52nd Conference
Synthesis and Analysis of Drugs 2024
Hradec Králové

BOOK OF ABSTRACTS

Compiled and edited by Andrea Bachtíková and Jan Zitko

Hradec Králové, 2024

Dear colleagues and friends,

The Organizing Committee is pleased to invite you to participate in the 52nd Conference Synthesis and Analysis of Drugs (SAL 2024) that will take place in Hradec Králové (Czech Republic) as an onsite event from September 19th to 20th, 2024. This year's conference will commemorate the 55th anniversary of the foundation of the Faculty of Pharmacy in Hradec Králové.

Synthesis and Analysis of Drugs (Syntéza a analýza léčiv) is an annual conference held alternately in the Czech and the Slovak Republic. It has a long tradition dating back to 1966. The conference covers all aspects of pharmaceutical chemistry and analysis, including adjoining fields such as biochemistry, pharmacology, molecular biology, bioorganic and bioinorganic chemistry, and related disciplines. The conference will be organized under the auspices and on the premises of the Faculty of Pharmacy, Charles University in Hradec Králové.

I believe the conference will be very pleasant and fruitful. There will be many opportunities to make new contacts and discuss current challenges in the fields of medicinal chemistry, pharmaceutical analysis, and other disciplines. Young scientists and PhD students are especially encouraged to present the results of their work. Dear colleagues, I look forward to meeting you at this traditional annual conference for experts from the Czech Republic, the Slovak Republic, and many other countries.

Prof. PharmDr. Martin Doležal, Ph.D.
chairman of the Organising committee
chairman of the Section of Synthetic Drugs, Czech Pharmaceutical Society

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POSTERS

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY

P01

SYNTHETIC APPROACH TO UNSYMMETRICAL ABAC-TYPE PHTHALOCYANINES VIA [3+1] INTERMOLECULAR CYCLIZATION

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Phthalocyanines (Pc) are a class of π-conjugated heteroaromatic macrocycles, consisting of four isoindole rings linked by nitrogen bridges. Upon introducing asymmetry, the molecular structure gets perturbated electronically allowing a fine-tuning of the physical properties. However, the chemistry of low-symmetric phthalocyanine has received mediocre attention than that of Pc's with D_{4h} symmetry. The mixed condensation reactions using multiple precursors limit the feasibility of the formation of low-symmetric Pc's owing to the identical molecular weight and properties of the product formed.[1]

Controlled formation of target Pc with a minimal amount of side products could be achieved by a [3 + 1] approach *i.e,* a base-promoted condensation of pre-linked trisphthalonitrile (ABA-trimer) and a free phthalonitrile (C) in the presence of a metal template (M).[2] We have successfully synthesised the subunit B of ABA-trimer with *tert*-butylthio linkers at the β-position and propoxy linkers at the α-position. Correspondingly, the propoxy linkers at the α-position of subunit B are connected to the α-position of phthalonitrile A, via a sulphur atom constituting the targetted trimer. Eventually, the ABAC-Pc is obtained via the cyclization reaction of ABA trimer with 4,5-bis(2,6-bis((1H-imidazol-1-yl)methyl)-4-methylphenoxy)phthalonitrile (C). Similarly, with improved selectivity and yield, this approach could be pursued for the preparation of low-symmetrical phthalocyanines having hydrophilic and/or lipophilic moieties attached covalently. Moreover, the pre-connected ABAtrimer and free phthaonitrile C can be appropriately chosen to generate exotic phthalocyanines.

The study was supported by ERC-CZ, Project Number: LL2318.

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P02

IN-CELL ACTIVATABLE PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY OF CANCER

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Conventional chemotherapeutic treatment of Cancer poses a threat to healthy cells, presenting the need for selective cell death. Although photodynamic therapy[1] of cancer is an efficient non-invasive therapy, an ideal photosensitizer (PS) is still on the lookout. Silicon phthalocyanines are macrocyclic tetramers with interesting photophysical properties and highly applicative singlet oxygen production capabilities which can be fine-tuned. Cathepsin B is a lysosomal cysteine protease that plays a pivotal role in tumour development and its overexpression is associated with tumour environments. Its dicarboxypeptidase activity makes it cleave a valine-citrulline labile bridge[2] at the C-terminus. In this work we develop an optimized photosensitizer for precision medicine, possessing desired water solubility, absorption in the biological window of tissues and high singlet oxygen production. It is modified further with ferrocene to make the PS a PET (photoinduced electron transfer)-controlled switch for in-cell activation.

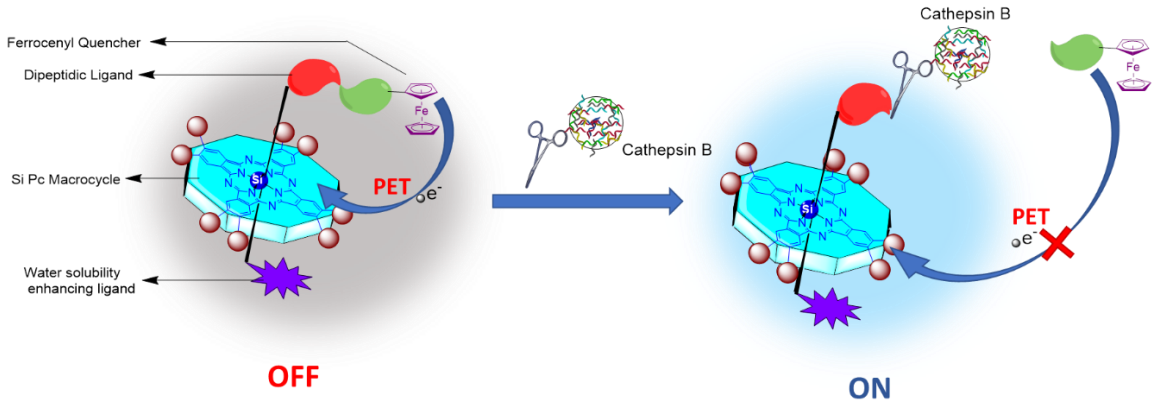

Fig 1: Schematic functioning of the antibody-drug conjugate with the cleavable Valine-Citrulline ligand.

The study was supported by ERC-CZ (Project LL2318) and Charles University (SVV 260 666).

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P03

SYNTHESIS, STRUCTURAL CHARACTERIZATION AND DNA/BSA INTERACTIONS OF NEW PALLADIUM(II) AND PLATINUM(II) COMPLEXES WITH *N*-BENZYLPHENOTHIAZINE

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In this study, we report the synthesis, structural characterization and DNA/BSA interactions of new palladium(II) and platinum(II) complexes, $[PdCl_2(N\text{-Bzphtz})_2]$ (**1**) and $[PtCl_2(N\text{-Bzphtz})_2]$ (**2**), where *N*-Bzphtz is *N*-benzylphenothiazine. These complexes were obtained in the reactions of MCl_2 (M = Pd^{2+} (**1**) and Pt^{2+} (**2**)) with *N*-Bzphtz ligand in 1 : 2 molar ratio. The reaction was carried out in acetonitrile at room temperature for the palladium(II) complex, while for the platinum(II) complex, the reaction mixture was stirred under reflux. The synthesized complexes were characterized by spectroscopic and electrochemical methods, while their crystal structures were determined by single-crystal X-ray diffraction analysis. The coordination sphere of the metal ion in these complexes is occupied by two monodentate *N*-Bzphtz ligands and two chloride ions. Moreover, the interactions of the synthesized complexes with calf thymus DNA (ct-DNA) and bovine serum albumin (BSA) were studied by fluorescence emission spectroscopy to evaluate their binding affinities towards these biomolecules.

The study was supported by the Science Fund of the Republic of Serbia, Grant No. 7730810, Value-added biologics through eco-sustainable routes – BioECOLogics. This research has also received funding from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreements No. 451-03-65/2024-03/200122, 451-03-66/2024-03/200122, 451-03-68/2022-14/200378, 451-03-66/2024-03/200125 and 451-03-65/2024-03/200125).

P04

MICELLE FORMATION OF HOMOLOGOUS 3-ALKOXYPHENYLCARBAMOYLOXYETHYL-PYRROLIDINIUM CHLORIDE IN AQUEOUS SOLUTION

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The investigation of the influence of a homologous series of pyrrolidinoethylesters substituted *3*-alkoxyphenylcarbamic acid[1] on the critical micellar concentration (CMC) values in aqueous solution at laboratory temperature was carried out by measuring the optical density[2] at the wavelength 450 nm. The CMC values decrease with increasing the number of carbon atoms n_c= 2, 3, 5, 6, 9, and 10 in the hydrophobic chain. The Gibbs free energy, necessary for the transfer of the methylene group (–CH_2) of the *3*-alkoxy substituted chain from the aqueous phase to the inner part of the micelle[3] was equal to -0,28 RT.

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P05

CHROMATOGRAPHIC SEPARATION OF DIBENZOFURAN FROM *CLADONIA STELLARIS* AND DIDEPSIDE FROM *PSEUDEVERNIA FURFURACEA*

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Lichens represent symbiotic associations between fungi and algae, or cyanobacteria, and produce important secondary metabolites. Products of secondary metabolism of lichens include multiple biological effects, including antibacterial, antiviral, antiproliferative, anti-inflammatory, antiangiogenic and antioxidant[1]. The presented study deals with the extraction, isolation and identification of secondary metabolites present in two lichen species *Cladonia stellaris* (Opiz) Pouzar & Vězda (CLA) and *Pseudevernia furfuracea* (L.) Zopf. (PSE). The methanolic extracts were evaporated to dryness on a rotary vacuum evaporator. The presence of usnic acid dibenzofuran in the CLA extract and didepside atranorin in the PSE extract was confirmed using the HPLC method. Based on the comparison of the chemical shifts of hydrogen and carbon atoms in the ^{1}H and ^{13}C NMR spectra in the standard and in the CLA extract, we can conclude dibenzofuran usnic acid is present in the CLA extract. The comparison of the chemical shifts of hydrogen and carbon atoms from the ^{1}D NMR spectra of the standard shows that didepside atranorin is present in the PSE extract[2].

The study was supported by Ministry of Education KEGA, Grant No. 003UVLF-4/2024.

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P06

PROLYL-tRNA SYNTHETASE INHIBITORS: A VIRTUAL SCREENING AND RATIONAL DRUG DESIGN APPROACH

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The ever-increasing rise in antimicrobial resistance highlights the urgent need to develop antimicrobials with novel mechanisms of action and calls for innovative research strategies. This study explores two distinct approaches to identify novel inhibitors of prolyl-tRNA synthetase (ProRS), a promising antimicrobial target which catalyzes the covalent attachment of proline to its cognate tRNA, and thus representing an essential step in protein synthesis. The first approach involves High-Throughput Virtual Screening (HTVS) of a 20-million-compound library from eMolecules. HTVS exploring several promising hits exhibiting favorable binding interactions, while some of these compounds demonstrated moderate activity against mycobacterial strains (e.g., *Mycobacterium tuberculosis* H37Ra; MIC = 62.25 μg/mL) and slight activity against several bacterial strains (e.g., *Staphylococcus aureus*; MIC = 125 μM). The second approach is based on previously published 3-aminopyrazine-2-carboxamide derivatives with confirmed activity against *Mycobacterium tuberculosis*.[2,3] The new derivatives replace the carboxamide linker with 1,2,3-triazole moiety using click chemistry. So far, 10 derivatives with various substituents on the benzene ring have been prepared, and their antimicrobial activity is yet to be tested.

The study was supported by Charles University (project GA UK No. 219823), SVV 260 666 and by "The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU."

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P07

BIOSYNTHESIZED SILVER NANOPARTICLES AS ANTIMICROBIALS AND ANTIOXIDANTS

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Nanoparticles (NPs) represent objects with a size of 1–100 nm. Due to small size and relatively broad surface, they dispose different properties in comparison to the same material with larger diameters. Silver nanoparticles (AgNPs) possess broad possibilities of use in various industries from agriculture to medicine. In the field of medicine, AgNPs are being studied for drug transport, screening of various diseases, and cancer therapy[1]. For increasing resistence of pathogens to conventional antibiotics, AgNPs are also studied as antimicrobial agents[2]. These facts lead to study of AgNPs as inhibitors of resistant bacterial strains by multiple mechanisms of action, including oxidative stress, DNA replication inhibition, or interaction with proteins and enzymes [10, 11].

Our study is focused on the green synthesis of AgNPs using an aqueous medium for extraction. We also studied the biological activities of prepared AgNPs using a selected set of water extracts of various plants, namely *Stachys recta* leaves, *Fallopia japonica* leaves, and *Berberis vulgaris* L. fruit. Namely, antioxidant, and antimicrobial properties were investigated.

The study was supported by project of the Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (2/0112/22).

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P08

SYNTHESIS AND APPLICATION OF NOVEL FLUORESCENT LABELING TAGS FOR OLIGOSACCHARIDE AND GLYCAN ANALYSIS

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Sample preparation is crucial for oligosaccharide and glycan analysis by chromatographic or electrophoretic methods.[1] Since native oligosaccharides and glycans lack chromophores or fluorophores, they require molecular modification for optical detection. Adding a charged group also enhances electrophoretic mobility and ionization efficiency for MS detection.

Standard oligosaccharides and *N*-linked glycans were derivatized with newly synthesized fluorescent and MS-active labels. After purification, the labeled compounds were separated by CE or LC and detected using fluorescence or MS. Novel labels based on 2-phenylpyridine derivatives were created, allowing tuning of fluorescence and MS properties. Compared to commercially available labels, these new labels showed higher reaction yields, tunable fluorescence, and better ionization efficiency.

This work showcases the development of novel fluorescent labels with fast reaction kinetics, high fluorescence yields, and improved MS detectability.

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P09

PRENYLATED XANTHONES – IMPORTANT SUBSTANCES OF MACLURA POMIFERA

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Maclura pomifera (Moraceae) is grown in Czech Republic as an ornamental tree. Based on the plant part, it contains various biologically active substances, especially flavonoids and xanthones,[1] often prenylated. Since prenylation significantly affects the biological activity of the compounds,[2] this work was focused on the isolation and identification of prenylated xanthones, interesting taxonomically as well as for the reported effects.[1]

In genus *Maclura*, they are mainly in the cortex of the root and stem, so alcoholic extracts were prepared from these plant parts, followed by liquid/liquid extraction and separation of the compounds by chromatographic methods. Using column chromatography, preparative TLC and HPLC, eight prenylated xanthones were isolated in quantities sufficient for further study. Identification of the compounds was carried out using spectroscopic methods. The incorporation of the prenyl substituent varies from compound to compound, with both a free variant and cyclization to a six or five-membered ring on the basic skeleton.

Since biological activity varies depending on the structure of the molecule, isolated xanthones will be subjected to selected biological activity tests. The results will help to assess the relationship between structure and activity and provide information on the potential therapeutic possibilities of these interesting compounds.

The study was supported by GAČR project GA23-04655S, Role of prenylation and glycosylation patterns in anti-inflammatory activity and metabolism of natural phenolic compounds

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P10

MICROWAVE-ASSISTED SYNTHESIS, PURIFICATION AND CYTOTOXIC ACTIVITY STUDY OF 1,3,5-TRIAZINE DERIVATIVES WITH AMINO ACIDS ON HT-29 CELL LINE

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Over recent years, hybrid molecules incorporating 1,3,5-triazine have shown promising antibacterial, antiviral, antiphlogistic, anticonvulsive, as well as anticancer activity by inhibiting many enzymatic pathways.[1] From the group of our systematically studied triazine derivatives, 1,3,5-triazines containing 4-aminobenzenesulfonamide and a pair of identical amino acids with non-polar (Ala, Tyr, Trp) and polar (Ser, Thr, Asn, Gln) side chains are investigated in the present work. The tested compounds were synthesized by a newly developed method using microwave irradiation. This procedure showed significantly shortened reaction time and higher or similar yields and purities of the products when compared to our conventional reflux conditions[2,3]. Separation and purification of the desired derivatives using semi-preparative HPLC methods was performed to obtain the purities over 98%. Chemical structures and purities were confirmed by NMR, IR spectroscopy and HPLC-DAD/MS. Then, the cytotoxic activity was screened on human cancer HT-29 cell line using the MTT assay.

This study was supported by the project VEGA 1/0514/22 and the grant of the Scientific Board of the Faculty of Pharmacy Comenius University Bratislava No. GVRFaFUK/1/2023.

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P11

COPPER AND ZINC COMPLEXES OF REDUCED SCHIFF BASES SHOWING MARKED BIOACTIVITY

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Schiff bases represent a category of compounds with manifold biological effects. The purposeful modification of their structure by varying the substituents on the amine and/or carbonyl components allows for directed alteration of their properties. They also act as versatile ligands in metal complexes. Several reduced Schiff bases containing fluoride substituents and their zinc and copper complexes have been prepared and structurally characterized by single-crystal X-ray diffraction. [1,2] Benzaldehyde derivatives containing fluorine substituents (as -F or -CF_3 groups) were selected as starting compounds due to the often profound effect of fluorine substituents on biological activity. The antimicrobial activities of the reduced Schiff bases and their zinc(II) and copper(II) complexes were evaluated in vitro, with one of the zinc complexes showing excellent antibacterial activity comparable to the antibiotic ciprofloxacin used as a standard. The cytotoxicity of the products in the HepG2 cell line was evaluated as well. The copper(II) complexes showed marked cytotoxic activities, considerably higher than the standard cisplatin. The cytotoxicity depended significantly on the substitution pattern. Furthermore, the affinity of the complexes towards bovine serum albumin and calf thymus DNA was established.

The study was supported by the Scientific Grant Agency of Slovak Republic VEGA (Project No. 1/0661/24 and 1/0429/21) and by the Research and Development Agency under the contract No. APVV-23-0349.

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P12

NOVEL SALICYLIC ACID-BASED DERIVATIVES AND TANDEM PEPTIDE CARRIERS AS TOOLS TO COMBAT INFECTIONS

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Infectious diseases are a looming threat to public health worldwide. Tuberculosis, with over 10 million casualties yearly, is one of the biggest. This is further stressed by the growing incidence of drug resistant strains. Strains resistant to multiple first-line antituberculotics are especially troubling. Novel antitubercular drugs with unique mechanisms of action are necessary to combat this epidemic and stop the spread of drug-resistant strains.

Derivates of salicylic acid have exhibited several interesting biological properties including antimicrobial effects. Based on previously described compounds,[1] we prepared two series of salicylamide derivates containing *N*-monosubstituted carbamate scaffold and screened them for antimicrobial activity. One series of compounds showed a broad effect on tested mycobacterial strains with small variation between individual compounds. Additionally, these compounds showed an antifungal activity comparable to fluconazole against *T. interdigitale*. A few of them also exhibited exceptional activity against G+ bacteria (MIC < 0.1 μmol l^{-1}).

Concurrently we report the synthesis of a novel peptide carriers to further enhance the effectiveness of small antitubercular molecules. By combining two kinds of cell-penetrating peptides into one sequence we aim to create a selective and highly efficient intracellular delivery system for new small molecule drugs.

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU. Supported by Ministry of Health of the Czech Republic, grant nr. NW24-05-00539.

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P13

PYRAZINAMIDE AND 1,2,3-TRIAZOLE: A PROMISING RELATIONSHIP

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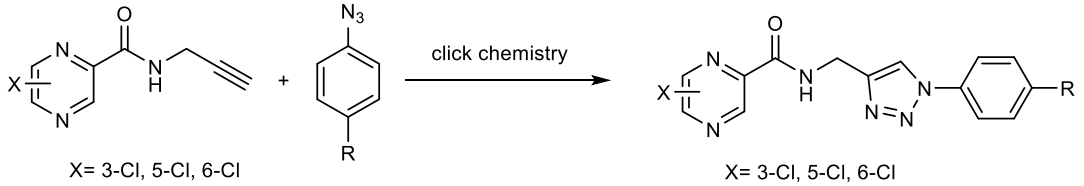
Pyrazinamide, a key antituberculotic agent, has been part of the 1st line treatment regimen of tuberculosis ever since its introduction in 1950.[1] Although tuberculosis can be treated, the course is lengthy (up to 6 months) and often complicated by antibacterial resistance.[1] To discover more potent antituberculotics, this work explores the combination of pyrazinamide with 1,2,3-triazole, a linker thoroughly reported in literature as part of the structure of many antimycobacterial agents.[2] The backbone of the synthesis of 1,2,3-triazole containing derivatives is so called "click chemistry" – a reaction combining two molecular building blocks as easily as a key inserted in its lock.[3] The specific click reaction consists of combining starting materials containing terminal alkynes with beforehand synthetized aromatic azides.[3] The reaction is catalysed by Cu(I), obtained by exposing a source of Cu (II) to a reducing agent, and results in dipolar cyclo addition of azides onto said alkynes.[3] This reaction has high yields, an easy workup, straightforward purification, and obtained compounds present interesting antimycobacterial activity (*M.Tb.* H37Ra MIC =15,625 µg.mL^{-1}, *M.Tb.* H37Rv MIC = 6,25 µg.mL^{-1}, if R= 4-OH and X= 5-Cl).



The study was supported by the project of Specific Academic Research (SVV 260 547) and by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU

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P14

SYNTHESIS OF FLUOROQUINOLON HYBRIDS AND THEIR PROSPECTS AS NEW ANTIMICROBIALS

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Overuse and misuse of antibiotics has been leading to the appearance of numerous resistant strains since the beginning of their utilization in clinical practice. However, it is possible to design new molecules based on the known ones and the aim of our investigation was to synthesize and study antibacterial properties of hybridized fluoroquinolones (FQ).

At first, the docking studies were carried out. Their results helped to identify the promising molecules, mainly among C-7 and C-3 FQ derivatives. Ciprofloxacin and norfloxacin were taken as core structures. Their C-7 position was modified using 1,2,3-triazole moiety through a developed synthetic procedure. Then, we studied introduction of the arylsulfonyl moiety into C-3 position with subsequent hybridization of C-7 and N-1. The ranges of new compounds were obtained with medium yields and their structures were confirmed using ^{1}H NMR, ^{13}C NMR, LC/MS spectroscopy and X-Ray diffraction studies.

FQs hybridized with 1,2,3-triazole moiety revealed moderate antimicrobial and antifungal activities, and new C-3 substituted arylsulfonyl derivatives showed a bit smaller activity, probably due to their lower solubility in common solvents. A few hit compounds were identified and selected for further investigations.

The study was supported by the Ministry of Health of Ukraine from the state budget according to the topic 'Molecular design and microbiological screening of innovative derivatives of fluoroquinolone antibiotics in order to combat resistant strains of microorganisms' (SRN: 0121U109239).

P15

CHITOSAN-KEFIRAN-BASED FILMS CONTAINING PLANT EXTRACT AS A POTENTIAL NON-TOXIC AND ANTIBACTERIAL WOUND DRESSING MATERIALS

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Due to the significant increase in patients struggling with wounds, developing new wound dressings with good physico-chemical and biological properties is now attracting much more attention[1-2]. In this study, we successfully fabricated chitosan and kefiran-based films cross-linked with a new dialdehyde kefiran obtained by our team. To improve the properties of these materials, we introduced plant extract into the biopolymer matrix. The test results showed that the obtained films have antimicrobial, antioxidant, anti-inflammatory, non-hemolysis, and non-toxic material. Moreover, they can bond with human serum albumin and fibrinogen. Therefore, the presented films have promising potential for practical wound-dressing applications.

The study was supported by the National Science Centre Poland grant UMO-2022/47/D/NZ7/01821.

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P16

ANTIMICROBIAL DERIVATIVES WITH IMPROVED PHYSICOCHEMICAL PROPERTIES – A HIT EXPANSION STUDY OF 2-AMINOOXAZOLES

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Antimicrobial drug resistance, combined with the scarcity of new highly active agents, remains a challenge in the treatment of infectious diseases. Regrettably, little progress has been made over the years and these issues are expected to persist in future. In our previous study[1], we explored a strategy to address the presence of some PAINS groups, hepatotoxicity or low water solubility common in drug discovery efforts. Building upon our success, here we present a hit expansion study of the most promising compounds in the hope of improving their antimicrobial activity while retaining favourable physico-chemical properties. So far, the synthesis yielded 29 derivatives of general structures as presented in **Figure 1**. All derivatives were tested against a panel of microbial species of clinical importance, including multidrug-resistant clinical isolates. Compounds showed higher activity against mycobacteria, especially *Mycobacterium tuberculosis* (best MIC = 0.39 µg/mL). None of the tested active derivatives were cytotoxic against Hep G2 cell line, thus representing promising compounds for further development.

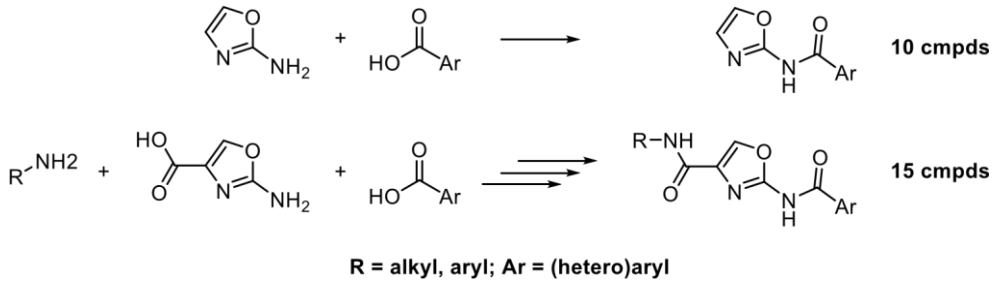

Figure 1. Synthetic diagram and general structures of the investigated derivatives

This project was supported by „The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.“

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P17

DESIGN OF NOVEL INHITORS OF PROLYL-TRNA SYNTHETASE WITH CHALLENGING SYNTHETIC PROCEDURES

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Inhibition of prolyl-tRNA synthetase (PRS) is associated with antitumor activity and suppression of autoimmune response. One of the unexplored but very promising areas is antimicrobial utilization. We began with a knowledge of proven inhibitor of human PRS[1] and attempted to create scaffold with higher affinity to the PRS.

This work continues in previous effort to find new inhibitors.[2] Our goal is to obtain similar interactions as ATP, specifically in the ribose binding site. We introduced polar functional group to our series, which made the synthesis much more challenging. The synthesized compounds will be tested on inhibition of prolyl-tRNA synthetase to find best achieved pharmacophore. Additionally, the compounds will be screened on their antimicrobial activity and cytotoxicity.

The study was supported by the Charles University, project GA UK No. 349721, by Ministry of Health of the Czech Republic, grant nr. NU21-05-00482, by Charles University project SVV 260 666 (Czech Republic), and by "The project National Institute of Virology and Bacteriology (Programme EXCELES, ID project no. LX22NPO5103) – Funded by the European Union – Next Generation EU."

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P18

SELECTIVITY OF N(2)-SUBSTITUTED OXOTRIAZINOINDOLE ALDOSE REDUCTASE INHIBITORS WITH THE INTERACTION PATTERN IN PRO301-ARG312 LOOP OF ALDEHYDE REDUCTASE

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Inhibition of aldose reductase (ALR2), the first enzyme of the polyol pathway, is a promising approach in the treatment of diabetic complications. In our previous study, 2-(3-**o**xo**t**riazino**i**ndol-5-yl)acetic acid derivatives (**OTI**s) were identified as ALR2 inhibitors with high efficacy and selectivity[1]. Selective inhibition of ALR2 related to ALR1 is crucial due to potential side and unwanted effects associated with several inhibitors of aldose reductase (ARIs). In this study, a series of novel *N(2)*-substituted oxotriazinoindoles was developed with the aim to investigate molecular interactions within the ALR2 inhibitor binding site. About twice increased inhibition efficacy of the most efficient derivative **14** (*N(2)*-CH_2CH_2COOH) compared to the unsubstituted lead **OTI** was obtained, yet at the expense of selectivity relative to anti-target aldehyde reductase (ALR1). To explain the major drop in selectivity, observed also in other *N(2)*-substituted derivatives, *in silico* molecular modeling approach revealed the role of extra interactions with the residues of Arg309, Arg312 and Met302 located in the additional *C*-terminal loop of ALR1 missing in ALR2, which can prevent or enhance binding in ALR1. These key findings will be used for development of the next generation of selective OTI inhibitors.

The study was supported by VEGA 2/0008/22, APVV-20-0411, APVV-20-0543, APVV SK-SRB-21-0047 and APVV SK-FR-22-0017.

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P19

A CLICKABLE AZAPHTHALOCYANINE DERIVATIVE FOR SUPRAMOLECULAR COMPLEXES WITH QUENCHERS

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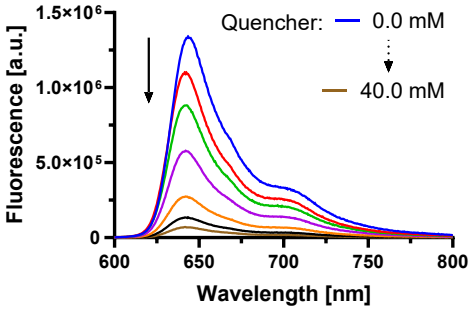
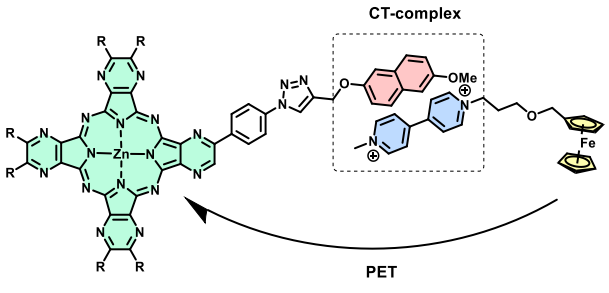
For development in the field of photosensitizers, it is desirable to study conventional and unconventional mechanisms of quenching to design new and improved analyte-activable fluorescence sensors and "smart" photosensitisers. It was reported, that photoinduced electron transfer (PET) between a strong electron donor, such as ferrocene, and phthalocyanine acceptor leads to quenching.[1] In our current project, we study the interactions of phthalocyanine close relatives, azaphthalocyanines, with ferrocene-based quenchers. Azaphthalocyanines contain nitrogen atoms that increase their electron-deficient character compared to phthalocyanine, what makes them more susceptible to PET with electron donors. In this project, we test quenching of fluorescence of an azaphthalocyanine derivative with ferrocene-based quenchers. The structure of both the azaphthalocyanine, and the quencher is designed to favour their interaction by additional supramolecular forces (charge-transfer complexes).





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P20

SYNTHESIS AND SOD-MIMETIC ACTIVITIES OF COPPER(II) COMPLEXES OF SCHIFF BASES DERIVED FROM 4-HYDROXY OR 4-METHOXYSALICYL ALDEHYDE AND AMINO ACIDS

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In our study, a wide series of Schiff base ligands derived from 4-hydroxy and 4-methoxysalicyl aldehyde (2,4-dihydroxybenzaldehyde or 2-hydroxy-4-methoxybenzaldehyde) and amino acids such as L-serine, L-threonine, β-alanine, γ-aminobutyric and 5-aminovaleric acid was prepared – altogether 7 ligands. These ligands were used in synthesis of copper(II) complexes using copper(II) acetate and copper(II) chloride. The SOD mimetic properties of the prepared complexes were measured by INT (superoxide radical scavenging)[1] and ABTS (cation-radical reducing)[2] methods. Complex solutions (1.4 - 2 × 10^{-4} M in DMSO/water mixtures) were used to measure antiradical activity calculated as percentage of radical transfer inhibition (INT) up to 57% and as percentage of radical quenching (ABTS) up to 100%. ABTS method was also used to determine IC50 values of SOD activity from a series of measurements with decreasing complex concentration. The IC50 were measured for 12 out of 14 complexes with range 103 – 10 μM with best activities observed for 5-aminovaleric acid derived ligand complexes.

The study was supported by the grant of Ministry of Education, Research, Development and Youth of Slovak Republic VEGA 1/0661/24 and Slovak Research and Development Agency APVV-23-0349.

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P21

SYNTHESIS OF DEXRAZOXANE ANALOGUES WITH HYDROXYLATED LINKER

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Anthracyclines (ANTs) such as daunorubicin or doxorubicin are very potent anti-cancer agents widely used in the treatment of various malignancies. However, their clinical usage is limited by their cardiotoxicity, which can result in severe and sometimes irreversible heart damage that can lead to heart failure. The mechanism of ANTs cardiotoxicity is primarily based on the interaction and poisoning of topoisomerase IIβ (TOP2B) in cardiomyocytes.[1]

The only clinically used drug in the prevention of ANT-induced cardiomyopathy is dexrazoxane (DEX). Recent data shown that catalytic inhibition of TOP2B is the actual mechanism of the cardioprotective action of DEX in the prevention of ANTs cardiotoxicity.[2]

Unfortunately, the solubility of dexrazoxane in water is very low. The aim of this work is to prepare an analogue of DEX (figure 1) with increased solubility in water by adding a hydroxymethyl group to the linker. Additionally, the hydroxyl group can be used to prepare prodrugs with enhanced hydrophilicity. These modifications can improve the solubility and bioavailability of the drug.

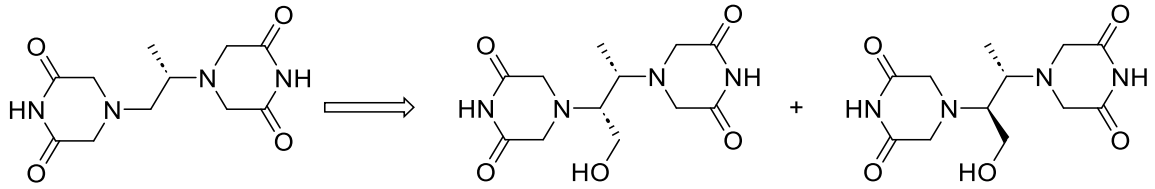

Figure 1: Scheme 1: DEX, and methylhydroxy-DEX

The study was supported by GAUK (no. 361422 and 155124)

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P22

IN SILICO ASSESSMENT OF SILVER(I) COMPLEX WITH *N*-METHYLPHENOTHIAZINE AGAINST SELECTED MICROBIAL STRAINS

MARKOVIĆ, V.R.,[1] BRANKOVIĆ, J.,[1] PETROVIĆ, V.,[1] ANDREJEVIĆ, T.P.,[1] STEVANOVIĆ, N.LJ.,[1] PANTOVIĆ, B.V.,[1] AŠANIN, D.P.,[2] GLIŠIĆ, B.Đ.[1]

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In the present study, the silver(I) complex with *N*-methylphenothiazine (*N*-Mephtz), $[Ag(N\text{-Mephtz})_4]CF_3SO_3 \cdot 1/3H_2O$, was evaluated *in silico* against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. For this purpose, prescreening was performed on thirty relevant protein structures, after which eight of them were highlighted as potential targets. Based on the obtained binding affinity values, in the case of *E. coli*, β-ketoacyl-ACP synthase III (**1hnj)** and 1,4-dihydroxy-2-naphthoyl-CoA synthase–MenB (**3t88**) were identified as potential targets of the $[Ag(N\text{-Mephtz})_4]CF_3SO_3 \cdot 1/3H_2O$ complex. Among *S. aureus* protein targets, the most favorable binding mode of this silver(I) complex was found for tyrosyl-tRNA synthetase (**1jij**), while four relevant *P. aeruginosa* proteins were identified as its potential antimicrobial targets, *i.e.*, (3R)-hydroxyacyl-ACP dehydratase –FabZ (**1u1z**), LasR ligand-binding domain (**2uv0**), 3-hydroxydecanoyl-Acyl Carrier Protein Dehydratase–FabA (**4b0c**), and caseinolytic protease ClpP2 (**7m1l**). Finally, it was shown that the $[Ag(N\text{-Mephtz})_4]CF_3SO_3 \cdot 1/3H_2O$ complex can interact with the sterol 14-alpha demethylase (CYP51) with the binding affinity value of -7.2 kcal/mol, which is slightly higher than that of the antifungal drug fluconazole (-7.0 kcal/mol).

The study was supported by the Science Fund of the Republic of Serbia, Grant No. 7730810, Value-added biologics through eco-sustainable routes – BioECOLogics. This research has received funding from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreements No. 451-03-65/2024-03/200122, 451-03-66/2024-03/200122 and 451-03-68/2022-14/200378).

P23

STRUCTURE–ACTIVITY RELATIONSHIP STUDY OF DINITROBENZYL-1,3,4-OXADIAZOLES, HIGHLY EFFICIENT ANTI-TUBERCULOSIS COMPOUNDS

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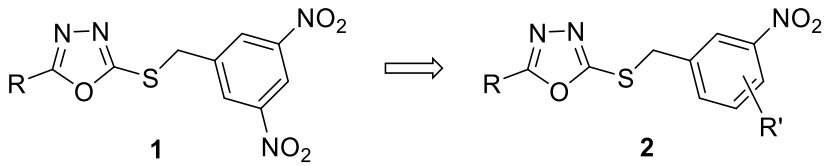
Mycobacterium tuberculosis (M.tb.) is a recalcitrant pathogen that is rife around the world, latently infecting approximately a quarter of the worldwide population. The presence of a nitro group in the structure of some drugs (delamanid, macozinone) is crucial for their activity against *M.tb.*, despite the fact that the presence of nitro group can be associated with a toxicity risk, such as hepatotoxicity, genotoxicity, mutagenicity.

5-Alkyl/aryl-2-(3,5-dinitrobenzylsulfanyl)-1,3,4-oxadiazoles **1** prepared and studied in our group showed excellent activity against both drug susceptible and drug-resistant *M.tb.* strains with minimum inhibitory concentrations as low as 0.03 μM (0.011-0.026 μg/mL) and were highly effective against non-replicating *M.tb.* SS18b strain. Lead compounds showed low cytotoxicity against various cell lines, including isolated human hepatocytes, despite the presence of two nitro groups in the molecules.[1,2]

For further development of these potent antimycobacterial agents we focused on replacing one nitro group of the 3,5-dinitrophenyl moiety with other (electron-withdrawing) substituents:



The study was supported by Program EXCELES, NPO (reg. No. LX22NPO5103).

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P24

ANTIPROLIFERATIVE AND CYTOTOXIC PROPERTIES OF GERANYLATED FLAVONOIDS FROM *PAULOWNIA TOMENTOSA* (THUNB.) STEUD. FRUIT IN HUMAN CANCER CELL LINES

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Paulownia tomentosa (Thunb.) Steud. (Paulowniaceae), a traditional Chinese medicinal plant, is a rich source of secondary metabolites, mainly geranylated flavonoids. These compounds are currently studied for their promising biological activities, such as antimicrobial, anti-inflammatory, antioxidant, and cytotoxic.

Our continuous research done on this plant leads to the isolation of a series of geranylated flavonoids using chromatographic methods. They were obtained from the chloroform portion of the ethanolic extract of immature fruit of *P. tomentosa*.[1,2]

Compounds were tested for potential antiproliferative activity using WST-1 assay in three human cancer cell lines (DU-145 prostate cancer cell line, THP-1 monocytic leukaemia cell line, and MCF-7 breast carcinoma cell line). Diplacone, as one of the most potent compounds from that series, was also shown to induce apoptosis in prostate cancer cell line.

The study was supported by Czech Science Foundation: GA23-04655S (Role of prenylation and glycosylation patterns in anti-inflammatory activity and metabolism of natural phenolic compounds).

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P25

BORONIC ACID FUNCTIONALIZED CHITOSAN COATED-MAGNETIC NANOPARTICLES FOR GLYCOPROTEIN BINDING

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Biomarkers are substances associated with the presence, development, or course of a disease that can be detected and quantified in the body. Biomarkers include plasma proteins, such as human serum albumin (HSA) and α-1-acid glycoprotein (AGP).[1] Serum HSA and AGP tests are often performed in diagnostic laboratories to assess patients' health status, diagnose diseases, and monitor the effectiveness of applied therapies. A significant challenge in working with free, non-immobilized proteins is the difficulty of separating them from the supernatant. This process typically requires techniques such as ultrafiltration, ultracentrifugation, and microdialysis. Recently, a substantial amount of literature has focused on carriers that can be used for the isolation, identification, and determination of protein concentration in biological samples. One example of such carriers is magnetic nanoparticles(AGP).[2] Many carriers for the immobilization or capture of HSA are well-known and described in the literature. However, there is a notable lack of research on the binding of α-1-acid glycoprotein. Therefore, the key goal of my work was to design and synthesize materials capable of rapid glycoprotein uptake. As a result of the research, magnetic nanoparticles coated with modified chitosan with free dihydroxyboryl groups were obtained. The obtained material was characterized (SEM, TEM, ATR-FTIR, DLS) and its ability to bind alpha-1-acid glycoprotein was tested in an environment of pH 7.4 and 9.0.

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P26

SYNTHESIS AND PHYSICOCHEMICAL PROPERTIES OF SOME BENZOFURAN DERIVATIVES

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Heterocyclic chemistry plays extraordinary roles in the field of current drug design and development. Compounds containing heterocyclic scaffolds, both of natural and synthetic origin, are (notably) biologically active and extensively used in clinical practice.[1] The benzofuran core might be considered one of crucial heterocycles, as being incorporated into a structure of many pharmacologically effective compounds.[2] Due to the wide range of beneficial biological activities connected with this moiety, synthesis, physicochemical and biological parameters determination and comprehensive structure–activity relationships have deeply attracted the interest of pharmaceutical / medicinal chemists, culminating in the discovery of several molecules that are effectively used as the treatment option(s) for many diseases.[3] The presented research focused on a multistep synthesis, spectral characteristics (^{1}H NMR, ^{13}C NMR, and IR) estimation and several physicochemical descriptors (solubility in various solvents, melting point values, and thin-layer chromatography analysis) determination of the compound containing a given privileged scaffold – (variously substituted)benzofuran-2-yl-(4-phenyl-/4-diphenylmethylpiperazine-1-yl)methanones.

The study was supported by an Excellent Grant of Comenius University Bratislava no. UK/3033/2024.

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P27

SYNTHESIS AND STABILIZATION OF SILVER NANOPARTICLES BY ZWITTERIONIC GEMINI SURFACTANTS

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Dialkylphosphocholines are groups of zwitterionic gemini compounds with a wide spectrum of biological properties such as cytotoxic, anticandidal and antiamoebal activity. Molecules of compounds used in our study have two dissymmetric alkyl chains of variable length ranging from 2 to 15 carbon atoms, while the sum of carbon atoms in both chains is being kept constant. These novel zwitterionics were used for the stabilization of silver nanodispersions. Synthesis and investigations of physical properties of phosphorus-based surfactants were studied recently[1] as well as they were applied as potent stabilizers of silver nanoparticles (AgNPs).[2] In our study, formation and composition of dialkylphosphocholine-capped AgNPs were analysed by the changes in the UV-VIS spectra due to the plasmon resonance effect. AgNPs size was determined using dynamic light scattering method and nanoparticle surface charge was analyzed by zeta potential measurements. As the results indicate, the ability of dialkylphosphocholine gemini surfactants to efficiently stabilize AgNPs strongly depends on the molecular structure of the used surfactants, especially on the length of both alkyl chains in zwitterionic surfactant molecule. The formation of stable zwitterionic surfactant-capped AgNPs will allow their passage through biological membranes and provides desired therapeutical effect related effective distribution of silver.

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P28

STRUCTURAL CHARACTERIZATION AND CYTOTOXICITY OF SILVER(I) COMPLEXES WITH *N*-METHYLPHENOTHIAZINE

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In the reaction of equimolar amounts of *N*-methylphenothiazine (*N*-Mephtz) and silver(I) salts, ($AgSbF_6$ for **1** and $AgPF_6$ for **2**) carried out in ethanol under reflux for 2 h, two mononuclear silver(I) complexes were obtained, $[Ag(N\text{-Mephtz})_4]SbF_6$ (**1**) and $[Ag(N\text{-Mephtz})_4]PF_6$ (**2**). The synthesized complexes were structurally characterized by spectroscopic (IR, NMR, UV-Vis) methods, while their crystal structure was determined by single-crystal X-ray diffraction analysis. In these complexes, four *N*-Mephtz ligands are monodentately coordinated to the Ag(I) ion through the sulphur atom, forming a catonic $[Ag(N\text{-Mephtz})_4]^+$ species with $[SbF_6]^-$ and $[PF_6]^-$ acting as counter anions for **1** and **2**, respectively. The cytotoxicity of the silver(I) complexes was evaluated on human fibroblasts (MRC5), human colorectal carcinoma (HCT116) and human lung carcinoma (A549) cell lines. The interactions of complexes **1** and **2** with calf thymus DNA (ct-DNA) and bovine serum albumin (BSA) were studied to evaluate their binding affinity toward these biomolecules. In this study, we have also performed fluorescence competition experiments with site markers for BSA to locate the bindig site of the investigated complexes to this protein.

The study was supported by the Science Fund of the Republic of Serbia, Grant No. 7730810, Value-added biologics through eco-sustainable routes – BioECOLogics and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreements No. 451-03-65/2024-03/200122, 451-03-66/2024-03/200122 and 451-03-68/2022-14/200378, 451-03-66/2024-03/200042).

P29

LOW-SYMMETRICAL PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY

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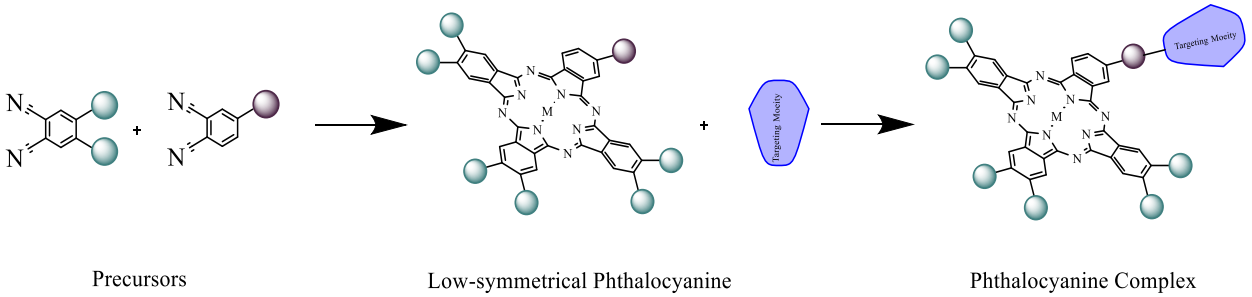
Photodynamic therapy is an effective form of cancer therapy involving a photochemical reaction with a light activatable molecule – a photosensitizer, light, and molecular oxygen. But optimal photosensitizer is yet to be developed. Phthalocyanines are aromatic macrocycles having 18 π-electron and have been shown to be optimal photosensitizers from the spectral and photophysical point of view[1].The macrocylic core can be modified to achieve better targeting, enhanced water solubility and to have increased singlet oxygen production.

In this project we aim to synthesize low-symmetrical phthalocyanine through the Linstead method using $Mg(BuO)_2$ as the initiator of the reaction with phthalonitrile bearing alkylsulfanyl or arylsulfanyl groups of different bulkiness as one of the precursors. Another precursor carries carboxyl or azide group. Introduction of one such functional group into phthalocyanine can be used for attaching to a targeting moiety. The alkylsulfanyl groups are known to have better shift in the absorption spectra and improved singlet oxygen production[2].



The study was supported by ERC-CZ Project Number- LL2318

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P30

MAGNETIC NANOPARTICLES COATED WITH MODIFIED CHITOSAN AND HEMOGLOBIN AS POTENTIAL DRUG CARRIERS IN PHOTODYNAMIC THERAPY (PDT)

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The research is focused on finding carriers for photosensitive drugs that can be used in photodynamic therapy (PDT) that belongs to the established methods of treating cancer[1]. For this purpose, magnetic nanoparticles coated with modified chitosan and hemoglobin were designed. The material was characterized in terms of chemical structure using infrared spectroscopy (ATR-FTIR) and X-Ray diffraction (XRD). The surface morphology was described based on images from scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM). The size of nanoparticles was determined using dynamic light scattering (DLS). The next step was to attach a photosensitive drug, chlorin e6, using chemical and physical adsorption. The material was characterized. The potential photosensitivity of nanoparticles with the drug was tested (based on the distribution of the singlet oxygen scavenger ADPA), finding that the presence of hemoglobin on the nanoparticles increases the photosensitive effect. The release profile of chlorin e6 from the material to which the drug was physically bound was also analyzed. It was noted that the presence of hemoglobin slowed down the release process of chlorin e6.

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P31

ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITY OF SILVER(I) AND GOLD(III) COMPLEXES WITH MICONAZOLE

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New silver(I) and gold(III) complexes with the antifungal drug miconazole (mcz), $[Ag(NO_3)(mcz)_2]$ (**1**) and $[AuCl_3(mcz)]$ (**2**), were synthesized by the reaction of $AgNO_3$ and $K[AuCl_4]$ with an equimolar amount of mcz in ethanol. The synthesized complexes **1** and **2** were characterized by mass spectrometry, IR, UV-Vis and ^1H NMR spectroscopy. Complex **1** contains two mcz and one monodentately coordinated nitrate anion. In the case of complex **2**, mcz is a monodentate ligand coordinated to the Au(III) ion *via* the triazole nitrogen atom, while the remaining coordination sites of this metal ion are occupied by chloride anions leading to the square-planar arrangement. The antimicrobial activity of complexes **1** and **2** was investigated against different bacterial and fungal strains, as well as their cytotoxic activity on the normal human lung fibroblast cell line (MRC-5). These complexes were assessed for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) H37Rv (ATCC 27294) strain. Coordination of mcz to Ag(I) and Au(III) ions led to enhancement of its activity against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* strains, while against the panel of *Staphylococcus aureus* and *Candida* species, only complex **2** has improved activity in respect to mcz. Both complexes **1** and **2** demonstrated good antitubercular activity, whereby **1** is twice potent than parent mcz drug.

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P32

NOVEL ISATINS DERIVATIVES

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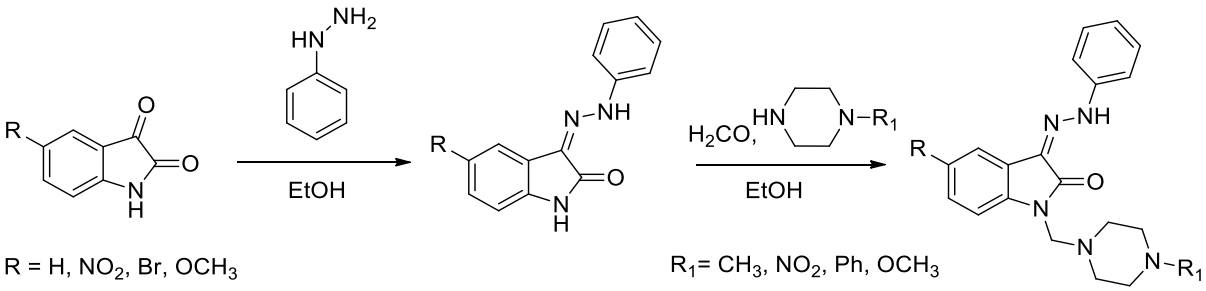

The research is focused on the synthesis, characterization and study of the biological properties of isatin derivatives as suitable candidates for new drugs. Isatin and its derivatives belong to the organic compounds used in chemical practice. They are used primarily in areas such as medicinal chemistry (e.g. as antibiotics, antimalarials, etc.), in the field of nanotechnology, development of analytical reagents and dyes, and in the field of synthesis of heterocyclic compounds and stereoselective procedures. The high application potential of isatin and its derivatives, their occurrence and the occurrence of their metabolites in plants and in the human body have aroused the great interest of chemists, doctors and pharmacists in the study of their chemical reactivity. [1] The aim of the work was the synthesis of new, not yet described in the literature, derivatives of 3-(phenylhydrazono)isatin with a methylpiperazine substituent in the N-1 position. [2] [3]

The study was supported by APVV-22-0133.

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P33

SYNTHESIS OF NEW DERIVATIVES WITH CARBAMATE AND ARYLOXYAMINOPROPANOL PHARMACOPHORE AS CHOLINESTERASE INHIBITORS WITH β-ANTIADRENERGIC ACTIVITY

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Today, carbamates make structural and/or functional part of many drugs and prodrugs approved and marketed for the treatment of various diseases such as cancer, epilepsy, HIV infection, and Alzheimer's disease.[1,2] β-adrenergic antagonists (β-blockers) with at least one chiral center are an exceedingly important class of drugs used mostly to treat cardiovascular diseases. At least 70 β-blockers have been investigated in history. Chemically, these drugs contain in their structure an aryloxyaminopropanol fragment.[3-5] The work deals with the synthesis of new derivatives, which contain in molecule the above mentioned pharmacophoric groups. It clarifies the structure activity relationship of selected derivatives which have undergone modification in the aryloxyaminopropanol chain (replacement Ar-O-CH_2(OH)-CH_2-NR^1R^2 for Ar-COO-CH_2(OH)-CH_2-NR^1R^2 group). The basic part of this chain was also chemically modified (-NR^1R^2); benzylpiperidine was replaced by various substituted benzylpiperazines. For selected derivatives, anticholinesterase activity were determined. The effect on β-adrenergic signaling was also tested.

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P34

STUDY OF PHYSICAL PROPERTIES OF SILVER NANOPARTICLES STABILIZED BY HEXADECYLPHOSPHOCHOLINE DERIVATIVES

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Silver nanoparticles (AgNPs) were prepared by chemical reduction from silver nitrate and stabilized with a group of zwitterionic surfactant molecules composed of two alkyl chains with unequal variable length. The hydrophilic part of surfactant molecule contains a positively charged ammonium and a negatively charged phosphate group. The class of cationic surfactants based on quaternary nitrogen and phosphorus turned out to be a potent stabiliser of silver nanoparticles.[1-3] The presented research is focused on the utilization of zwitterionic dialkylphosphocholines as stabilizers of AgNPs because of their smaller toxicity and better biocompatibility. Moreover, zwitterionics are important from the point of view of biological applications. Besides cytotoxic and antineoplastic activity alkylphosphocholines also possess antiprotozoal properties. In our study, dialkylphosphocholine-capped AgNPs are characterized by multiple physical methods such as UV-VIS spectrometry, zeta potential measurements for surface charge determination, and dynamic light scattering for hydrodynamic size of AgNPs. Our results indicate that the efficiency of dialkylphosphocholine gemini surfactants in stabilizing AgNPs strongly depends on surfactant molecular structure, particularly on the difference in length of both alkyl chains in zwitterionic surfactant molecule. Moreover, the performed experiments indicate that interchanging the location of both alkyl chains of unequal length in surfactant molecule dramatically affects their stabilizing efficiency of AgNPs.

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P35

POLYPHARMACOLOGICAL DRUG-LIKE MOLECULES FROM THE 2,5-DIMETHYL-4-OXO-3,4-DIHYDROTHIENO[2,3-d]PYRIMIDINE-6-CARBOXAMIDE SERIES

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A considerable number of biologically active molecules are capable of binding to several targets in the body. Therefore, there is a question modern drug discovery is about modifications of privileged scaffolds, which are characterized by a wide spectrum of pharmacological activity.

Thieno[2,3-d]pyrimidine heterocyclic system, which is a fragment of the molecules of a large number of antimicrobial agents, with the potential to interact with bacterial TrmD, is among those scaffolds. On the other hand, this heterocyclic system is close in structure to purine and may be attractive from the point of view of interaction with adenosine receptors. In order to broaden the range of potential biologically active compounds, we conducted the synthesis of 2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides. The key stage was the preparation of ethyl 2,5-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate within the one-reactor procedure using 1,1-dimethoxy-N,N-dimethylmethanamine with futher cyclization of the resulting amidine with ammonium acetate. Subsequently, the corresponding amides were synthesized from the acid obtained on the basis of hydrolysis of the ester.

The best parameters of antimicrobial activity were found for unsubstituted benzylamide. At the same time, the slightly less active at *in vitro* screening 4-methylbenzylamide showed better placement parameters in the active site of TrmD isolated from *Pseudomonas aeruginosa*. It is interesting that this particular amide also turned out to be the best ligand for the A_{2A} adenosine receptor active site in comparison to Istradefylline as a reference ligand.

The study was supported by The Ministry of Health Care of Ukraine for the State Budget grant on the topic "Molecular modeling and synthesis of innovative pyrimidine derivatives as promising agents for the treatment of neurodegenerative diseases" (State registration number: 0124U002006).

PHARMACEUTICAL ANALYSIS AND BIOANALYTICAL CHEMISTRY

P36

DNA/BSA INTERACTIONS OF PALLADIUM(II) COMPLEXES WITH PHENOTHIAZINE AND *N*-METHYLPHENOTHIAZINE

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The potential use of palladium-based drugs in the treatment of some types of cancers is well recognized, whereby the interactions of palladium(II) complexes with biomolecules play an important role in the mechanism of their antitumor action.[1] Considering this, in the present study, we have investigated the binding affinity of phenothiazine (phtz) and *N*-methylphenothiazine (*N*-Mephtz) and their palladium(II) complexes, *trans*-[$PdCl_2$(phtz)$_2$] (Pd1) and *trans*-[$PtCl_2$(*N*-Mephtz)$_2$] (Pd2) to calf thymus DNA (ct-DNA) in the presence of an intercalative agent ethidium bromide (EthBr) and minor groove binder Hoechst 3325 (Hoe) by fluorescence emission spectroscopy. Moreover, fluorescence competition experiments with site markers, eosin Y, ibuprofen and digitoxin, for bovine serum albumin (BSA) were performed to determine the binding site of the investigated compounds to this protein.

The study was supported by the Science Fund of the Republic of Serbia, Grant No. 7730810, Value-added biologics through eco-sustainable routes – BioECOLogics. This research has received funding from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreements No. 451-03-65/2024-03/200122, 451-03-66/2024-03/200122 and 451-03-68/2022-14/200378).

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P37

ANALYSIS OF IMPURITIES AND CONTAMINANTS IN PHARMACEUTICAL PRODUCTS

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Identification and accurate determination of naturally occurring impurities and/or contaminants is an essential part of analyses of pharmaceutical products which has to meet mandatory strict criteria according to the various regulatory authorities.[1]

The first part of the presented work focuses on the transfer and further optimization of an HPLC-PDA method for the determination of impurities in a mucolytic drug. The initial method, obtained from a commercial laboratory, encountered issues in our laboratory, particularly with insufficient resolution between the peaks of individual impurities.[2] It was discovered that selectivity could be significantly improved by adding methanol to the mobile phase. The final mobile phase consisted of methanol/acetonitrile/10 mM ammonium acetate in a 10/30/60 % v/v/v ratio.

The second part of the study was focused on optimizing a chromatographic method for detecting impurities in pharmaceutical formulations, where the initial HPLC-PDA-MS approach faced challenges with effective analyte ionization due to an alkaline mobile phase. To resolve this, a UPLC-PDA-MS/MS method was developed, utilizing an acidic mobile phase that improved ionization in positive electrospray mode, where separation efficiency and the sensitivity of impurity detection were significantly enhanced. This approach led to the successful identification of key contaminants, including 2-hydroxy-methylpropiophenone, which was confirmed through detailed MS analysis and elemental composition reports. It concluded that the impurities were transferred from printed foil to the medication during packaging, highlighting the importance of material compatibility in pharmaceutical production.

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P38

OPTIMIZATION AND VALIDATION OF A RAPID HPLC-ELSD METHOD FOR CARBOHYDRATES ANALYSIS

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HPLC in the HILIC (hydrophilic interaction liquid chromatography) mode is often used in pharmacy to separate and determine polar analytes (in our case, saccharides on a polyol stationary phase).[1] However, a conventional UV–detector is unusable due to a lack of chromophores; alternative detectors, such as a refractometric detector, would require isocratic elution, and therefore, an evaporative light scattering detector (ELSD) can be used to advantage.[2] Nowadays, environmentally friendly chemistry is quite a topic of discussion. That is why we performed experiments with a shorter column.[3] In this paper, column HALO-Penta HILIC 75x4.6 mm with particle 2.7 μm (AMT) was used for the optimization of separation mixture of common saccharides present in food and food beverages (fructose, glucose, saccharose, lactose, maltodextrins). To observe analyte retention, ribose, and melezitose were added to the saccharide mixture. An HPLC Dionex 3000 (Thermo) with an ELSD Varian 380-LC (Varian) was used. A multivariable approach in combination with Artificial Neural Networks (ANNs) was used for optimization. Central composite design with three parameters: column temperature (10–40°C), mobile phase flow rate (0.2–1 $mL.min^{-1}$) and gradient ramp (2-5 $\%.min^{-1}$) was used. Sixteen HPLC methods were created with combinations of the three inputs. Retention time, peak area, and peak high of ribose, glucose, saccharose, and melezitose were used as outputs. The best match of predicted outputs with experimentally obtained data was chosen as optimal chromatographic conditions for our sugar mixture separation. This chromatographic method was further validated by ICH Q2 (R2) guideline.

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P39

OPTIMISATION OF MIDDLE-UP QUANTIFICATION OF INFLIXIMAB IN PHARMACEUTICAL MATRIX BY CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Infliximab (IFX) is a chimeric mouse-human therapeutic monoclonal antibody (mAb) against tumor necrosis factor (anti-TNFα), commonly used in treatment of inflammatory bowel disease. IFX is, structurally, a large protein of size approximately 150 kDa consisting of two types of subunits – two heavy (~50 kDa) and two light (~25 kDa) chains connected by disulfide bonds. Typically, bottom-up approach is employed for mAb analysis, where mAbs are enzymatically digested into smaller peptides and subsequently analysed. This work makes use of an alternative, middle-up (MU) approach, where the mAb disulfide bonds are reduced by a reducing agent into heavy and light chains. An MU procedure is used together with capillary electrophoresis-mass spectrometry (CE-MS) for quantitative analysis of IFX in pharmaceutical matrix. Stock solution of IFX reference standard (Sigma-Aldrich, US) (1 mg/mL in water) was prepared and IFX disulfide bonds were reduced by tris(2-carboxyethyl)phosphine (TCEP). The reduced IFX was subsequently analysed by Agilent 7100 CE system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent, Santa Clara, US). Background electrolyte systems (BGE) containing 0,5 M and 1 M formic acid and acetic acid in the range of 2 – 4 M were tested together with different separation voltages applied (18 – 25 kV). Various concentrations of IFX stock solutions (0,01 – 0,5 mg/mL) were analysed to plot a calibration curve. The obtained results show that the most suitable BGE for analysis of reduced mAb IFX is 1 M formic acid and the optimal separation voltage to be 25 kV. Furthermore, a calibration curve was plotted from the acquired data showing that the proposed proof-of-concept quantitative method requires further optimisation.

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P40

RAPID DETERMINATION OF NIMESULIDE IN PHARMACEUTICAL FORMULATIONS BY CAPILLARY ZONE ELECTROPHORESIS (CZE)

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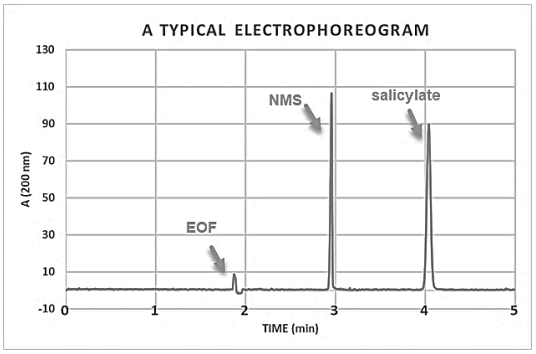
Nimesulide is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis, and primary *dysmenorrhoea* in adolescents and adults above 12 years old[1]. In several countries, nimesulide has been withdrawn from the market due to concerns about the risk of hepatotoxicity; however, it is still available in many countries, esp. in Europe and Asia, even as an over-the-counter (OTC) drug.

A rapid method for determining nimesulide by CZE in various dosage forms (powder, tablets, gel) was developed. Experimental conditions were revised and simplified.[2] Under optimized conditions using sodium salicylate as an internal standard, the run time was less than 5 minutes. Also, the sample preparation is straightforward: tablets/powder are homogenized in a mortar, suspended in methanol, and filtrated. In gel samples, the filtration step can be omitted. The method was applied to samples of three products available in the Czech market: Nimesil (sachets), and Aulin (tablets, gel), including the content uniformity test (*Pharmacopoeia 11.0*) of Nimesil sachets.



An example of electropherogram of nimesulide and salicylate under optimized conditions

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P41

CHIRAL SEPARATIONS OF NEW POTENTIAL PHARMACOPHORES: NIDO-$[7,8\text{-}C_2B_9H_{12}]^-$ AND $[Co(C_2B_9H_{11})_2]^-$ DERIVATIVES

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Anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ are known for their stability, water solubility, and low toxicity, finding applications in catalysis, synthetic, and medicinal chemistry. Despite the vast investigations of these compounds, their chirality is currently underexplored, hindering the development of new pharmaceuticals. Addressing this knowledge gap is crucial to understand the possibly different pharmacological activity and toxicity of drugs based on individual enantiomers of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$.

In this presentation, the investigations of the chromatographic behavior of derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ are discussed using HPLC and SFC using polysaccharide-based columns and beta-cyclodextrin-based columns. The anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ were previously unseparated in HPLC due to ionic interactions with the silica-gel surface, which were mitigated by addition of counterions or chelating agents, achieving chiral separation on the beta-cyclodextrin-based chiral stationary phase. The zwitterionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ were separated on the same column without the evidence of strong ionic interactions with the surface of the silica-gel.

After the initial experiments, we tested multiple chiral stationary phases and chromatographic modes in HPLC and SFC. In general, SFC methods using polysaccharide-based columns are preferred for zwitterionic and anionic derivatives of $[Co(C_2B_9H_{11})_2]^-$ and zwitterionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$. However, for anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$, RPLC using a bromated-beta-cyclodextrin-based chiral stationary phase is the method of choice.

In conclusion, this work lays the foundation for chiral separations of multiple structural types of derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$, essential for analysis of enantiomeric purity and development of chiral pharmaceuticals.

This work was supported by the Charles University (SVV 260 666).

P42

RP-HPLC METHOD FOR DETERMINATION OF SALBUTAMOL SULFATE AFTER APPLICATION FROM A PMDI TYPE OF INHALER INTO THE PHARMACOPOEIAL GLASS APARATURE

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This work presents the study of the release of antiasthmatic salbutamol sulfate from the pMDI type of inhaler into the pharmacopoeial glass apparatus, which represents a model of human respiratory tract, while the physiological inhalation is imitated by a vacuum pump. The aim of the experiment was to determine the amount of the drug trapped in the inhaler and three compartments of the glass apparatus after application of one dose. 25 applications were analyzed, while a trend was observed when the least amount of salbutamol was captured in the inhaler (2.9 %), then in the upper separation chamber (10.9 %), the throat (38.6 %), and the largest portion of drug dose was captured in the lower separation chamber (47.6 %), which represents lower respiratory tract and lungs, and thus the main site of drug's therapeutic effect.

Quantitative analysis was carried out using Dionex UltiMate 3000 System. A Symmetry® C18 column (4.6 × 250 mm, 5 μm) and a mobile phase consisting of methanol and aqueous solution of phosphoric acid (7.3 mmol L^{-1}) in a volume ratio of 70:30 were used. The flow rate of the mobile phase was 1 mL min^{-1}, the column temperature was maintained at 40 °C and detection was performed at a wavelength of 229 nm. As part of the method validation, linearity, detection limit, quantification limit, intraday and interday precision, accuracy, specificity, selectivity and robustness were evaluated.

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-23-0508.

P43

DETERMINATION OF RIFAMPICIN AND ITS METABOLITES IN CELL MEDIUM

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Rifampicin (RIF) is a macrocyclic antibiotic used in the treatment of tuberculosis. In addition, RIF is known to induce cytochrome P450 enzymes, especially the 3A4 isoform. Due to this property, RIF is used as a model compound in pharmacological studies. RIF is primarily metabolized into 25-desacetyl RIF through enzymatic deacetylation and into RIF quinone via nonenzymatic autooxidation.[1] Other metabolites or degradation products, such as 3-formyl RIF and RIF N-oxide, have also been reported.[2] Several HPLC methods for detecting RIF and RIF quinone in human plasma or urine have been published[3]. Still, there remains a need for a straightforward LC-UV method for the determination of RIF and its metabolites.

In our study, we developed an LC-UV method for the detection of RIF and its metabolites. The separation was performed on an ACE Excel3 C18-AR column (3 µm, 100 × 3 mm). The mobile phase consisted of ammonium acetate (30 mM, pH 6.30) (A) and methanol (B). The gradient was as follows: 0-9 min (65 → 90% B), 9-11 min (90% B), 11-11.1 min (90 → 65% B), and 11.1-15 min (65% B). This chromatographic method was effectively used for the quantification of RIF and its metabolites in a pharmacological study examining the potential of RIF and its derivatives to activate the pregnane X receptor. Additionally, we will discuss the stability of RIF and RIF quinone in cell medium, both in the presence and absence of cells.

The study was supported by SVV project (260 666, Czech Republic).

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P44

BRIDGING MICROSAMPLING WITH MICROEXTRACTION FOR DOXORUBICIN AND DOXORUBICINOL DETERMINATION

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Volumetric Absorptive Microsampling (VAMS) facilitates repeated blood collection from a single animal, minimizing overall animal use in preclinical studies. VAMS devices stand out by relying less on hematocrit, elevating precision, and making sample collection more straightforward than conventional dried blood spots. [1] Electromembrane extraction (EME) is a relatively new extraction technique based on hollow fiber microextraction. The application of direct current voltage in EME accelerates analyte isolation, leading to increased recoveries within a shorter time, and it also facilitates the use of a 96-well format. [2]

This study aimed to establish a methodology for assay of doxorubicin (DOX) and its metabolite, doxorubicinol (DOXol), from blood absorbed onto VAMS tips using EME followed by UHPLC-MS/MS analysis. The primary focus was on optimizing sample preparation. Various EME parameters (supported liquid membranes, voltage, time, donor and acceptor solutions) were systematically explored to attain optimal recovery. After optimization, the method was fully validated. Additionally, protein precipitation, as a conventional extraction method, was developed and compared to EME. The method's applicability was confirmed by analyzing VAMS samples taken after DOX administration in pharmacokinetic studies involving mice and rabbits.

The study was supported by Charles University (GAUK 232223 and SVV 260666).

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P45

EXTRACTS FROM THE MEDICINAL MUSHROOM CORDYCEPS SINENSIS AND THEIR ABILITY TO REDUCE SILVER IONS

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An entomopathogenic fungus, Cordyceps sp. has been known to have numerous pharmacological and therapeutic implications, especially, in terms of human health making it a suitable candidate for ethno-pharmacological use.[1] An ever growing list of symptoms remedied using *Cordyceps sinensis* include respiratory, renal, liver, nervous system, cardiovascular diseases, cancerous tumors, decreased libido and even stress, fatigue and aging.[2] Silver nanoparticles have attracted vast research interest because of their potential functions in many fields such as biomedical, environmental, electronic, catalysis, and antimicrobial areas. Silver nanoparticles prepared from *Cordyceps sinesis* extracts exhibit outstanding antibacterial activity, which is needed for many medical and biological applications.[3]

This work deals with the preparation of silver nanoparticles using extracts prepared from the fungus *Cordyceps sinensis*. The extracts were prepared from samples of the fungus that were cultured on two different substrates; pea and maize sown. Maceration and ultrasonication were used in the preparation of the extracts. The formation of silver nanoparticles was monitored spectrophotometrically in the region of 400-500 nm using 10.0 mmol L^{-1} $AgNO_3$ solution and elevated temperature over a period of 1 hour. As shown from the measurements, for all extracts, there was a reduction of silver ions by the polysaccharides present in the *Cordyceps sinensis* extracts, which was reflected by a significant increase in absorbance in the measured area. As revealed from the measurements, the change of substrate during the preparation of the extracts is an important factor affecting the formation of silver nanoparticles.

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PLENARY LECTURES

PL-1

THE DESIGN OF PDT CHROMOPHORES

RÜDIGER FAUST

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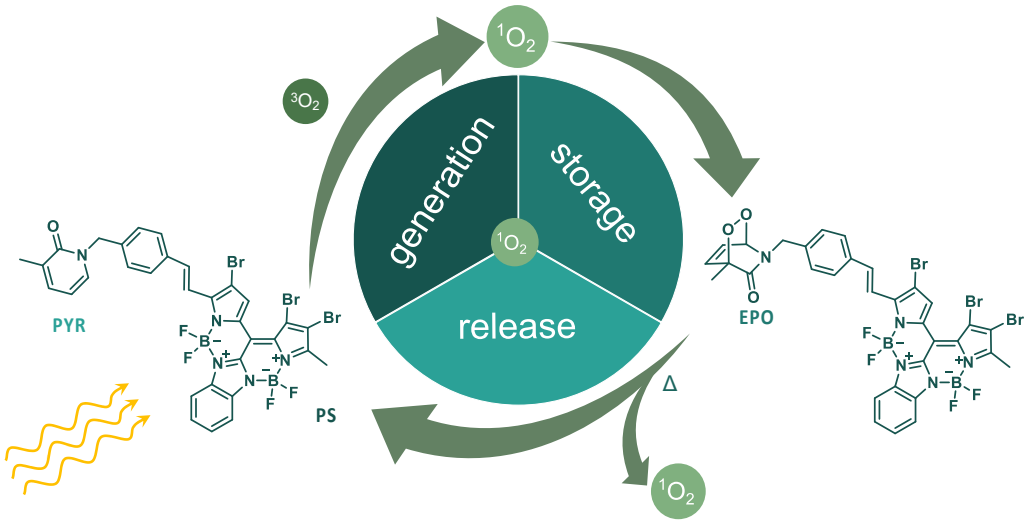
Photodynamic tumour therapy (PDT) is a minimally invasive treatment for various forms of cancer that utilizes the ability of some chromophores to produce cytotoxic singlet oxygen $^1O_2$ when stimulated by light of a suitable wavelength. While PDT has certain advantages over conventional therapies (e.g. it is a local therapy with limited dark toxicity) it also has limits, inter alia with respect to the oxygen environment: Many tumors tend to develop in hypoxic regions, which significantly reduces the efficiency of photosensitization. In addition, PDT itself further increases the hypoxic state of the tissue.

We are currently exploring concepts for a delayed for singlet oxygen provision in the design of molecular catch-and-release systems. At the core of these concepts lies the covalent attachment of pyridone units on photosensitizing chromophores such as subphthalocyanines or the so-called BOIMPY chromophores shown in the Figure below. The photosensitization process generates a pyridone endoperoxide from which singlet oxygen can be released by a thermal process. presentation will describe our synthetic entry into this field and will highlight recent progress of this ongoing research.



PL-2

AUTOMATION OF MATRIX PRECIPITATION FOR FOOD ANALYSIS

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Matrix precipitation is a frequent preparative step used before the analysis food and biological samples by liquid chromatography. Inefficient removal of particles or precipitable matrix components (salts, proteins, fibers, …) can cause column blockage or inhibit solid phase extractions. The typically manual protocols differ mostly by the inducing agent, e.g., chaotropic organics, metal salts, or aprotic solvents. This is followed by sample filtration or centrifugation to separate the formed precipitate. Herein, we show centrifugation-less deproteination based on homogeneous liquid-liquid microextraction, automated by the Lab-In-Syringe technique [1,2].

The developed methodology was applied to milk and serum using selected pharmaceuticals as model analytes [3,4] and coupled online to HPLC and LC-MS/MS, respectively with quantitative analyte recovery, high operational reproducibility, and operation in parallel to the running chromatographic separation. System design, operation principle, method optimization, technical challenges, and application are shown together with an outlook on current research.

The study was supported by the project EFSA-CDN co-funded by ERDF (No. CZ.02.1.01/0.0/0.0/16_019/0000841).

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PL-3

DEVEOPMENT OF THE PECAM1 COVALENT BLOCKER AS A NOVEL THERAPIUTIC APPROACH FOR ANTI-AUTOIMMUNE DISORDERS TREATMENT

GETTER, T.,[1] MARGALIT, R.,[2] KAHREMANY-HOFFMAN, S.,[1] LEVY-NISSIM, L.,[1] BLUM, E.,[1] KHAZANOV, N.,[1] LAHAV, R.,[2] ZILBER, S.,[3] SENDEROWITZ, H.,[1] BRADFIELD, P.,[4] IMHOF, B. A.,[4,5] ALPERT, G.,[2] GRUZMAN A.[1]

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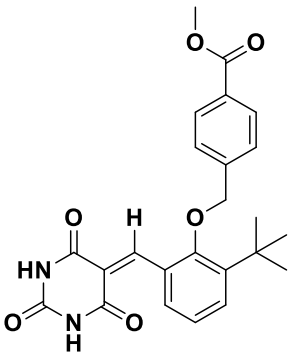

GT-73

Leukocyte transendothelial migration from the blood to the tissues is one of the most important steps in launching an inflammatory immune response (its cellular component). However, misregulation of this process can lead to devastating autoimmune diseases. We designed a molecule (GT-73) with an ability of covalent binding to active form of PECAM1 (CD31) (Platelet and Endothelial Cell Adhesion Molecule 1). PECAM1 is one of the key players in the regulation of the leukocyte penetration througth the endothelial barrier. We showed that in vitro GT-73 completely blocked human monocyte, T-lymphocytes, B-lymphocytes and neutrophils transendothelial migration, without any toxic effects on immune or endothelial cells (IC_{50}=2.4 μM). In vivo, GT-73 exhibited significant therapeutic effects in inflammatory bowel disease (IBD)/Crohn's disease, multiple sclerosis, fatty liver disease, acute respiratory distress syndrome, lupus and rheumatoid arthritis mouse models. GT-73 was active in pharmacological relevant doses (10-30 mg/kg) and in several routes of administration and almoust complitlyb elimnated the symptoms of thouse disorders in mice. The compound was not active in atopic dermatitis and diabetis type one mouse models. A detailed acute and chronic toxicity profile, including GLP toxicity of GT-73 did not reveal any toxic effects[1-3].

The study was supported by "AltA-ZuZ LTD", Israel; "MesenFlow LTD", Switzerland; and "Silverskate Bio LTD", Israel/Japan.

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PL-4

GENE THERAPY FOR CYSTIC FIBROSIS

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Recent advancements in the field of genetic engineering with the development of highly efficient biomedical technology tools and strategies notably renewed the enthusiasm for gene therapy of multiple severe (difficult-to-treat) diseases / disorders or rare diseases including the ones characterized by an alteration at the gene level.[1] Cystic fibrosis (CF) is a life-shortening inherited autosomal recessive disease caused by various mutations in the cystic *fibrosis transmembrane regulator* (*CFTR*) gene encoding a cystic fibrosis transmembrane conductance regulator (CFTR) protein.[2] Versatile therapeutic interventions for CF have been proposed, developed or approved for clinical use generally focused on *i)* non-gene therapy strategies aiming small molecule drugs (CFTR modulators) and their suitable combinations which improve trafficking and processing of the mutated CFTR protein, and *ii)* gene therapy strategies including the use of antisense oligonucleotides, RNA interference technology for regulating *CFTR* gene expression (modulation of gene expression approaches by interaction with cellular mRNA), CRISPR-based gene editing strategies, base editors as well as prime editors (genome editing technologies).[3–5] Current lecture briefly considered several aspects of both non-gene and gene therapeutical tools with special attention paid to the latter ones – their enormous potential in addressing the genetic root of the disease, promises, challenges, pittfalls and future directions.

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SHORT COMMUNICATIONS

SC-1

J-DIMERS OF PHTHALOCYANINES

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Phthalocyanines (Pcs) are macrocyclic dyes that tend to form aggregates based on the π-π stacking of the planar core. Such aggregates are called H-aggregates and typically are characterized by blue-shifted Q-band and non-fluorescent nature. On the other hand, several papers have reported formation of slipped J-dimers with retained fluorescence and red-shifted absorption Q-band, however, the examples are extremely rare.[1-3] Our work has been focused on various aza-analogues of Pc (AzaPcs) that formed the slipped J-dimers on the basis of coordination of peripheral substitutes (various dialkylamino substituents or heteroaryls) with central cation (Zn(II) or Mg(II)). Unequivocal dependence between dimerization constant and bulkiness of peripheral binding sites was observed. The J-dimers can be disassembled to monomers with changes to shape and intensity of fluorescence and also in production of singlet oxygen using various external stimuli – increased temperature, presence of external coordinating ligand (see figure below) or protonization of the binding site by acids.

The work was supported by Czech Science Foundation, grant Nr. 23-06177S

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SC-2

ASSEMBLY OF PHTHALOCYANINE DERIVATES INTO J-DIMERS

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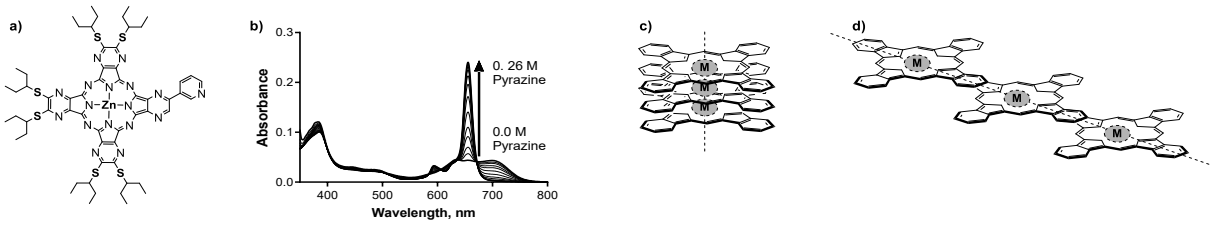
Phthalocyanines (Pcs) are synthetic macrocyclic dyes formed by four isoindoline units connected by azomethine bridges structurally close to porphyrins. Due to their extended 18 π-conjugated system, they show unique photophysical properties, and they have been largely investigated in various fields, such as fluorescence sensors or photosensitizers in photodynamic therapy (PDT).[1] However, these properties are related mostly to the monomeric form of the Pcs only. Aggregation of the Pcs is usually an unfavorable phenomenon. The planar Pc core tends to aggregate due to π-π stacking interactions. The most common H-type aggregates (Figure 1c) align molecules into a sandwich-like arrangement, resulting in increased absorption at blue-shifted wavelengths and strongly decreased fluorescence emission. However, J-aggregates (Figure 1d) give rise to red-shifted absorption bands and retain fluorescent properties.[2] In this work, we synthesized unsymmetrical Pc derivatives containing one ligand (coordinating moiety, e.g., pyridyl) that formed slipped J-dimers upon coordination to the central cation of the second Pc molecule in non-coordinating solvents.



The study was supported by Charles University (SVV 260 666).

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SC-3

DERIVATIVES OF 3-AMINOPYRAZINE-2-CARBOXAMIDE AS INHIBITORS OF PROLYL-tRNA SYNTHETASE

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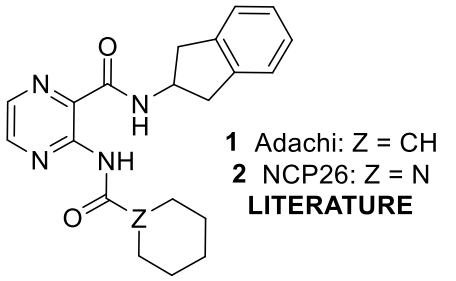
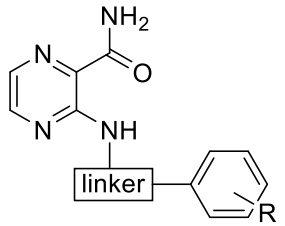
Prolyl-tRNA synthetase (ProRS), a member of the aminoacyl-tRNA synthetases (aaRSs) family, is essential for the growth and survival of all cells. The aaRSs are part of both eukaryotes and prokaryotes, but their significant evolutionary divergence offers the possibility to develop selective inhibitors. 3-Aminopyrazine-2-carboxamide derivative (**1**) is a reported ATP-competitive inhibitor of human ProRS (hsProRS).[1] A nitrogen isostere **2** (NCP26) has a strong potential for the treatment of multiple myeloma[2], but also as an antimalarial agent.[3] In our laboratory, we prepared several series of structurally related derivatives (**3**) and based on the type of the linker and substituent R obtained compounds with affinity to hsProRS,[4] or compounds with antimycobacterial activity (with prpbable binding to mycobacterial ProRS).[5,6] The structure-activity relationships will be discussed.



1 Adachi: Z = CH
2 NCP26: Z = N
LITERATURE



IN-HOUSE 3

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SC-4

AUTOMATION OF LONG-TERM PROCESSES

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Advantages of universal manipulation of sample and reagents were applied in the past for the liberation of drugs using Franz diffusion unit(s) in a sequential injection (SIA) system. In this talk, an overview on recent development in this field will be given with respect to two areas where automated monitoring can significantly deepen knowledge based on the real-time information. Kinetic profiles of active substances trapped in nanofiber materials made of different polymers and produced by means of different technologies were examined.[1]

Similar flow-programming setup based on the SIA concept was proposed for on-line monitoring of cell membrane permeation.[2] The permeation kinetics of the fluorescent marker rhodamine 123 was studied in a fully automated mode by sampling the apical and basolateral compartments of a 3D printed permeation module. The effect of the P-glycoprotein transporter inhibitor on the efficiency of a marker transport across a cell monolayer was investigated in detail. Finally, a 3D printed module was used for the automation of toxicity assays based on the release of *Metridia* luciferase upon interaction with toxic substances and thus changes in cell membrane permeability were studied.[3]

The study was supported by the project New Technologies for Translational Research in Pharmaceutical Sciences (NETPHARM, project ID CZ.02.01.01/00/22_008/0004607), co-funded by the European Union.

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SC-5

ANALYSIS OF SELECTED DRUGS IN WHOLE BLOOD COLLECTED WITH A VOLUMETRIC ABSORPTIVE MICROSAMPLING DEVICE

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Preclinical research plays a crucial role in developing new drugs. However, ethical concerns about using animals in research have led to the 3Rs (replacement, reduction, and refinement) principles. Microsampling aligns with the 3Rs by reducing the number of animals needed for a study via collecting low blood volume from a single animal multiple times, which is particularly beneficial for small rodents. It also provides the advantage of gentle sample collection and improved data quality. Volumetric absorptive microsampling (VAMS) is a reliable method for collecting precise whole blood volume and minimizes the impact of hematocrit on the assay. Analytes are commonly extracted from VAMS tips by direct desorption, but microextractions have a high potential for this purpose and offer an ethical and environmentally friendly sample cleanup solution. This presentation focuses on developing various sample extraction protocols to isolate selected drugs from whole blood microsamples collected with VAMS devices (10 µl, Mitra®, Neoteryx) for preclinical studies. After isolation, the extracts are analyzed using UHPLC-MS/MS. Doxorubicin, a clinically used anticancer drug, and its metabolites doxorubicinol, which are commonly used in preclinical cancer/cardio-oncology studies, were isolated from the VAMS tips using electromembrane extraction in a 96-well plate format. The cardioprotective agents, dexrazoxane, and its more potent analog ICRF-193 were extracted from whole blood microsamples using ultrasonic-assisted desorption to organic solvent. The reliability of the developed procedures was demonstrated through the analysis of real samples from PK studies with athymic nude mice.

The study was supported by Czech Science Foundation, (No. 23-06558S) and /NETPHARM (ID CZ.02.01.01/00/22_008/0004607), co-funded by the European Union.

SC-6

HPLC AND SFC AS USEFUL TOOLS FOR CHIRAL SEPARATION OF CARBORANES - PROMISING COMPOUNDS IN DRUG DEVELOPMENT

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Boron cluster compounds represent a distinctive category of highly stable abiotic molecules. In medicinal chemistry, these clusters are being investigated as innovative isosteres of phenyl rings in medicinal chemistry.[1] Unfortunately, the chirality of boron clusters has been largely overlooked for the past sixty years. Our research group has recently developed efficient methods for enantiomeric purity evaluation of these compounds. We conducted a thorough investigation into the primary factors hindering the successful chiral separations of anionic carboranes in HPLC. Our findings indicate that cyclodextrin-based stationary phases are effective in separating enantiomers of anionic carboranes. Notably, we have achieved the enantioseparation of 7,8-dicarba-nido-undecaborate($^{1-}$) ions in HPLC for the first time.[2] Our research also demonstrates that polysaccharide-based chiral selectors can facilitate the chiral separation of anions and zwitterions under reversed-phase conditions. Moreover, SFC has emerged as a valuable technique for rapid chiral baseline separations, capable of completing the process within 10 minutes. This method substantially outperforms HPLC in terms of the number of separated chiral carboranes, resolution, and analysis time. [3,4]

In summary, our findings provide essential insights into the development of chiral methods for anionic and zwitterionic carboranes, with significant implications for drug development. Furthermore, these results have broader applications in any field where the chirality of these intriguing molecules is a concern.

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SC-7

DEXRAZOXANE - NEW INSIGHT INTO THE MECHANISM OF ACTION OF THIS CLINICALLY USED DRUG

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Anthracyclines (ANTs) like doxorubicin (DOX), daunorubicin (DAU) and epirubicin rank among the most effective anticancer drugs. However, their clinical use is hampered by cardiotoxicity. Particularly feared are chronic forms, which are largely irreversible and manifest themselves as a congestive heart failure and dilated cardiomyopathy. Moreover, mechanisms of ANT cardiotoxicity are not fully elucidated.

Dexrazoxane (DEX) is the only cardioprotective drug approved for clinical use against ANT cardiotoxicity. Its cardioprotective effects have been well established in experimental settings, but, surprisingly, its mechanism of action is still not fully understood. Moreover, the absence of any other cardioprotective agent active against chronic ANT cardiotoxicity in vivo complicates the elucidation of DEX mechanism of action and thus blocks the rational development of new potent cardioprotective drug. Traditionally, DEX has been considered as a pro-drug of metal chelating agent ADR-925 which should prevent ANT-induced oxidative stress and myocardial damage by iron chelation with subsequent disruption of ROS formation. But DEX is also catalytic inhibitor of topoisomerase II, which is also a target of ANTs action.

The goal of our research project was to elucidate the mechanism of action of DEX, to investigate its structure-activity relationships and to develop another effective cardioprotective agent against ANT-induced cardiotoxicity based on the data obtained.

This work was supported by the NTER-EXCELLENCE project (reg. no. LUAUS24335) and by the project OncoPharm (CZ.02.01.01/00/23_021/0008442).

SC-8

DEVELOPMENT OF COVALENT MODULATORS OF MACROMOLECULES: NOVEL ALKYLATING AGENTS AGAINST GLIOBLASTOMA

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Glioblastomas represent 15% of brain tumors; it is the most common and aggressive cancer beginning within the brain. There is no cure for GBM. Untreated gliobastoma patients have a median survival of 3 months. Currently the only FDA approved drug for use against GBM is Temozolomide (TMZ) which has been found to increase life span by a few months when paired with tumor resection and radiotherapy. TMZ is only useful in high doses, and GBM often becomes resistant to the drug over time.

Michael acceptors are potential DNA alkylators that have been used for cancer chemotherapy. Based on the structure of TMZ we designed and synthesized a set of novel, structurally simple, tetrazole-containing cyclic and acyclic Michael acceptors as potential drug candidates using a combination of novel and classical synthetic methods. Some of the prepared tetrazole-containing Michael acceptors exhibited significant anticancer activity in vitro exceeding TMZ potency by a factor of 10 with considerably lower toxicity toward normal cells. These compounds can be used as candidates for further development of anti- glioblastoma drugs. SAR (structure activity relationship) studies were conducted for optimization of activity and specificity. However, the biological molecular mechanism of action is still unclear and extensive biological studies are required.

This study was supported by Bar-Ilan University President PhD fellowship and by Adama Ltd, through the Adama Scholarship.

SC-9

REPLACEMENT OF NITRO FUNCTION BY FREE BORONIC ACID IN NON-STEROIDAL ANTI-ANDROGENS

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With the aim to find new compounds for androgen deprivation treatment to combat prostate cancer, a series of new potential flutamide-like antiandrogens was designed and synthesized. In this work we present the possibility of replacement the characteristic feature of nitro group by a boronic acid functionality, in addition, the compounds were variated in the acyl part, in the linker as well as in the substitution of the benzene ring in the general scaffold 4-nitro-3-trifluoromethylanilide of non-steroidal androgens. Compound 4-(5-chloropyrazine-2-carboxamido)-2-fluorophenylboronic acid exhibited higher activity against LAPC-4 than the standards flutamide and bicalutamide and even lower toxicity against non-cancerous cell line HK-2. The initial *in-silico* study did not support the covalent bonding to androgen receptor, which was confirmed in an NMR binding experiment. The structure-activity relationships obtained in this study might show directions for further research in the field of non-steroidal antiandrogens.

The study was supported by Charles University project SVV 260 666 (Czech Republic). "COOPERATIO" program, research area Pharmaceutical Sciences.

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SC-10

DESIGNING AND OPTIMIZING NRF2 ACTIVATORS FOR ENHANCED DERMO-PROTECTION IN OXIDATIVE STRESSRELATED PATHOLOGIES

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Nrf2 (nuclear factor erythroid 2-related factor 2) is a crucial transcription factor regulating the cellular defense response under oxidative stress. Anti-oxidative protection ability of Nrf2 in human pathological conditions, such as Alzheimer's disease, Parkinson's disease, psoriasis, acute dermatitis, multiple sclerosis, macular degeneration etc. has been widely investigated. Among all human tissues, skin is extremely sensitive to oxidative stress. The high sensitivity to oxidative stress is related to exposure to environmental harmful factors such as ultraviolet light (UV) and the subsequent production of reactive oxygen species (ROS). Taking into consideration that oxidative stress is a key event in the pathogenesis of skin damage and inflammation, this pathological condition may therefore be a suitable target for therapeutic intervention. The activity of Nrf2 is tightly regulated by a negative regulator termed Keap1 (Kelch ECH-associating protein 1). The Keap1-Nrf2- ARE complex is responsible for cytoprotective effects against endogenous and exogenous stress caused by ROS. Nrf2 binds to ARE (antioxidant response element) in the regulatory regions of target genes, and Keap1, a repressor protein, binds to Nrf2 and promotes its degradation by the proteasome. To enhance Nrf2 activity, disruption of the Keap1−Nrf2 interactions at the protein−protein interface is necessary.

Thus, we hypothesized that compounds that interact with Keap1 at its putative binding site with Nrf2 have the potential to disrupt Nrf2 binding. In this way, free Nrf2 might transmigrate to nuclei and lead to an activation of the transcription of anti-oxidative stress response genes and the expression of the corresponding proteins that will course neuro- and dermo-protective effect.

We developed two Nrf2 activators that showed very significant antioxidant in vitro and ex vivo effects in neurons, keratinocytes, and human skin samples. Although both compounds have good efficacy and potency in the pharmacological concentrations, they contain a metabolically unstable imine bond

as well as potentially unstable phenol groups. To increase their metabolic stability, we designed and synthesized a series of their derivatives with increased metabolic stability containing amide bond instead of the imine one, as well as acetyl protected hydroxyl groups instead of the phenols. This increased metabolic stability and lipophilicity thereby improved membrane and skin penetration while retaining an ideal balance between the polar and unipolar groups thereby resulting in structurally and functionally optimized compounds which exhibited significant protective effects.

SC-11

NOVEL HETEROCYCLIC COMPOUNDS AS ANTIMICROBIAL AGENTS

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Heterocyclic compounds represent the most diverse scaffolds with unique structural properties that offer various pharmacological activities. Current advances in synthetic methodologies enable the effective synthesis of highly functionalized heterocycles to fine-tune their chemical and biological properties. During the last few years, we have been involved in synthesizing compounds with potential antituberculosis effects.[1-4] We have developed several promising compounds against *Mycobacterium tuberculosis* inhibiting the mycobacterial ATP synthase[1,2] or mycobacterial virulence factor Zmp1.[3,4] During the development of these compounds, we also came across interesting heterocyclic compounds exhibiting nanomolar activities against the parasite *Trypanosoma brucei*. We believe our results might open up new avenues for designing selective antimicrobial agents addressing the urgent need for novel selective and effective antibiotics in the face of antibiotic resistance.

The study was supported by grants JG_2019_002 and IGA PrF 2024 028 from Palacký University in Olomouc, FWO grant no. G066619N, project ELIXIR CZ no. LM2022123 from the Ministry of Education, Youth and Sports of the Czech Republic.

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SC-12

DEVELOPMENT OF DINITROPHENYL-CONTAINING ANTITUBERCULOSIS AGENTS

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Tuberculosis (TB) remains one of the deadliest diseases in the world, and the situation has not changed significantly in recent years. There were 1.45 million deaths and 10 million new TB cases in 2018, and 1.3 million deaths and 10.6 million new TB cases in 2022.[1] However, the growing concern is related to the increasing number of drug-resistant TB cases, with MDR-TB rising from 187,000 in 2018 to 410,000 in 2022. Therefore, the demand for new drugs with a novel mechanism of action remains high.

Recently, our group discovered 3,5-dinitrobenzylsulfanyl-1,3,4-oxadiazoles, which showed high activities against both drug-susceptible and drug-resistant *Mycobacterium tuberculosis* strains in both replicating and non-replicating states, had low toxicity against proliferating cell lines and isolated human hepatocytes, and didn't inhibit the growth of bacteria and fungi.[2] Intensive study of the structure-activity relationships has shown that the position of both nitro groups on the benzyl moiety as well as the position of the dinitro-phenyl fragment in the molecular scaffold can have a crucial influence on the activity of the compounds as well as on their mechanism of action.[3]

The study was supported by Czech Health Research Council (project NU21-05-00446)

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SC-13

SYNTHESIS AND BIOLOGICAL ACTIVITY OF STYRYLQUINOLINE DERIVATIVES

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Advances in medical sciences have significantly contributed to the progress in the treatment of many diseases; however, there remains an urgent need to explore new therapeutic approaches. We are observing an alarming increase in bacterial and fungal infections. Therefore, the search for new drugs remains one of the primary challenges in contemporary science.

In our research group, we have been focusing for years on the discovery of new, active derivatives that exhibit strong antifungal and antibacterial properties, along with high selectivity towards normal cells. These efforts have led to the development of a series of styrylquinoline derivatives. We conducted *in vitro* screening of synthesized compounds against various fungal and bacterial strains.[1] Subsequently, the antifungal activity of selected compounds was further evaluated by determining the MIC values for a wild-type *Candida albicans* strain and *cdrΔ* single and double mutants. It was found that cells with *CDR1* deletion exhibited a significant increase in sensitivity to styrylquinolines, suggesting that these compounds are substrates of *Cdr1p*. This indicates a potential mechanism of action as inhibitors of this transporter. Additionally, synergistic effects with fluconazole were investigated.[2,3]

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SC-14

NOVEL INHIBITORS OF HUMAN CARBONIC ANHYDRASE

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Carbonic anhydrases (CA, EC 4.2.1.1) are metalloenzymes catalyzing the reversible hydration of CO_2, thereby affecting the pH and related physiological processes in various organisms. In human bodies, 15 different isoforms of CAs can be found, including two tumor-associated: hCA IX and hCA XII (human CA). These two isoforms are responsible for up-regulated metabolism, growth, and survival of cancer cells. They are involved in spreading metastasis, and correlated with resistance towards chemotherapy or radiotherapy.[1] Given the above, it is clear that CA inhibitors are very promising compounds in cancer treatment. However, for now, their crucial limitation is their selectivity towards specific isoenzymes.

A new series of inhibitors of tumor-associated CA was designed and synthesized, specifically 1,3,5-triazines containing aminobenzenesulfonamide, piperazine, aniline, and other structural moieties. New compounds' inhibitory activity and selectivity were determined against hCA IX, hCA XII, and hCA II.

The study was supported by grant number MUNI/G/1002/2021.

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SC-15

VITAMIN D SUPPLEMENTATION WITH THE INFLUENCE OF ARTI (CASE REPORT)

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Low serum 25-hydroxyvitamin D concentrations are associated with a higher susceptibility to acute respiratory tract infections (ARTIs). The aim of this case study is to present the association between vitamin D levels, supplementation and the incidence of ARTIs.

A 23-year-old female patient with vitamin D deficiency successfully increased her vitamin D level from 45.60 nmol/l to 85.91 nmol/l (reference range 75-200 nmol/l) by supplementation. However, surprisingly, in the following period, instead of the expected level of 120 nmol/l, a decrease in vitamin D level to 70.04 nmol/l was observed, although the patient continued taking supplementation. Further investigations revealed that the patient had been suffering from common symptoms of acute respiratory tract infection during the time of supplementation. Determination of total vitamin D levels was performed with the chemiluminescence kit ACCESS 25(OH) Vitamin D on a Unicel® DxI 800 instrument.

This case study demonstrates the complex relationship between vitamin D levels, proper supplementation and ARTI. The observed decline in vitamin D levels during supplementation and ongoing acute respiratory tract infection suggests that respiratory infections may significantly affect vitamin D metabolism.

The study was supported by the Ministry of Health, Czech Republic conceptual development of research organization (Faculty Hospital in Pilsen-FNPl, 00669806), BBMRI-CZ: Biobank net-work – a versatile platform for the research of the etiopathogenesis of diseases Z.02.1.01/0.0/0.0/16_013/000167 and LM2015089, and by the "Cooperatio" Program, research area Pharmaceutical Sciences.

SC-16

INHIBITORY EFFECTS OF SOME CHALCONES ON ACHE ENZYME

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Natural compounds are essential in the discovery of new therapeutic agents. Chalcone derivatives are one of the most studied flavonoid-derived structures.[1] Under acidic or basic conditions, chalcone (1,3-diaryl-2-propenone) derivatives can be classically synthesized by Claisen Schmidt condensation.[2-4] Chalcone and its analogs have therapeutic importance, such as anticancer, anti-inflammatory, antioxidant, antimicrobial, antituberculosis, antimalarial, and antiallergic properties. Reports indicate that it possesses numerous essential bioactivities.[1] Researchers have also investigated some antipsychotic phenothiazines as potential cancer therapeutics. For instance, studies have found promising activity of chlorpromazine against endometrial, lung, colorectal, and breast cancer.[5] In addition to neuroleptic effects, phenothiazine derivatives have pharmacological effects such as antiemetic, antihistamine, analgesic, and anthelmintic.[6] Additionally, it has essential bioactivities such as anticancer,[7] antibacterial,[8] cholinesterase inhibitor,[9] antimalarial,[10] antituberculosis,[11] and anti-HIV.[12] Importantly, AChE is a promising tumor suppressor.[13] Given this information, we aimed to synthesize five chalcones bearing phenothiazine rings, elucidate their chemical structures, and investigate their inhibitory activities on the AChE enzyme.

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SC-17

PHENOTHIAZINE BASED D-A SYSTEMS: FROM STRUCTURAL AND PHOTOPHYSICAL TO BIOLOGICAL PROPERTIES

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