cycle arrest, and senescence, activating their transcription[1]. Notably, p53 shares a high degree of homology with two other proteins, p63 and p73. Collectively, the three proteins are known as the p53 family, and all participate in transcriptional regulation. While p53 primarily functions as a tumor suppressor, p63 and p73 are key regulators of differentiation and development. Furthermore, current research indicates that p63 and p73 are ancestors of p53, with p63 being the most ancient of the three[1].

Recent findings also reveal that p53 can interact with heme. This interaction induces conformational changes within the structure of p53, impairs its DNA-binding ability, and ultimately results in the nuclear export and proteasomal degradation of the protein[2,3]. While the consequences of this interaction have been extensively studied in p53, no detailed investigation was performed with p63 or p73. All members of the p53 family share many similarities with the most striking being the presence of DNA-binding domain[1]. This region is conserved across the p53 family and is proposed to be critical for the protein-heme interaction[2,3].

In our present study, we examine the structural and functional aspects of the heme-binding ability in both p53 and

its evolutionary predecessor, p63. We focus on determining the heme-binding sites within these proteins and identifying the critical regions for this interaction. The results of this study not only propose novel perspectives on the role of heme as a signaling molecule but also provide insights into the mechanisms of tumorigenesis and developmental disorders in the context of pathological conditions characterized by heme and iron overload.

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ANTI-INFLAMMATORY EFFECTS OF PALM^{11}-PRRP31 IN A RAT MODEL OF LIPOPOLYSACCHARIDE-INDUCED ACUTE INFLAMMATION

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Lipopolysaccharide (LPS) is the main structural element of the outer membrane of Gram-negative bacteria. LPS is capable of inducing endotoxemia as well as acute inflammation. The administration of LPS in rats in experimental settings has been widely used as a valuable model to induce acute inflammatory responses mainly by activating the Toll-like receptor 4 (TLR4) signaling pathway[1]. Prolactin-releasing peptide (PrRP) is an anorexigenic neuropeptide that is produced and acts in the brain. Recently, we designed a more stable lipidized PrRP31 analog, known as palm^{11}-PrRP31, which can act centrally after peripheral administration[2].

The major aim of this project is to evaluate the anti-inflammatory potential as well as mechanism of action of palm^{11}-PrRP31 in rat model of LPS-induced acute inflammation.

24 Wistar Kyoto (WKY) rats were divided randomly into four groups. Rats were pre-treated intraperitoneally with saline, or palm^{11}-PrRP31 for 7 days and then administered with saline or LPS (saline/saline group, saline/LPS group, palm^{11}-PrRP31/saline group and palm^{11}-PrRP31/LPS group). Body weight and food intake were monitored. Released cytokines and chemokines were determined by enzyme-linked immunosorbent assay (ELISA) kit and ProcartaPlex Assay from plasma, liver and hypothalamus samples. Protein expression associated with the TLR4 signaling pathway was detected in the liver by western blots.

The results showed that the palm^{11}-PrRP31 mitigate LPS-induced weight loss and anorexia, emphasizing its potential protective effects. The peptide also modulates key cytokines and chemokines in plasma liver and hypothalamus, showing broad-spectrum of anti-inflammatory properties. Furthermore, the study demonstrated a significant suppressive effect on the activation of TLR4 signaling pathways during LPS-induced inflammation.

In conclusion, these results contribute valuable insights into the immunomodulatory properties of palm^{11}-PrRP31. The study suggests a potential for applications in inflammatory disorders.

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CELL-MEDIATED MULTILAYERED 4th GENERATION BIOMIMETIC SCAFFOLD FOR SINGLE-STEP TOTAL SKIN SUBSTITUTE: FROM LABORATORY TO CLINICAL APPLICATION

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Patients with deeply damaged skin over a large body surface area lack a mechanical barrier, immune protection, and other essential skin functions. In severe cases, this condition can lead to multiorgan dysfunction or other serious complications. Conventional treatments such as autologous split-thickness skin graft (STSG) are not feasible for these patients because they lack donor sites. There are some commercial skin substitutes, but they are often acellular, require overlapping with STSG, or are only applicable as a temporary solution[1]. Therefore, a total skin substitution is the only option for them.

It has been shown that cellular skin substitutes ensure faster healing and vascularization of the wound. Suitable cells for the skin substitutes are mesenchymal stromal cells (MSCs), which have anti-inflammatory properties, suitable differentiation capacity, and promote angiogenesis[2]. Our goal was to create a biomimetic skin substitute that contains three

native layers of skin (epidermis, dermis, hypodermis) and is applied in only one step.

We designed and optimized a biodegradable and biocompatible collagen/chitosan foam as a scaffold[3]. Next, we differentiated MSCs into fibroblasts and preadipocytes, which were then seeded on a scaffold mimicking the dermis and hypodermis. Isolated keratinocytes were seeded on top of the scaffold and served as a layer of epidermis.

Complete 3D skin substitutes cultured *in vitro* were evaluated by histological analysis and compared with vital skin samples. The distribution of individual cell types and layers was determined by immunohistochemistry staining. The first successful *in vivo* testing of skin substitutes in a pig model was also performed.

This work was supported by the Czech Health Research Council under the project no. NU22-08-00454 and by funds from the Faculty of Medicine, Masaryk University – MUNI/A/1598/2023.

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TRUNCATED VITRONECTIN WITH E-CADHERIN ENABLES THE XENO-FREE DERIVATION OF HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are able to differentiate into any cell type of a human body, have self-renewal abilities and very high proliferative activity[1]. These unique abilities enable their use in cell therapy, disease modelling, and drug development.

The derivation of hESCs is usually performed using an animal or human cell feeder layer, which is undefined and potentially contagious, and because of these difficulties, there is a tendency to replace feeders with xeno-free defined substrates in recent years[2].

We used truncated vitronectin with E-cadherin as a defined xeno-free substrate for the derivation of hESCs for the first time, derived three hESC lines, and confirmed their undifferentiated state, hESC morphology, and standard karyotypes together with their potential to differentiate into three germ layers (ectoderm, mesoderm, and endoderm)[3]. We confirmed for the first time that truncated vitronectin with E-cadherin is a defined xeno-free substrate that is suitable for the derivation of hESCs involved in cell therapies.

This research was supported by funds from the Faculty of Medicine, Masaryk University –MUNI/IGA/1332/2021, MUNI/A/1301/2022 and MUNI/A/1598/2023. Supported by Ministry of Health of the Czech Republic, Grant No. NU22-08-00629. *All rights reserved.*

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SYNTHESIS AND APPLICATION OF MAGNETIC MOLECULARLY IMPRINTED POLYMERS IN PRETREATMENT OF PERSONAL CARE SAMPLES

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Molecularly imprinted polymers (MIPs) are synthetic materials with a predetermined affinity for a given analyte. MIPs on the surface of magnetic particles are a promising tool for the extraction of substances from complex samples. They combine magnetic properties and selectivity for target molecules[1]. The aim of work was to fabricate MIP on the surface of a magnetic carrier for selective pretreatment of D-panthenol in cosmetic samples (body milk, hair water). MIPs were prepared on the surface of a magnetite and the polymerization mixture included a monomer (methacrylic acid, acrylamide or 4-vinylpyridine), a porogen (methanol), an initiator (azisobutyronitrile), and a cross-linker (ethylene glycol dimethacrylate)[2]. MIPs were characterized from several aspects to select the most suitable. The process of adsorption kinetics was described by pseudo 2nd order model which implied a chemisorption of D-panthenol onto MIPs particles. According to the values of the swelling ratio MIPs swells more in water than in methanol. The adsorption of D-panthenol on MIPs is fitted by Freundlich isotherm, indicating sorption occurred on heterogeneous sites. FTIR analysis showed complete removal of the template from the cavities of the prepared MIPs. MIPs were five-times reusable without significant decrease in adsorption efficiency (< 5 %, RSD < 2.5 %). The most suitable MIP (monomer: 4-vinyl-pyridine) was applied as adsorbent for magnetic solid phase extraction. Extractions reached recovery higher than 80% (RSD < 5.0 %). The extracts were analyzed by HPLC-DAD method which was characterized by LOD value of 1.1 μg/mL. Compared to traditional SPE in column, the proposed

procedure is more advantageous due to saving time and solvents, and it is appropriate for more viscous types of samples.

This work was supported by Early stage grant 09I03-03-V05, number 23-04-01-A.

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SELECTIVE ELECTROPHOTOOXIDATIONS OF BENZYL ALCOHOLS IN THE PRESENCE OF COMPETITIVE OXIDABLE HETEROATOMS

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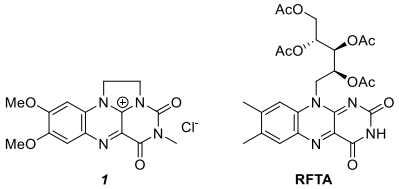
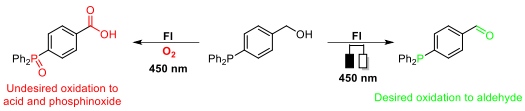
Recently, photooxidations have been an efficient tool in organic synthesis with a wide range of applications[1]. The well-known catalysts in photooxidations are flavins and their analogues (isoalloxazines, alloxazines, and flavinium salts). The basic structure of isoalloxazines represents riboflavin tetraacetate (RFTA) which is an efficient catalyst for easily oxidizable groups (*e.g.* benzyl alcohols), but for more challenging substrates (e.g. electron-poor benzyl alcohols) flavinium salts, *e.g.* salt ***1***, are more powerful (Scheme 1)[2].



Scheme 1. **Structure of isoalloxazinium salt *1* and RFTA.**

However, "pure" photocatalysis suffers from using O_2 as a regenerating agent and because of the deactivation of the catalyst and the lack of selectivity due to the overoxidation of functional groups. The solution to this problem could be another regenerating agent (e.g. electric potential[3] or acetonitrile[4]) and an inert atmosphere. Electrophotocatalysis with flavins has been tested on the oxidation of cyklohexylalcohols, but it requires the presence of thiourea as a mediator[3]. Herein, we have developed an electrophotocatalytical setup without any mediator only in the presence of catalyst and substrate, which will be applied in the selective oxidation of alcohol group alongside other oxidizable heteroatoms (S, P) in the same molecule (Scheme 2).



Scheme 2. **Classical photooxidation (on the left) in comparison with selective electrophotooxidation (on the right).**

This work was supported by the Czech Science Foundation (reg. No. 23-06465S).

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JOURNEY TOWARD THE FIRST TOTAL SYNTHESIS OF ENIGMAZOLE C – SYNTHESIS OF FRAGMENT B

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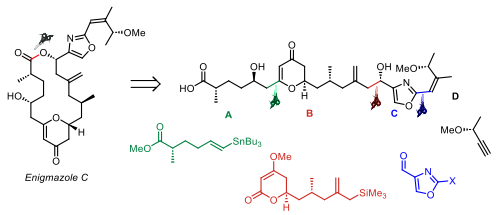
Enigmazoles A-E are newly isolated macrocyclic lactones from recently identified marine fungi of the genus *Homophymia*. Over the past 20 years, marine sponges have become a never-lasting source of new types of substances, especially macrolides, with interesting structures and fantastic biological activity, the most important biological activity being antitumor and neuroprotective activity. Probably the most important substances from these marine sponges are homophymins A-E, homophymamides, pipecolidepsins and callipetins – in short, high-molecular peptides with cytotoxic and anti-HIV activities.

Interestingly, however, our project focuses in general on macrolides and this subdivision of it to Enigmazole C in particular. The long-term aim of our study is to prove that there is a synergistic effect between high-molecular-weight peptides and macrolides (macrocyclic lactones). Thus, we wish to prove that macrolides recently isolated in the presence of high-molecular peptides enhance their observed biological activity. As a model substrate, recently isolated Enigmazole C was chosen to serve as a model macrolide for this purpose. In this contribution, our synthetic efforts directed towards enigmazole C synthesis will be presented.



Scheme 1. **Retrosynthesis of Enigmazole C.**

This work was supported by the European Regional Development Fund-Project "Centre for Experimental Plant Biology" (no. CZ.02.1.01/0.0/0.0/16_019/0000738) and by the IGA of Palacky University (IGA_PrF_2022_012 and IGA_PrF_2022_022).

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PREPARATION AND OPTIMIZATION OF MOF AND SILICONE POLYMER COMPOSITE FOR SAFE MEDICAL MATERIAL

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MOFs are highly porous crystalline materials characterized by a large specific surface area[1]. The main advantage of MOFs is usually their easy synthesis and availability[2]. MOFs are biocompatible, biodegradable and heat resistant structures. It is in terms of these properties that MOFs are more effective than gold and silver nanoparticles, graphene and than some carbon materials in many known applications[3]. MOFs can be referred to as porous coordination materials. Their structure consists of two parts. The first one is organic ligands linked by strong coordination bonds and the second inorganic part consists of metal-based nodes. The structure of MOFs then forms open crystalline frameworks that have permanent porosity[4]. An important property of MOFs is that they can release antibacterial metal ions or organic linkers from their structure[5].

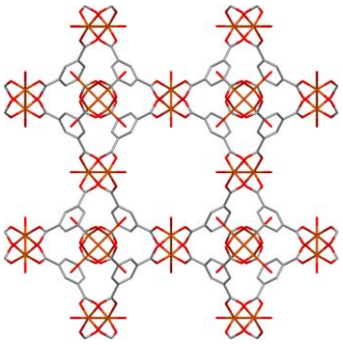

Fig. 1. **X-ray image of CuBTC MOF, view along the crystallographic axis *a* (from[5]).**

The aim of this work was to study a composite material based on an antibacterial metal-organic framework, MOF. Biomedical application was selected as the application of the composite material to be further studied. Specifically, the use of the polymeric material as an implant or tissue replacement. We want to improve this polymeric material by adding MOF with antibacterial effects. For this work, the MOF CuBTC was selected, which is known for its antibacterial properties and has been studied in a number of applications.

A composite material based on polydimethylsiloxane (PDMS) containing different concentrations of MOF CuBTC was prepared. Characterization of the individual samples was performed using infrared spectroscopy, Raman spectroscopy, X-ray structural analysis, scanning electron microscopy, BET analysis, mechanical testing and most importantly antibacterial testing. Due to the positive results of the antibacterial tests, the resulting composite will be the subject of further research in terms of its use as an antibacterial biomaterial.

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CE-MS ANALYSIS OF INSULIN-LIKE GROWTH FACTOR 1 IN PHARMACEUTICAL PREPARATIONS

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Insulin-like growth factor-1 (IGF-1) is a 70-amino acid single chain polypeptide synthesized and secreted by the liver in response to pituitary growth hormone. IGF-1 has found application in diagnostics as a biomarker of growth hormone disorders and as a therapy for growth failure in children and adolescents. Due to its strong anabolic effects, it is often abused by athletes for doping purposes in various pharmaceutical forms, including tablets, capsules, and injectable solutions.

Here, we developed on-line capillary zone electrophoresis-mass spectrometry (CE-MS) method hyphenated with electrospray ionization for determination of IGF-1 in pharmaceutical matrices. We achieved an efficient separation and well-shaped peaks with favorable migration times (<15 min) by optimizing the background electrolyte (500 mM formic acid + 5% acetonitrile) and a separation voltage (+30 kV). Highly efficient, accurate, repeatable, sensitive (sub μg/mL levels) and selective analysis of IGF-1 was achieved with further optimization of a sheath liquid composition (0.1% formic acid in 50% methanol, v/v/v), sheath liquid flow rate (0.6 μL/min) and mass spectrometry conditions (nebulizing gas pressure 4 psi, drying gas temperature 300 °C and flow rate 8 L/min, capillary voltage +4 kV). Optimized and validated CE-MS method was successfully applied for the determination of IGF-1 in injectable solutions (Increlex®) and its presence was also confirmed in nutritional preparations (tablets and liquid colostrum).

To the best of our knowledge, this is the first work that uses validated CE-MS method for determination of IGF-1 in pharmaceutical matrices and reveals the potential of capillary electrophoresis for its use in drug quality control laboratories with benefits such as high separation efficiency, high-speed analysis, low sample consumption, as well as environmental and cost aspects compared to the more established liquid chromatography methods.

This work was supported by the projects VEGA 1/0483/20, VEGA 1/0514/22, UK/3181/2024, and UK/58/2023 and carried out in the Toxicological and Antidoping Center at the Faculty of Pharmacy Comenius University Bratislava.

DIAGNOSIS OF PERIPROSTHETIC JOINT INFECTION: NEW POTENTIAL BIOMARKERS IN FOCUS

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A serious complication accompanying total joint arthroplasty (TJA) is periprosthetic joint infection (PJI). It appears that bacteria can more easily adhere to implant surfaces and eventually form a biofilm, making them resistant to immune system response and antibiotics. This infection can than expand to the bone, destroying it in the process[1]. Early diagnosis of PJI is thus crucial to prevent further complications and preserve patients' quality of life.

Although several standardized definitions of PJI diagnosis and biomarkers in PJI diagnosis have emerged in the last decade, new biomarkers are urgently needed for early diagnosis of PJI as well as for "grey zone" infections with a lack of traditional infection-related biomarkers and negative culture. Unfortunately, presence of implant entails presence of periprosthetic byproduct, namely wear particles, derived from the material of implant[2]. These particles may trigger the same anti-infection programs as bacteria[3], making distinguishing between real bacterial and aseptic inflammation challenging.

In this study, we focused on novel potential biomarkers of PJI from a group of membrane receptors TREM1, IREM-2, TLT-2 and CD88 on synovial fluid (SF)-derived immune cells. SF can be sampled either at the outpatient service from the patient with TJA with joint effusion, or during the revision operation. SF-derived immune cells in patients with TJA without infection (n=8) and patients with proven or suspected PJI (n=8) were analysed with the use of 14-colour flow cytometry (BDFACS Aria fusion; BD Bioscience) and novel bioinformatical approaches available in FlowJo v10.9.1 (BD Bioscience).

First, total immune cell content was established (Fig. 1A). SF from PJI patients contained higher abundance of neutrophils ($CD15^+CD16^+$), less abundance of monocyte-macrophage lineage ($CD15^-$HLA-DR^+) cells with emphasis on low abundance of myeloid dendritic cells ($CD14^-CD16^-$) and high CD4+/CD8+ ratio comparing to the patients with TJA without infection. Out of the four tested markers, two of them showed high potential for distinguishing PJI. Namely, TREM1 (triggering receptor expressed on myeloid cells 1) has been found mostly on neutrophils with higher expression in suspected/proven PJI patients. On the other hand, CD88, also known as C5aR complement receptor, was expressed on monocyte-macrophage lineage and in lower abundance in TJA patients without infection (Fig. 1B).

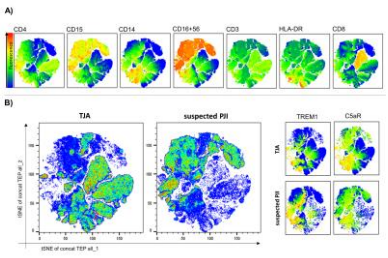

Fig. 1. **[A] Identification of immune cell subsets, [B] Immune cells subpopulations and their distributions in synovial fluid in patients with TJA without infection (n=8) and patients with TJA with PJI (n=8). Immunophenotypes are displayed after t-SNE dimensionality reduction, subgroups are concatenated.**

Immune cells in SF are usually present in high abundance and with proper analytical methods, results can be available in the matter of hours. This study may contribute to the deeper understanding of PJI and obtained results can help to refine diagnosis of PJI.

Acknowledgement: NU21-06-00370, MH CZ – DRO (FNOl, 00098892), IGA_LF_2024_013.

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PHOTOCHEMICAL REACTIONS BATTLES: *o*-QUINONE METHIDES OR ELSE?

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o-Quinone Methides (*o*-QMs) stand out as short-lived, highly reactive electrophilic intermediates with a wide range of applications in organic synthesis, pharmaceutical industry, materials science and biology, among other fields[1]. In fact, flash photolytic techniques are often used to monitor the progression of these reactions due to their rapid nature[2]. However, the methods used to generate *o*-QMs *in situ* involve undesirably high temperatures, long reaction times and catalysts which cause side reactions[3]. This study aims at (i) understanding the reactivity and electronic properties of *o*-QM precursors through cooperating of different photochemically active functional groups (e.g., ortho-nitrobenzyl[4], meta-effect-based systems[5]), (ii) shifting the absorption maxima of *o*-QMs from UV (~350 nm) to the visible range by introducing electron-donating (EDGs) and electron-withdrawing groups (EWGs) in a push-pull arrangement[6], and (iii) probing various monochromophoric systems designed for variable photochemistry controlled by external factors (e.g., solvent, excitation wavelength, chemical additives). Ultimately, our study seeks to overcome the limitations of *o*-QM photochemistry and the findings of this study may help to expand the scope and versatility of *o*-QM applications.

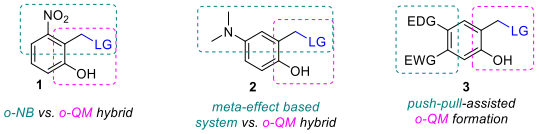

Fig. 1. **Designed cooperative o-QM-based systems.**

This work was supported by the Czech Science Foundation (reg. No. 22-20319S).

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NANOSTRUCTURED NITINOL IN LIVING SYSTEMS: A RAT-BASED BIOCOMPATIBILITY STUDY

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Nitinol is a metallic biomaterial formed by alloying nickel and titanium. Many different characteristics set nitinol apart from other metals and alloys, including its biocompatibility, superelasticity, strength, shape memory, fatigue resistance, and corrosion resistance. This makes nitinol widely used in medical applications, such as orthodontic appliances, vascular stents, joint replacements, and fracture fixation.

Specific health risks are associated with the presence of metal due to interactions between live tissues and implanted metal components. Metal material degradation is unavoidable and can lead to complications. When an implant is damaged (by corrosion or wear), material degradation products (micro- and nanoparticles, ions, inorganic salts, and metal oxides) are released. These products can affect the behaviour of

surrounding cells and induce loosening and osteolysis as well. Various surface modifications are used to improve metal properties, such as extending implant life, reducing inflammation, reducing ions/nanoparticles, and enhancing biocompatibility.

The main goal of this *in vivo* study is to better understand the processes of healing and inflammation brought on by metal ion release.

The experiment was carried out on female Wistar rats. Nitinol disc (with or without nanotube coating) 10 mm in size and 1 mm thick was placed between the *latissimus dorsi* and *scapula*. The control group underwent an incision without the insertion of a metal implant. After the monitoring period (24 hours, 72 hours, 2 weeks, and 4 weeks), blood samples and muscle samples from direct contact with the metal were collected. ICP-MS was used to measure the kinetics of heavy metal ion release into the tissue and circulation, and RT-qPCR was used to examine the expression levels of selected genes that play essential roles in the inflammatory response. The $2^{-\Delta\Delta CT}$ method was used to calculate changes in the expression of target genes.

The data showed a high degree of variability due to varied muscle interactions with metal. Larger amounts of Ti and Ni ions in muscle tissue are related to the formation of a Ti oxide layer on the metal surface and its direct contact with the tissue. When NiTi with a nanotube coating was used, higher levels of Ti ions were tolerated in the muscle tissue, and healing was not affected in any way.

This research was supported by GA20-11321S. Part of this work was carried out with the support of RECETOX Research Infrastructure (ID LM2018121, MEYS CR, 2020-2022).

THE DEVELOPMENT OF AN ALTERNATIVE DRUG FOR STROKE TREATMENT USING STRUCTURAL PROTEOMICS

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The structural proteomic approach, employing hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS), supported by bioinformatics and computational modelling can unravel a plethora of information about protein dynamics.

In our research, we focus on understanding the dynamics of a potential thrombolytic drug, Staphylokinase (SAK), which is extremely promising and cost-effective[1] as currently, Alteplase stands as the sole FDA-approved thrombolytic drug for the treatment of acute ischemic stroke besides having significant limitations such as intracranial bleeding, short half-life, low clot permeability, and potential neurotoxicity[2].

We conducted a comparative analysis of two SAK mutants (SAKm) denoted as SY155 and THR174, using HDX-MS (Fig. 1) These mutants were designed through a rational protein engineering approach. We were able to observe noteworthy conformational changes in SAKm compared to SAKwt that were complemented by biochemical tests, including thrombolytic activity assays.

In conclusion, these results have been used to ascertain the optimal template for the enhancement of the SAK's activity as a potent thrombolytic agent.

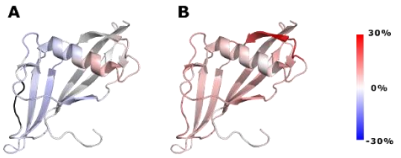

Fig. 1. **3D models of SAK illustrate variations in deuterium uptake (red-maximum, blue-minimum, and black-uncovered peptides) of SAKm compared to the wild-type. (A) SAK SY155 exhibits overall decreased solvent accessibility despite higher deuteration observed in the α-helix region while (B) SAK THR144 is more flexible with a significant increase of deuteration in the loop due to K74Q mutation and exhibits higher solvent accessibility.**

This work was supported by the Czech Science Foundation, project no. 22-09853S, MH CZ - DRO (MMCI, 00209805) and Brno Ph.D. talent, JCMM.

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BACTERIAL ENDOSPORES DISPLAYING RBD DOMAIN OF SARS-COV-2 SPIKE PROTEIN

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Bacillus subtilis is a Gram-positive rod-shaped bacterium and its spores are considered to be efficient vehicles for the surface display and delivery of heterologous proteins[1,2]. In this work we prepared recombinant spores with the receptor binding domain (RBD) of Sars-CoV-2 virus displayed on the surface of spores using fusion with CotZ and CotY[3]. Figure 1 presented an ideal model of fusion of RBD with the spore crust protein CotY. CotZ and CotY are morphogenetic proteins of the spore crust[4]. The use of CotZ and CotY proteins make them a suitable anchor for the spore surface display technique

because of their localization and abundance. In addition, we placed a flexible linker peptide GGGGS between the anchor and target proteins while focusing on the construction of fusion proteins of both types, the SARS-CoV-2 spike glycoprotein RBD to the N-terminal and C-terminal of the spore coat protein CotZ or CotY. Using SDS-page method the pattern of released proteins did not show large differences between the strains. The amount of proteins gradually decreased during the sporulation process, which only confirmed the gradual increase in the number of spores during sporulation. Western blot analysis showed a possible fusion of both crust proteins (CotZ and also CotY) with RBD and also the possibility of fusion in both positions (N-terminal, C-terminal). However, there were differences between the strains during the sporulation process. Strains with RBD in C-terminal positions had bands in higher molecular weights, which most probably represented oligomers of fused proteins, whereas in the remaining two strains were rather bands in l*ower molecular weights, which predicted degradation of fused proteins*. Immunofluorescene microscopy showed fluorescence signal around all recombinat spores, whereas there was only slight fluorescence signal for wild type strain, which might be caused by direct adsorption of antibodies on the surface of spores. We also directly adsorbed non-recombinantly produced RBD on the spore surface and it was fixed strongly enough that it cannot be removed by vigorous spore washing. Fluorescence microscopy showed that intensity of fluorescence of spores with RBD adsorbed on the surface was higher than those of the recombinant strains, which may be due to more accessible binding of the primary antibody to the spore surface, since adsorbed proteins were not incorporated into the spore surface[3]. When considering whether such systems could be used for the development of an oral vaccine, several factors speak in their favour. Firstly, spores are extremely resilient and stable and can withstand unfavourable conditions such as extreme temperatures desiccation, freezing and thawing as well as harsh gastrointestinal environment caused by gastric acids[5]. Secondly, *B. subtilis* is considered as a generally safe microorganism and is also used as a probiotic and feed supplement product for humans and animals, which why they are ideal vehicle for oral administration of vaccines[6]. Last but not least, other studies have shown the benefits of using spores as vaccines because they can stimulate the immune response with a strong adjuvant affect[7]. These results indicate that both types of systems could be used for the development of new oral vaccines not only against Sars-Cov-2 virus, but also against other newly emerging life-threatening viruses and bacteria.

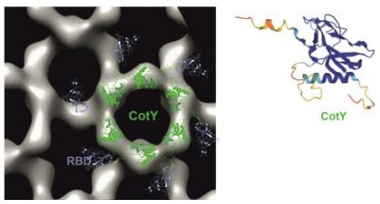

Fig. 1. **Model of the CotY-RBD fusion protein structure. Left picture represents three-dimensional 2D surface crystal structure of CotY into which the 3D structure of CotY is superimposed. The RBD domain of the SARS-CoV-2 spike protein structure is adapted from Protein DataBank structure 6M0J. Right picture represents monomer structure of the CotY. Pictures of the CotY protein are predicted using AlphaFold[8].**

This work was supported by VEGA – Grant No. 2/0001/21 from the Slovak Academy of Sciences, a Grant from the Slovak Research and Development Agency under contract APVV-18-0104 and APVV-22-0303 to IB.

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DYSREGULATED MICRORNA EXPRESSION IN SELECTED MONOCLONAL GAMMOPATHIES

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Monoclonal gammopathies are a group of both benign and malignant diseases characterized by increased production of monoclonal immunoglobulin due to clonal expansion of malignantly transformed plasma cells (PCs). Diseases in this group comprise, for example, multiple myeloma (MM), extramedullary disease (EMD), and plasma cell leukemia (PCL). In MM, PCs infiltrate the bone marrow, whereas in EMD, the cells lose their dependence on the bone marrow and form bone-related or soft tissue lesions[1]. Plasma cell leukemia is in turn characterized by the presence of PCs in the peripheral blood. Treatment continues to be difficult as

patients relapse or develop refractory disease. Tumor microenvironment, which also includes exosome-mediated intercellular communication, is thought to play an important role in the pathogenesis of the disease or the development of drug resistance.

Exosomes are lipid bilayer-coated vesicles of 30-100 nm in size, which are classified as extracellular vesicles (EVs) and are found in almost all body fluids (peripheral blood, breast milk, urine, etc.). Their contents include proteins, lipids or nucleic acids, while the composition of the exosomes reflects the cell of origin. Bioactive molecules can be transported between cells via exosomes and subsequently induce changes in biological processes in the recipient cell by various mechanisms[2]. An example is microRNA (miRNA), i.e. non-coding RNA molecules of about 20–25 nt in length. In particular, they are involved in the post-transcriptional regulation of gene expression, thereby influencing many biological processes, both physiological and pathological. Previous research by us and others suggests that the expression level of specific miRNAs can potentially be used as a biomarker (minimally invasive in the case of peripheral blood analysis as part of the concept of so-called liquid biopsies)[3]. However, in this work, we focused on the characterization of the bone marrow microenvironment in these patients in terms of miRNAs from exosomes and small EVs.

We first isolated small EVs (qEVoriginal Size Exclusion Columns, Izon Science) and then miRNAs (miRNeasy Micro Kit, Qiagen) from bone marrow plasma of MM, EMD, and PCL patients. Subsequently, we prepared libraries (CleanTag™ Small RNA Library Prep Kit, TriLink BioTechnologies) from a subset of the processed samples (specifically 8 MM, 6 EMD, 8 PCL). The Qubit dsDNA HS Assay (Thermo Fisher Scientific) and High Sensitivity D1000 ScreenTape Assay (Agilent) were used for quantification and quality control; based on the results, some of the libraries were adjusted prior to sequencing using the Pippin Prep system (Sage Science). Libraries were pooled and sequenced on the Illumina platform using the NextSeq 500 Reagent kit v2 and flow-cell with 50 bp single-end reads. By this means, we identified several differentially expressed miRNAs, 6 of which were validated on a larger, independent cohort (24 MM, 32 EMD, 8 PCL) by qRT-PCR (TaqMan® Advanced miRNA cDNA Synthesis Kit & Assays, Applied Biosystems). ROC analysis was performed for miRNAs whose relative expression was significantly different. The findings were correlated with clinical data. A statistically significant difference in expression between disease groups was confirmed for selected miRNAs.

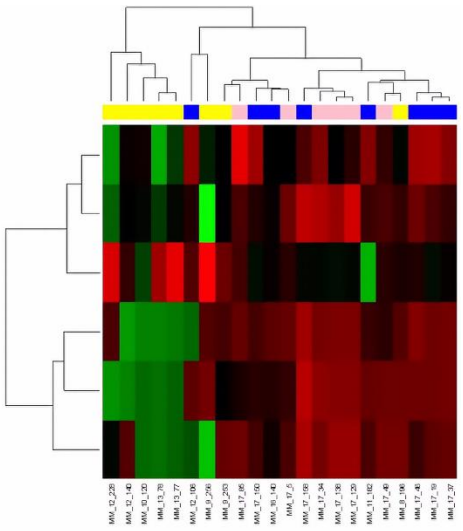

Fig. 1. **Heat map showing differentially expressed microRNA in MM (blue), EMD (pink), PCL (yellow).**

Supported by the Czech Ministry of Health (NU21-03-00076), the Masaryk University (MUNI/A/1370/2022), the University Hospital Brno (FNBr, 65269705), and the project National Institute for Cancer Research (EXCELES, ID Project No. LX22NPO5102) - Funded by the EU – Next Generation EU.

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DEVELOPMENT OF TANDEM SEQUENCE FOR THE CONSTRUCTION OF POLYCYCLIC SCAFFOLDS

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Chemical synthesis stands as a pivotal approach for obtaining complex molecules, including life-saving drugs. Despite its successes, significant attention has been directed towards devising more efficient methods to synthesize these valuable molecules, focusing on atom-, step-, and redox-economical approaches[1]. One of the most potent strategies involves the utilization of catalytic cascade reactions, enabling the formation of multiple bonds in a single step, thus creating intricate molecular scaffolds from simple building blocks[2].

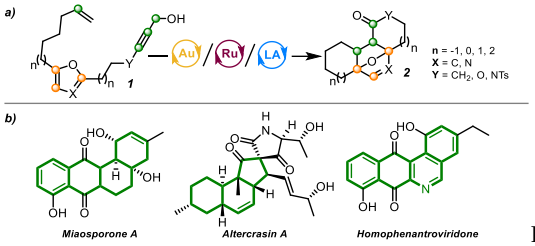

Fig. 1. **a) Meyer-Schuster rearrangement/RCM/TADA tandem sequence, b) Selected natural products.**

In this study, our focus has been on developing an innovative synthetic platform capable of rapid assembly of polycyclic scaffolds *via* a sequence involving the Meyer-Schuster rearrangement, Ring-closing metathesis, and transannular Diels-Alder tandem reactions (Fig. 1a)[3]. The process commences from basic linear precursors ***1*** and, through a series of cascade reactions utilizing gold, ruthenium, and Lewis acid catalysts, yields polycyclic systems ***2*** efficiently. Additionally, the synthetic platform represents a concise strategy for synthesizing a range of polycyclic natural products known for their potent bioactive properties, including antimicrobial or cytotoxic effects (Fig. 1b)[4]. Therefore, our strategy holds promise in providing a versatile and efficient pathway to these important compounds, which could lead to the discovery of novel therapeutics.

The financial support by the Experientia Foundation (Start-Up grant 2024-2026) is gratefully acknowledged.

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DEAZAALLOXAZINES AS POWERFUL MODERN CATALYSTS FOR PHOTOREDUCTIONS

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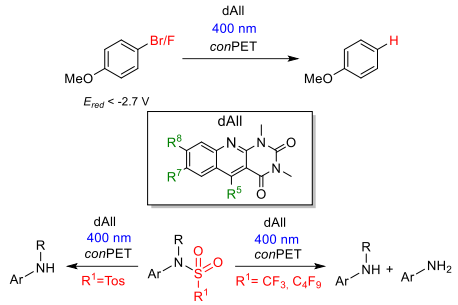
Deazaalloxazines are unexplored members of the flavin family. These derivatives have promising properties in the field of photocatalysis, because they are characterized, upon excitation, with reducing power comparable to that of lithium[1]. Such properties indicate potential applications in reductive photoredox catalysis. However due to absorption in the UV region only, unsubstituted deazaalloxazines have not been tested in photochemistry yet[2].

Using different approaches, we have successfully prepared series of deazaalloxazines with various substituents in positions 5, 7 and 8 (Scheme 1). We found a suitable modification of the structure for photochemical properties such as absorption and stability during irradiation.

The prepared derivatives have been tested in the dehalogenation of the challenging bromo- and fluoroarenes possessing electron-donating methoxy group (Scheme 1)[1]. Deazaalloxazines have also been applied in the photoreductive desulfonylation of sulfonamides to the parent amines and they have even mediated the cleavage of perfluoroalkanesulfonamides, which has not been previously achieved photochemically (Scheme 1)[3]. We have achieved of scaling up our method under using same condition.



Scheme 1. **Photoreduction catalysed by deazaalloxazines.**

This work was supported by the Czech Science Foundation (reg. No. 24-11386K) and by grant of Specific university research (reg. No. A2_FCHT_2023_025).

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NANOGEL-COATED PHOTON-UPCONVERSION NANOPARTICLES FOR IMMUNOCHEMICAL ASSAYS

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Photon-upconversion nanoparticles (UCNPs) outperform other luminescence labels with background-free emission, narrow emission peaks in the visible and near-infrared region, and large anti-Stokes shifts[1]. The use of UCNPs in analytical chemistry and biological fields is of increasing importance. To fully utilize the properties of UCNPs in aqueous dispersions, the research and engineering

of hydrophilic shells or surface modifications are absolutely necessary[2]. Here, we report a novel synthesis of polyacrylic nanogel-coated photon-upconversion nanoparticles (UCNP-NGs) for use in Upconversion-Linked Immunosorbent Assay (ULISA). Tm^{3+}-doped UCNPs with 68 nm size are encapsulated into the shell of polyacrylic nanogel in one synthesis step by utilizing water in oil microemulsion. The addition of ammonium persulfate catalyzes radical polymerization taking 90 min at laboratory temperature. Acrylic nanogel is present as a hydrophilic shell with a thickness of 3.8 ± 2.5 nm. UCNP-NGs provide bright emission at 802 nm making them suitable for both analog and digital bioaffinity assays[2,3]. UCNP-NGs are successfully conjugated to biotin, and streptavidin via carbodiimide activation chemistry, and synthesis yield is characterized by absolute quantification[4]. Then an indirect competitive ULISA for aflatoxin B1 determination is developed. Finally, three commercial samples of rolled oats are tested by ULISA for aflatoxin B1 presence. In comparison to Enzyme-Linked Immunosorbent Assay (ELISA), ULISA eliminates the need for enzyme-catalyzed signal amplification allowing for instant detection and contributes to the lower limit of detection[1].

JW and AH acknowledge the grant 24-11183S (Czech Science Foundation), and support from UIACH (RVO: 68081715).

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THIOL LABELLING BY IMPROVED METHANETHIOSULFONATE-BASED BODIPY DYES

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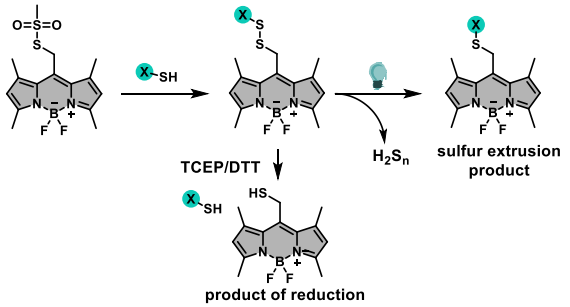
Thiols are reactive functional groups important in redox homeostasis (as free thiols) and protein folding (concealed as disulfides)[1]. Thanks to their higher reactivity compared to *O*- or *N*-nucleophiles, thiols are often used as reactive handles for the labelling of biomolecules. Thiol labelling is a crucial tool for understanding the structure of proteins and the function of thiols in cellular processes and homeostasis.

Common labelling methods often benefit from the reactivity of thiols with haloalkyl derivatives or maleimides, which often suffer from unwanted nonspecific side reactions. A more selective alternative uses mentahethiosulfonates (MTS), which are known for their selective and fast reactivity with free thiols. However, disulfides formed in this reaction can be readily reduced back to thiols, disconnecting the labelled conjugate[2].

Recently, we discovered a unique reactivity of disulfides directly attached to the *meso*-position of BODIPY, efficiently providing thioethers after irradiation with visible light (Scheme 1). Thioethers are more stable than disulfides and do not undergo unwanted reduction. This method improves the MTS-based labelling chemistry by enhancing the product stability without compromising the labelling selectivity.



Scheme 1. **Reactivity of MTS-based BODIPY dye and of disulfide product upon irradiation or under reducing conditions.**

This work was supported by the Czech Science Foundation (project Nr. 22-20319S).

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iPSC-DERIVED HUMAN INTESTINAL ORGANOIDS: A NEW PERSONALIZED MODEL TO STUDY IBD AND INTESTINAL FIBROSIS

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Inflammatory bowel diseases (IBD), such as ulcerative colitis or Crohn's disease (CD), are characterized by recurrent intestinal inflammation that compromises the integrity of gut epithelial barrier. An extremely dangerous complication that accompanies the outbreak of CD is intestinal fibrosis[1]. During the development of fibrosis, an excessive deposition of

extracellular matrix occurs which leads to the formation of strictures[2]. Currently, no effective therapies to treat this condition exist. Furthermore, no model is available that can be used to help researchers efficiently study fibrosis. The function of intricate intestinal mechanisms may be better understood with the use of human intestinal organoids (HIOs). The aim of this project was to develop personalized organoids from CD patients using patient-specific iPSCs to investigate intestinal fibrosis *in vitro*.

Blood-derived induced pluripotent stem cells (iPSCs) were obtained from the iPSC Core at Cedars-Sinai Medical Center. iPSC cells were maintained in mTeSR1 media. iPSCs were seeded into Matrigel-coated plates and directed to generate HIOs in multistep protocol. To stimulate definitive endoderm development, iPSCs were cultured in RPMI 1640 medium containing differentiation-promoting factors for three days. For four following days, cells were cultured in DMEM/F12 medium supplemented with FGF4 and CHIR99021 to form hindgut structures. Finally, the epithelial spheres became visible. These organoid-shaped structures were harvested, embedded in Matrigel, and cultured in HIO media[3]. HIOs were passaged regularly every week. Subsequently, HIOs were collected and dissociated into single cells using TrypLE Express. Magnetic-activated cell sorting (MACS) was used to separate mesenchymal and epithelial cell cultures. Epithelial cell culture was used to generate epithelial-only organoids (eHIOs). Both cell types, eHIOs and mesenchymal cells (MSCs), were then cultured separately and treated with TNFα or TGFβ to induce inflammatory and fibrotic conditions. After 24 h, expression of inflammation and fibrosis related genes was determined using RT-qPCR. Moreover, immunocytochemistry (ICC) protocol was used to section, stain and visualize the 3D structure of newly developed eHIOs using fluorescence microscopy.

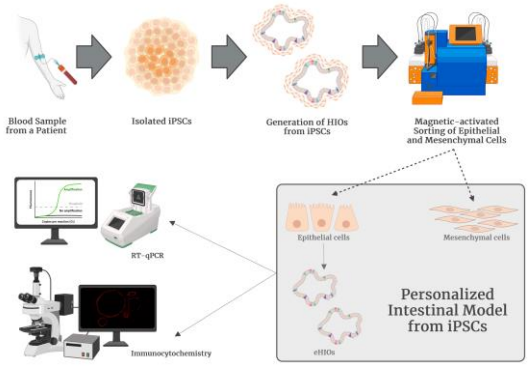

Fig. 1. **Development of personalized intestinal model from iPSC.**

Obtained iPSCs were successfully directed to form patient-specific HIOs containing both epithelial and mesenchymal cell types. To separate these two cell types, MACS was used to develop purified cell cultures of each. RT-qPCR results showed significant increase in expression of inflammation or fibrotic genes, such as *COL1A1*, *COL4A1*, *MMP9*, or *MMP10* in eHIOs and MSCs. Therefore, we were able to stimulate response of inflammatory and fibrogenic stimuli in this model. Using ICC, successful 3D formation of eHIOs was also confirmed.

A new approach to develop a personalized intestinal model was successfully implemented, resulting in forming of patient-specific HIOs, eHIOs and MSCs to study IBD and intestinal fibrosis.

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SYNTHESIS OF SELECTED CATELLATOLACTAMS, INSIGHTS INTO THEIR BIOSYNTHESIS, AND BIOLOGICAL ACTIVITY AGAINST NEMATODES.

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Ansamycins[1] is a family of secondary bacterial metabolites that show antimicrobial activity against various Gram-positive and several Gram-negative bacteria and antiviral activity against bacteriophages and poxviruses[2]. The biological activity of such compounds is strongly related to their unique structure consisting of a combination of a rigid aromatic part and a bridged aliphatic chain. Taking into account such background, it was surprising that recently isolated new types of ansamycin, catellatolactams A−C (***1−3***, isolated from rare actinomycete of the genus *Catellatospora*)[3] (Fig. 1A) had literally *no* biological activity.

This observation led us to hypothesize that the missing benzoquinone-like scaffold (***4*** and ***5***, respectively) is responsible for such an observation (Fig.1B). Based on the analysis of the proposed biosynthesis of such compounds, we have identified intermediates ***4*** and ***5*** that contain benzoquinone scaffolds as a possible biosynthetic precursor to natural products ***1-3***. The objective of the project is (a) to prepare catellatolactam A (***1***) and transform it into compound ***2*** with the help of laccase or peroxidase (via putative intermediates ***4*** and ***5***), and (b) to evaluate the biological activity of compounds ***4*** and ***5***. The contribution presents our latest results.

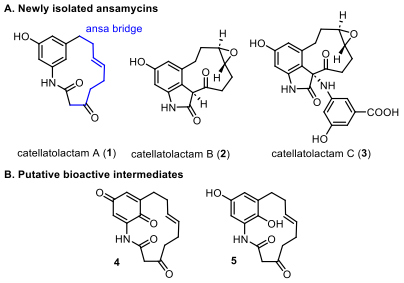

Fig. 2. **A. Structures of catellatolactams A-C (*1*–*3*) possessing no known biological activity. B. Proposed structures of presumably biologically active catellatolactam (biosynthetic) precursors.**

This work was supported by the IGA of Palacky University (IGA_PrF_2023_20).

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INHIBITION OF GALECTINS WITH LACTOSYL ANILIDES

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Galectins are proteins from the lectin family that selectively bind D-galactosides such as lactose and LacNAc. Over the years, interest in their inhibition has been growing, as they are responsible for several cell functions such as cell adhesion, communication, and other functions, which influence pathological processes as cancer and virus cell entry[1,2]. Recently, there have been several attempts to bring a glycomimetic drug targeting galectins to the market[3].

We have developed a new synthetic strategy towards glycosyl amines and amides[4] and proposed a simple two-step procedure for the preparation of lactose-based mimetics from available substrates. First results provided us with few hit compounds and their crystal structure in galectin-3. These were followed up by a second series of compounds based on a thorough *in silico* screening. The computational methods proved to be a great tool for the design of galectin inhibitors, as the theoretical results corresponded well with laboratory measurements.

Best motifs discovered in our study were combined with known derivatization, resulting in high-affinity compounds on a thiolactose scaffold. This new type of glycomimetics provided results comparable with those of known inhibitors.

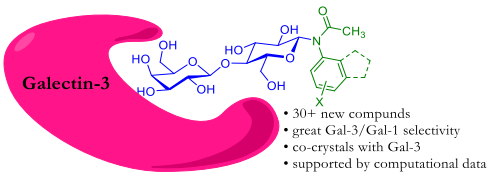

Fig. 1. **General representation of prepared compounds in Gal-3.**

This work was supported by the Czech Science Foundation (grant number 22-17586S).

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REMARKS AND NOTES

CZECH CHEMICAL SOCIETY SYMPOSIUM SERIES • ročník/volume 22 (2024), čís./no. 1 • ISSN 2336-7202 (Print), ISSN 2336-7210 (On-line) • ISSN 2336-7229 (CD-ROM) • evidenční číslo MK ČR E 21999 • Vydává Česká společnost chemická jako časopis Asociace českých chemických společností ve spolupráci s VŠCHT Praha, s ČSPCH a ÚOCHB AV ČR za finanční podpory Rady vědeckých společností ČR, Akademie věd ČR, Nadace Český literární fond a kolektivních členů ČSCH • IČO 444715 • Published by the Czech Chemical Society • VEDOUCÍ REDAKTOR/EDITOR-IN-CHIEF: V. Vyskočil • REDAKTOŘI/ EDITORS: J. Barek, E. Benešová, P. Drašar, P. Holý, P. Chuchvalec, M. Jurášek, Z. Kolská, B. Kratochvíl, J. Masák, J. Podešva, P. Šmejkal, V. Vyskočil; Webové stránky: P. Drašar • TECHNICKÁ REDAKTORKA/EDITORIAL ASSISTANT: R. Řápková • Redakce čísla (ISSUE EDITOR) M. Jurášek, R. Řápková, Z. Pecková, P. Drašar • ADRESA PRO ZASÍLÁNÍ PŘÍSPĚVKŮ/ MANUSCRIPTS IN CZECH, SLOVAK OR ENGLISH CAN BE SENT TO: Chemické listy, Novotného lávka 5, 116 68 Praha 1; tel./phone +420 221 082 370, e-mail: chem.listy@csvts.cz • PLNÁ VERZE NA INTERNETU/FULL VERSION ON URL: http://www.ccsss.cz • TISK: TG TISK s.r.o., 5. května 1010, 563 01 Lanškroun • SAZBA, ZLOM: ČSCH, Chemické listy • Užití tohoto díla se řídí mezinárodní licencí Creative Commons Attribution License 4.0 • • Cena výtisku / Sigle issue price 180 Kč • This journal has been registered with the Copyright Clearance Center, 2322 Rosewood Drive, Danvers, MA 01923, USA, where the consent and conditions can be obtained for copying the articles for personal or internal use • Pokyny pro autory najdete na http://www.ccsss.cz , zkratky časopisů podle Chemical Abstract Service Source Index (viz http://cassi.cas.org/search.jsp) • Molekulární námět na obálce: Vladimír Palivec • Dáno do tisku 22.4.2024.