51st Conference

Synthesis and Analysis of Drugs

51. Konferencia

Syntéza a analýza liečiv

Bratislava

September 7 & 8

2023

51st Conference

SYNTHESIS AND ANALYSIS OF DRUGS

2023

Faculty of Pharmacy

Comenius University in Bratislava

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Dátum výroby materiálu: apríl 2023. SK_EDU-05-2023_V01_press

Dear Researchers

It is our great pleasure to welcome you to the 51st conference Synthesis and Analysis of Drugs, hosted under the auspices of Faculty of Pharmacy, Comenius University in Bratislava. It is an established international conference dedicated to all aspects of pharmaceutical chemistry and analysis. The conference is held alternately in the Czech and in the Slovak Republic and cherishes a long tradition dating back to 1971. We hope that it will be a platform to gather and disseminate the latest knowledge in pharmaceutical chemistry and related disciplines. We would particularly like to encourage the junior scientists and students to profit from the knowledge and the know-how offered by experienced experts in this current and exciting scientific field. We are grateful to all contributors for presenting their valuable research.

The conference is dedicated to Professor Jozef Čižmárik on the occasion of his 80th birthday.

Ing. Ladislav Habala, PhD.
on behalf of the Organizing Committee

The contributions in this booklet have been edited by the editors only to the minimum extent necessary (spelling and formatting); their scientific content is the sole responsibility of the contributing authors. The definite version of the abstracts, including Short Communications, will be published in a special issue of the journal *European Pharmaceutical Journal*.

Undergraduate students are welcome to attend the conference presentations, their admission to all lectures is free.

CONFERENCE PROGRAMME

All lectures take place in the Room 151

Thursday September 7			
Arrival, registration of participants	10:00 –		
Conference opening	13:00		
Plenary lectures *Session chairs:* *Dr. Habala, Doc. Valentová*	13:20	L27	CRYSTALLIZATION-INDUCED DIASTEREOMER TRANSFORMATION: A COST-EFFECTIVE ROUTE TO DIASTEREOSELECTIVELY PURE DRUGS – THE CASE OF APREPITANT (*P. Bobal, Masaryk University Brno, CZ*)
	14:00	L24	AMINOPEPTIDASE N AS A POTENTIAL DRUG TARGET (*O. Farsa, Masaryk University Brno, CZ*)
	14:40	L8A	DRUGS IN THE ENVIRONMENT (*R. Opatřilová, Masaryk University Brno, CZ*)
Coffee break	15:20 -15:40		
Short presentations *Session chairs:* *Dr. Miklášová, Dr. Lintnerová*	15:40	S10	UREASE INHIBITION AS A TOOL AGAINST PATHOGENIC MICROBES: THE ROLE OF METAL COMPLEXES (*L. Habala, Comenius University Bratislava, SK*)
	15:55	S34	COPPER(II) AND ZINC(II) COMPLEXES OF REDUCED SCHIFF BASES: SYNTHESIS, STRUCTURE DETERMINATION AND BIOLOGICAL ACTIVITY (*B. Oboňová, Comenius University Bratislava, SK*)
	16:10	S35	BIOLOGICALLY ACTIVE DECAVANADATES (*L. Krivosudský, Comenius University Bratislava, SK*)
	16:25	S30	OPTIMIZATION OF CAPILLARY ELECTROPHORESIS – MASS SPECTROMETRY METHOD FOR ANALYSIS OF MONOCLONAL ANTIBODIES (*J. Havlikova, Comenius University Bratislava, SK*)
	16:40	S7	LEVOMENTHOL IN LIQUID MEDICINES PREPARED IN A PHARMACY AND ITS DETERMINATION *(M. Sýkorová, Comenius University Bratislava, SK*)
Poster session	17:00 – 18:00		
Welcome evening	19:00		Multimedia Room

Friday September 8

Plenary lectures *Session chairs:* *Doc. Hrčka Dubničková* *Doc. Andriamainty*	9:00	L38	PROGRAMMED AND IMMUNOGENIC CELL DEATH MECHANISMS INDUCED BY METAL-BASED DRUGS IN TUMORS (*E. Fischer-Fodor, Institute of Oncology, Cluj-Napoca, RO*)
	9:40	L39	PYRAZINAMIDE DERIVATIVES: ANTIMICROBIAL ACTIVITY AND BEYOND (*J. Zitko, Charles University Hradec Králové, CZ*)
	10:20	L40	NATURAL METABOLITES IN CURRENT PHARMACOTHERAPY (*P. Mučaji, Comenius University Bratislava, SK*)
Coffee break	11:00 -11:20		
Short presentations *Session chairs:* *Dr. Miklášová, Dr. Lintnerová*	11:20	S36	DETERMINATION OF STEROIDAL SAPONINES IN *TRIBULUS TERRESTRIS* FOOD SUPPLEMENTS BY LC-MS/MS METHOD (*J. Valentová, Comenius University Bratislava, SK*)
	11:35	S15	ADVANCING DIAGNOSTIC CAPABILITIES OF FLUORESCENT PROBES: UNVEILING THE STRUCTURE-BINDING AFFINITY RELATIONSHIP IN IMAGING AMYLOID β PLAQUES FOR ALZHEIMER'S DISEASE (*J. Kladnik, University of Ljubljana, SI*)
	11:50	S16	NOVEL 1,3,5-TRIAZINYL AMINOBENZENESULFONAMIDES AS POTENT CARBONIC ANHYDRASE INHIBITORS (*E. Havránková, Masaryk University Brno, CZ*)
	12:05	S20	STRUCTURAL ANALYSIS OF DITERPENOIDS ISOLATED FROM THREE PLECTRANTHUS S.L. SPECIES (*M. Gáborová, Masaryk University Brno, CZ*)
	12:20	S31	APPLICATION OF SPECTROSCOPIC TECHNIQUES FOR EVALUATING FUNGAL VIABILITY (*P. Paračková, Comenius University Bratislava, SK*)
Closing of the conference	12:35		

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S34 Oboňová B., Habala L., Litecká M., Herich P., Bilková A., Bilka F.: COPPER(II) AND ZINC(II) COMPLEXES OF REDUCED SCHIFF BASES: SYNTHESIS, STRUCTURE DETERMINATION AND BIOLOGICAL ACTIVITY

S35 Krivosudský L., Somasundaram D. J., Ebrahimi A.: BIOLOGICALLY ACTIVE DECAVANADATES

S36 Valentová J., Kepičová S., Švajdlenka E., Pagáč T.: DETERMINATION OF STEROIDAL SAPONINES IN TRIBULUS TERRESTRIS FOOD SUPPLEMENTS BY LC-MS/MS METHOD

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P2 Ungvarská Maľučká L , Csöllei J., Mokrý P.: SYNTHESIS AND STUDY OF BIOLOGICAL PROPERTIES OF NEW CARBAMATES WITH A MODIFIED BASIC FRAGMENT IN THE ARYLOXYAMINOPROPANOL CHAIN

P3 Onuscakova M., Pizova H., Kollar P., Bobal P.: SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITY OF NEWLY DESIGNED HYDROXAMATES AS POTENTIAL HDAC INHIBITORS

P4 Lintnerová L., Litecká M., Valentová J.: SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITIES OF COPPER(II) COMPLEXES OF LIGANDS DERIVED FROM 4-METHOXYSALICYLALDEHYDE AND β-ALANINE AND γ-AMINOBUTANOIC ACID

P5 Salanci E., Andriamainty F., Malík I., Shafieikazerooni P., Stopková L.: A STUDY OF THE MICELLIZATION OF CARBISOCAINIUM CHLORIDE IN AQUEOUS SOLUTION USING THE OPTICAL DENSITY METHOD

P6 Miklášová N., Bastami L., Fischer-Fodor E., Valentová J., Svitková B.: ANTIPROLIFERATIVE METAL COMPLEXES OF THE FLUORINATED CURCUMIN DERIVATIVE: 1,7-BIS(4-FLUOROPHENYL)HEPTA-1,6-DIENE-3,5-DIONE

P8B Brhelová E., Scheer P., Hložková J., Doležalová J., Mikulík R., Thalerová S., Aksu A.D., Grayston A., Rosell A., Opatřilová R.: THROMBOLYSIS *IN VIVO*

P9 Korcová J., Húserková M., Lintnerová L., Valentová J.: DNA CLEAVAGE AND CYTOTOXIC ACTIVITY OF COPPER (II) COMPLEXES BASED ON REDUCED SCHIFF BASES DERIVED FROM SALICYLALDEHYDE AND AMINO ACIDS.

P11 Kapustíková I., Garaj V., Kemka M.: ASSESSMENT OF LIPOPHILICITY OF NEWLY SYNTHESIZED CARBONIC ANHYDRASE INHIBITORS USING REVERSED-PHASE HPLC AND SCHRÖDINGER COMPUTATIONAL PLATFORM

P12 Vesely H., Bobal P.: NEW FLUOROPHORES FOR THE DETECTION OF GLYCANS

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P14 Dokupilová K., Dokupilová S., Kostovčíková E., Mučaji P., Bittner Fialová S.: PHYTOCHEMICAL ANALYSIS OF THE WATER-ETHANOL ROOT EXTRACT OF *DIPSACUS FULLONUM* L.

P17 Kurin E., Bittner Fialová S., Mučaji P., Trajcikova E., Nagy M.: GREEN TEA AND PEPPERMINT IN MIXTURE SYNERGISTICALLY ENHANCE THEIR ANTIOXIDANT CAPACITY

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P32 Slíž K., Olešová D., Piešťanský J., Mikuš P.: SIMPLE AND SENSITIVE ANALYSIS OF CLENBUTEROL IN URINE MATRICES BY UHPLC–MS/MS METHOD WITH ONLINE

P33 Strhársky T., Pindjaková D., Jamrichová J., Lavičková J., Kadlecová V., Kleinerová K., Kos J., Goněc T., Jampílek J.: SYNTHESIS OF ANTIBACTERIAL CINNAMAMIDES

P37 Mikláš R., Hasaralejko C., Šuštiak M.: SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF QUATERNARY AMMONIUM HOMOCHIRAL SULFONAMIDES

PLENARY LECTURES

L8A

DRUGS IN THE ENVIRONMENT

Radka Opatřilová

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Drugs of different chemical structures are becoming increasingly important potential environmental contaminants as pharmacotherapeutic options develop. Their effects on microorganisms, plants and animals are still being discovered and described. Drugs have been present in nature since they were first prepared synthetically.

One of the first synthetic drugs is Acetylsalicylic acid (patent, Bayern, 1899). Its high consumption for treatment may have caused that as early as 1977 Acetylsalicylic acid was detected on the effluent of a sewage treatment plant (Missouri River), in an amount of 8.64 (0.55-28.69) kg over a 10-month period[1].

Paracetamol has also been detected in the environment and is readily accumulated worldwide (concentrations in the units or tens of ng/l). Bacteria have already been described in the literature that are able to use Paracetamol as a carbon and energy source due to specialized systems and metabolic pathways[2]. In contrast, studies[3] have found that snakes die after consuming Paracetamol.

World consumption of Diclofenac exceeds 2,400 tonnes per year. A study[4] described renal and gastrointestinal disturbances in vertebrates. The authors[5] described the extinction of up to 95% of the African vulture population after consumption of Diclofenac-treated livestock.

Thanks to state-of-the-art instrumental methods, we can detect and quantify even ultra-low concentrations of pharmaceutical substances in the environment and study the influence of different chemical structures on environmental constituents. By setting up the right pharmaceutical management, we can then ensure a reduction in contamination and potential changes in a wide range of pharmaceuticals that we use in the context of our lifestyle.

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Keywords: drugs, detection, effect, environment

L24

AMINOPEPTIDASE N AS A POTENTIAL DRUG TARGET

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Aminopeptidase N (APN) is a broad specificity zinc metallopeptidase with many functions that do not always depend on its enzymatic activity. Among others, it is involved in tumor angiogenesis and methasthazing and also serves as a cellular receptor of some coronaviruses. Some APN inhibitors, such as bestatin or tosedostat, were used or tested as anti-cancer drugs in the past. Within the past two decades, we have prepared several series of potential APN inhibitors. Some of them reached interesting values of inhibitory activity and were also successfully tested for anti-proliferation activity in cancer cell lines. We also performed QSAR studies with APN inhibitors prepared by us and other authors.

This work was supported by the project of Masaryk University MUNI/IGA/0932/2021.

Keywords: aminopeptidase N, inhibitors, antitumor activity, anti-infectious activity

L27

CRYSTALLIZATION-INDUCED DIASTEREOMER TRANSFORMATION: A COST-EFFECTIVE ROUTE TO DIASTEREOSELECTIVELY PURE DRUGS – THE CASE OF APREPITANT

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The pharmaceutical industry produces a large amount of waste per 1 kg of active compound, which is much higher compared to other industries, resulting in more than 3 million tons of waste per year. The reason for it is that the preparation of active pharmaceutical ingredients (API) in most cases requires multi-step synthetic and purification processes. Very often these processes contain resolution techniques for the separation of homochiral organic molecules or the isolation of desired diastereomers. Most pharmaceutically active ingredients are known to be chiral, and one enantiomer or diastereomer is generally preferred over the racemic mixture.[1] Therefore, the implementation of novel cost-effective methods for the isolation of a single stereoisomer is highly desired in pharmaceutical production.

One of the efficient methods for the isolation of single stereoisomers from the reaction mixture is called crystallization-induced stereoisomer transformations (CIST). CIST can, in principle, be divided into two main categories: crystallization-induced enantiomer transformations (CIET) and the much more common crystallization-induced diastereomer transformations (CIDT).[2,3] The use of crystallization-induced diastereomer transformations is presented on the industrial production of the drug aprepitant **1**.

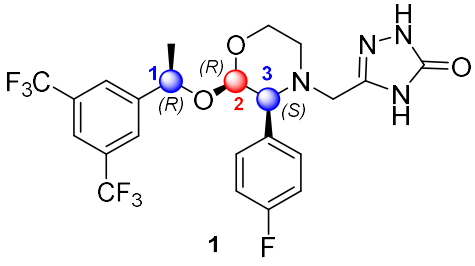
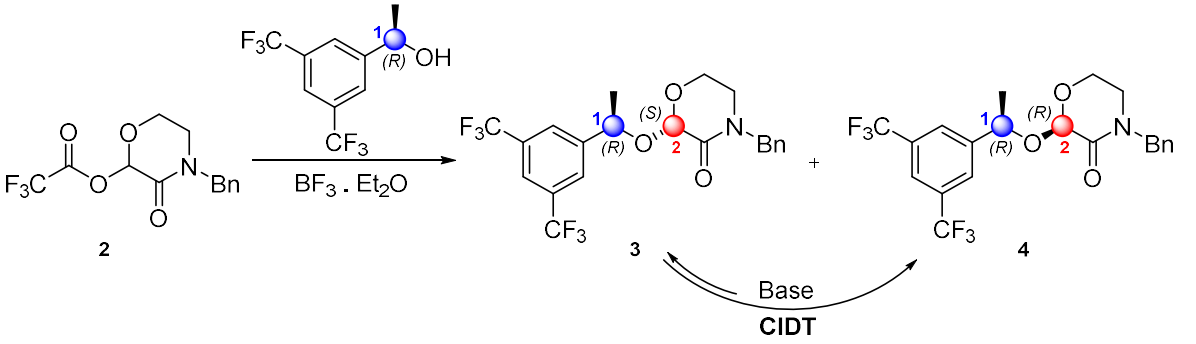


Figure. The structure of aprepitant **1**.

Aprepitant **1** is an orally active human antagonist of neurokinin NK_1 receptors, chemically known as 3-{[(2*R*,3*S*)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-morpholin-4-yl]methyl}-4,5-dihydro-1H-1,2,4-triazol-5-one (Figure), developed by Merck, and marketed with a trading name of Emend, used for the prevention and treatment of acute and delayed nausea and vomiting in adults related to anti-cancer chemotherapy. The aprepitant molecule contains three stereogenic centers, of which two are part of the morpholine skeleton. Several synthetic strategies have been developed for the preparation of aprepitant **1**, [4-7] among which the synthesis starting from enantiopure (1*R*)-1-[3,5-bis(trifluoromethyl)-phenyl]ethan-1-ol was found to be efficient and scalable.

Few methods of the synthesis (1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethan-1-ol have been reported mainly from corresponding acetophenone. From them, we have selected the catalytic asymmetric transfer hydrogenation developed by Noyori [8,9] using (1*S*,2*R*)-*cis*-1-aminoindan-2-ol and dichloro(*p*-cymene)Ru(II)dimer as chiral ligand and metal source for reduction. As a safe and benign hydride source, propane-2-ol has been used as a stoichiometric reductant. The enantiopure alcohol was prepared with an average yield of 94 % and about 90 % e.e. The enrichment via recrystallization through DABCO inclusion complexes from *n*-heptane and subsequent DABCO removal led to a purity of alcohol of approximately 99 % e.e.[10] The overall yields from starting 1-[3,5-bis(trifluoromethyl)phenyl]ethan-1-one to (*R*)-alcohol was in the range of 67 – 76 %. The second building block racemic 4-benzyl-3-oxomorpholin-2-yl 2,2,2-trifluoroacetate **2** was prepared from *N*-benzylaminoethanol and aqueous glyoxylic acid followed by acylation with TFAA.



Scheme. Trans-acetalization and crystallization-induced diastereomer transformation (CIDT) processes towards (R,R)-diastereomer **4**.

The crucial step of the synthesis of aprepitant **1** involves a Lewis acid-mediated trans-acetalization coupling of 4-benzyl-3-oxomorpholin-2-yl 2,2,2-trifluoroacetate **2** with enantiopure (*R*)-1-(3,5-bis(trifluoromethyl)phenyl) ethanol that provided a 45:55 mixture of acetal diastereomers **3** and **4** which was converted to a desired *R,R*-isomer **4** in 72 % yield via a crystallization-induced diastereomer transformation (CIDT), involving base-catalyzed equilibration in solution. The (*R,R*)-diastereomer **4** is less soluble in the reaction medium and crystallizes out. On the other hand, (*R,S*) diastereomer **3** is racemized in the solution by base-mediated deprotonation with lipophilic potassium salt of tetrahydrolinalol. The addition of 4-fluorophenylmagnesium bromide to intermediate **4** was almost quantitative. The adduct was after quenching hydrogenated at ambient temperature and pressure of 1.5 atm in the presence of palladium on charcoal. The enantiomerically and diastereomerically pure aprepitant **1** was finally prepared by reaction with triazolinonyl chloride.

Although there are certain drawbacks and limitations of this method, crystallization-induced diastereomer transformation (CIDT) is widely used in the synthesis and industrial production of active pharmaceutical ingredients (API) due to its efficiency and cost-effectiveness, in which crystallization is combined with racemization into one pot deracemization process leading to enantiomerically and/or diastereomerically pure products in almost theoretical yield.

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Key words: CIDT, crystallization-induced diastereomer transformation, aprepitant, active pharmaceutical ingredients

L38

PROGRAMMED AND IMMUNOGENIC CELL DEATH MECHANISMS INDUCED BY METAL-BASED DRUGS IN TUMORS

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The metallodrugs development after the first studies on cisplatin in 1965 gave rise to metal-based treatment regimens in cancer care, several clinical trials are ongoing and nowadays the preclinical testing of active metal compounds benefit from unprecedented abundance of investigational methods. Still, few new molecules reached the biopharmaceutical pipelines and the antitumor drug market.

Recent studies proved that metal compounds able to induce programmed cell death in tumour cells will more likely became prodrugs. Some of the metal drugs display complementary biologic effect to the monoclonal antibody-based targeted therapy such VEGF or EGFR inhibitors, PARP inhibitors, immune checkpoint inhibitors. More, if they can simultaneously modulate the antitumor immune response in the favour of the host immune system, they could serve as adjuvants of immune therapy.

In this respect, we identified active Pt (II) complexes with curcuminoid ligands, proapoptotic Re(I) and Rh(II) dendrimers, selective Ga(III) compounds and ferrocene derivates which damage the tumor cells DNA, immunogenic Pd(II) complexes having the potential to modulate the cytotoxic CD8+ and the helper CD4+ T lymphocytes expression and immune activation. The metal-based compounds, free or encapsulated in targeted nanostructures, if didn't prove to be apoptosis inducers, were re-evaluated for their capacity to trigger two "metallomic" programmed cell death pathways: ferroptosis and cuproptosis, seeking evidence that beyond the intracellular accumulation and DNA binding, metal-based drugs exert a fine-tuning on the cells physiological trace metal elements distribution, leading eventually on growth inhibition and exposes tumours to immune cells attack.

Despite of the increasing use of biological drugs in cancer care, in the era of the precision medicine the metal-based drugs have still a central role in the treatment of malignant diseases.

This work was supported by the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding Grant no. PN-III-P4-PCE-2021-1572, PCE105/2022.

Keywords: metallodrugs, cancer chemotherapy, apoptosis, cellular immunity

L39

PYRAZINAMIDE DERIVATIVES: ANTIMICROBIAL ACTIVITY AND BEYOND

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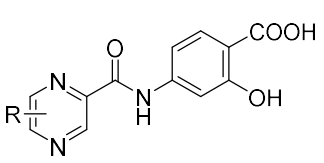
In the opening parts of the lecture, we will recapitulate the latest theories of the mechanism of action of the first-line antitubercular pyrazinamide (pyrazine-2-carboxamide, PZA). We will present our new antimicrobial derivatives of PZA, including hybrid compounds consisting of PZA and 4-aminosalicylic acid fragment (**I**),[1] and simple derivatives of 3-aminopyrazine-2-carboxamide (**II**). Compounds of general structure **II** were designed as inhibitors of mycobacterial prolyl-tRNA synthetase.[2] We will also present the results of our hit-expansion study on antistaphylococcal compound **III**, which might be considered a derivative with inversed carboxamide linker.



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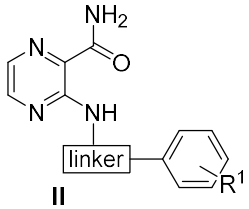

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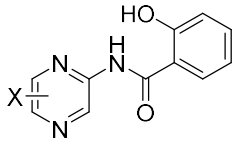

III

References:

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This research was supported by the Ministry of Health of the Czech Republic, grant nr. NU21-05-00482, and by „The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.“

Keywords: antimicrobial, drug design, prolyl-tRNA synthetase, pyrazinamide

L40

NATURAL METABOLITES IN CURRENT PHARMACOTHERAPY

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The WHO estimates that 80% of the world's human population primarily uses traditional medicine in healthcare. Selection of plant species based on empirically obtained knowledge and use in folk medicine (ethnomedicine/ethnopharmacology), botanical relatedness of plants selected according to the point or availability of the analyzed material, or (non-)existence of data on bioactivity or on content substances, knowing that the process of developing a new drug is lengthy, expensive, and uncertain.

The main advantages of natural substances include structural diversity and the fact that less than 0.1% of microorganisms in soil have been studied so far; only about 70,000 fungi out of an estimated 2-5 million species have been identified, or only about 800,000 out of a total of about 20 million insect species have been identified. The limiting factors of using natural substances are their toxicity, bioavailability, solubility, and isolation in the necessary quantity. Cell cultures and biotechnology are, therefore, increasingly used in plants.

Even in the era of biological drugs, natural substances do not lose their relevance. There are currently more than 100 ADCs (antibody-drug-conjugate) in various stages of clinical trials (from preclinical to phase III studies), containing warheads from natural sources derived from terrestrial and marine eukaryotic and prokaryotic organisms.

Botanical drugs - extracts approved by the FDA (Veregen, crofelemer) - are also entering the market. One of the reasons for using mixtures is that many disorders have a multifactorial etiology, and additive or synergistic effects can occur between the mixture's components, making the extract more effective than isolated pure compounds.

The Grants supported this work: VEGA 1/0226/22 and APVV-20-0017.

Keywords: secondary metabolites, phytotherapy, botanical drugs

SHORT TALKS

S7

LEVOMENTHOL IN LIQUID MEDICINES PREPARED IN A PHARMACY AND ITS DETERMINATION

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Levomenthol - (1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)-cyclohexanol is many years used as active pharmaceutical substance in pharmaceutical formulations. They are prismatic or acicular, colourless, shiny crystals practically insoluble in water, very soluble in ethanol (96 per cent). Quality control procedures have been developed and validated for levomenthol 1% and 10% alcohol solutions (ethanol 60 per cent) prepared in pharmacies.

As control methods for the identity tests of levomenthol and ethanol 96% in Solutio levomentholi ethanolica 1% and 10%, the official methods of the current European Pharmacopoeia: Relative Density (2.2.5), Specific Optical Rotation (2.2.7), Refractive Index (2.2.6) were used. For the determination of the levomenthol content of a medicinal product, procedures have been developed using optical rotation with Kruss digital polarimeter and absorption spectrophotometry in the visible region after the chemical reaction of levomenthol with salicylaldehyde (L-SAL) in H_2SO_4 on calibration with the external standard method and reaction of levomenthol with 4-dimethylaminobenzaldehyde (L-DMAB) in H_2SO_4 at 100°C on one point calibration. The stability of the color products formed was monitored by measuring the absorbance at 562 nm (L-SAL) and 547 nm (L-DMAB) as a function of time and the presence of water. The absorption spectra were recorded by a qualified spectrophotometer Shimadzu UV 1800.

The developed determination methodologies were validated in the range of 80-120% of the concentration level of levomenthol in the formulation using the parameters: precision (SD or RSD) as repeatability, intermediate precision, reproducibility, accuracy as recovery, linearity and range. All validation parameters met the criteria that were defined by the regulatory authority. Identity and assay procedures were developed and approved by the Pharmacopoeia Committee SUKL for the monograph of the drug in the Slovak Pharmaceutical Codex, 3rd edition.

Keywords: levomenthol , absorption spectrophotometry UV-VIS , optical rotation, relative density, analysis of drugs

S10

UREASE INHIBITION AS A TOOL AGAINST PATHOGENIC MICROBES: THE ROLE OF METAL COMPLEXES

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Urease (EC 3.5.1.5) is an enzyme containing and dependent on nickel ions in its active centre. It is widespread in nature, found in bacteria, fungi, algae, plants, and other species. The enzyme is also exprimated in several types of pathogenic bacteria and is an important virulence factor in these microorganisms. Prominent among them is *Helicobacter pylori*, an important human pathogen responsible for a variety of adverse medical conditions, the most severe among them being peptic ulcer and gastric cancer. Thus, development of novel inhibitors of urease represents an important challenge for medicinal chemistry.

Metal complexes are among the most efficacious inhibitors of urease [1]. Their inhibitory activity depends on the central metal atom as well as on the type of ligands and their arrangement. Bismuth compounds have long been used in the treatment of peptic ulcers and *Helicobacter pylori* infections, with urease inhibition playing an important role in their activity. Many other metal ions exert marked antiurease activity. As part of our work on the inhibition of enzymes by metal complexes we have been studying the inhibition of urease by complexes with various central metal atoms. The highest inhibition was achieved by copper complexes. The studies are also of theoretical importance as they provide interesting insights into the mechanism of enzyme inhibition by metal complexes.

References:

[1] L. Habala, F. Devínsky, A. E. Egger, *Metal complexes as urease inhibitors*, J. Coord. Chem., 2018, 71, 907–940.

This study was supported by the Grant VEGA 1/0145/20.

Keywords: urease, enzyme inhibition, metal complexes, bioinorganic chemistry

S15

ADVANCING DIAGNOSTIC CAPABILITIES OF FLUORESCENT PROBES: UNVEILING THE STRUCTURE-BINDING AFFINITY RELATIONSHIP IN IMAGING AMYLOID β PLAQUES FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid β (Aβ) plaques in the brain, which contribute to cognitive decline. Fluorescent dyes have become valuable tools for imaging Aβ plaques, allowing their visualization and examination. These dyes have specific properties, such as high photostability and solvatofluorochromic properties, which allow accurate detection of Aβ plaques. Advances in the synthesis and design of novel fluorescent probes have improved their specificity and binding affinity to Aβ aggregates and offer potential as diagnostic tools for the early detection and study of AD.[1,2,3]

Therefore, we focused on the synthesis of a series of novel fluorescent probes. The probes consisted of a central π-system (phenylethynyl or phenyl) end-capped with electron-donating (EDG) and electron-withdrawing groups (EWG). By incorporating different functionalities, we successfully modulated the optical properties and binding affinity of the probes to Aβ *in vitro, in cellulo,* and *ex vivo* (Figure 1).

Our results show that the synthesized probes exhibited selective binding properties to Aβ fibrils, and their interactions with the fibrils were supported by docking studies and molecular dynamics simulations. These results provide valuable insights into the potential application of these probes as diagnostic tools for AD. This study highlights the crucial relationship between structure-optical

properties and structure-binding affinity in the development of highly effective fluorescent probes for optical imaging of Aβ plaques.
Although our results are promising, further investigation and optimization are required to fully exploit the diagnostic capabilities of these probes and to advance their application in AD basic research and clinical diagnostic. Continued research efforts will facilitate the exploration of their full potential and enhance their effectiveness as valuable tools for AD diagnosis.

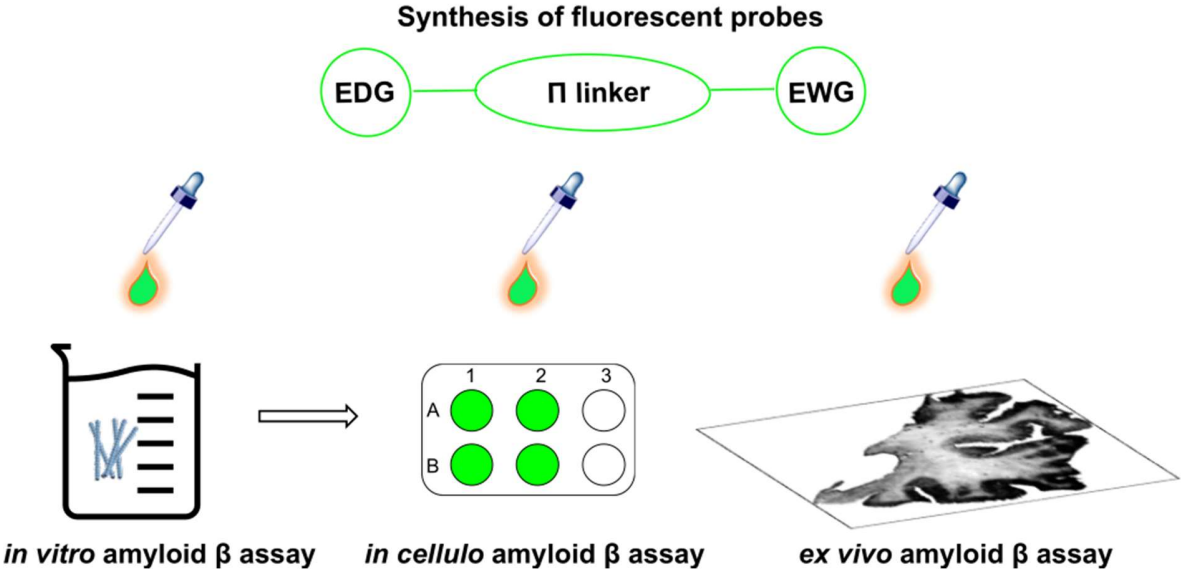

Figure 1: Simplified structure of fluorescent probes together with executed biochemical assays.

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1. H. Zetterberg et al., *Biomarkers for Alzheimer's disease–preparing for a new era of disease-modifying therapies*, Mol. Psychiatry, 2021, 26, 296–308.
2. Y. Fua, N. S. Finney, *Small-molecule fluorescent probes and their design*, RSC Adv., 2018, 29051–29061.
3. L. Rejc et al., *Design, syntheses, and in vitro evaluation of new fluorine-18 radiolabeled tau-labeling molecular probes*, J. Med. Chem., 2017, 60, 8741–8757.

Keywords: Alzheimer disease, amyloid β, diagnostic tool, fluorescent probes.

S16

NOVEL 1,3,5-TRIAZINYL AMINOBENZENESULFONAMIDES AS POTENT CARBONIC ANHYDRASE INHIBITORS

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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 pathogenic bacteria, CAs play an essential role in survival and growth. Inhibition of bacterial CAs leads to growth retardation, growth defects and makes bacteria vulnerable to host defense mechanisms. Bacterial CAs are, therefore, very promising targets in the search for new antibiotics. In humans, 15 different isoforms of CAs can be found, including two tumor-associated (hCA IX, hCA XII). Given the above, it is clear that carbonic anhydrase inhibitors can be drugs for a whole range of diseases. However, a fundamental problem is their selectivity towards a specific isoenzyme.
A series of 1,3,5-triazinyl aminobenzenesulfonamides substituted by aminoalcohol, aminostilbene, and aminochalcone structural motifs were synthesized as potential CAs inhibitors. The compounds were tested against vancomycin-resistant *Enterococcus faecalis* (VRE) isolates. To evaluate the selectivity of the compounds against bacterial CAs towards human CAs, the inhibitory activity of compounds against tumor-associated hCA IX and hCA XII, hCA VII isoenzyme present in the brain, and physiologically important hCA I and hCA II were determined. Tested compounds had only a negligible effect on physiologically important isoenzymes.
In conclusion, newly prepared compounds have great potential as antibacterial agents with high activity and, at the same time, with high selectivity for bacterial CA compared to metabolically important hCA isoenzymes (e.g., hCA I, hCA II) found in the human body.

This research was supported by grant number MUNI/G/1002/2021.

Keywords: carbonic anhydrase, inhibitors, 1,3,5-triazine, sulfonamide

S20

STRUCTURAL ANALYSIS OF DITERPENOIDS ISOLATED FROM THREE *PLECTRANTHUS S.L.* SPECIES

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The genus *Plectranthus s.l.* (Lamiaceae) consists of three distinct genera: *Coleus*, *Plectranthus s.s.*, and *Equilabium*, which can be genetically and morphologically distinguished from one another (Paton et al., 2019). Due to its significant contribution to traditional medicine, particularly in treating digestive, respiratory, genitourinary, and dermatological disorders, there has been extensive research on the phytochemical composition of *Plectranthus s.l.* All these researches have revealed that diterpenoids are the most prominent group of secondary metabolites found in these plants (Lukhoba et al., 2006).

Our current study focused on exploring the methanolic extracts derived from the aerial parts of *C. comosus*, *C. forsteri* 'Marginatus', and *P. ciliatus*. Through our investigation, we successfully isolated fourteen diterpenoids from these extracts, which belong to the abietane, *ent*-clerodane, and *ent*-kaurane classes. Notably, three of these diterpenoids were identified as new natural products, and we also re-evaluated the structure of a known diterpenoid.

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[1] Lukhoba CW, Simmonds MSJ, Paton AJ. *Plectranthus*: A review of ethnobotanical uses. J. Ethnopharmacol. 2006; 103:1-24.

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The study was supported by Grant Agency of Masaryk University (MUNI/A/1688/2020) and the NKFIH, Hungary (K-134704).

Keywords: diterpenoid, NMR, 2D NOESY, *Plectranthus*, spirocoleon

S30

OPTIMIZATION OF CAPILLARY ELECTROPHORESIS – MASS SPECTROMETRY METHOD FOR ANALYSIS OF MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAbs) are a growing group of biopharmaceuticals that are used in therapy of various types of diseases. Structurally, mAbs are large proteins that may potentially cause an immune reaction. Therefore, quality control is required to ensure mAb safety when introduced into organism during treatment. Multiple analytical techniques are used for mAb quality control including electromigration techniques such as capillary electrophoresis (CE). CE has been shown in recent years as suitable for analysis of proteins due to high efficiency separation, low operating costs, low sample and solvent consumption. CE coupled to mass spectrometry (MS) detection represents a powerful tool for analysis of proteins such as mAbs. This work is focused on optimization of CE-MS separation and detection conditions for quantitative analyses of various types of mAbs, involving infliximab and bevacizumab. It also demonstrates potential of the developed analytical method for future pharmaceutical applications.

Keywords: monoclonal antibodies, capillary electrophoresis, mass spectrometry, method optimization

S31

APPLICATION OF SPECTROSCOPIC TECHNIQUES FOR EVALUATING FUNGAL VIABILITY

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Microscopic filamentous fungi represent one of the most significant factors causing the contamination of cleanroom surfaces, pharmaceuticals and healthcare products. Filamentous fungi can produce a wide variety of enzymes capable of inducing different degradation processes, and they are known as producers of many mycotoxins and allergens.

Early detection and characterisation of contamination make it possible to apply relatively non-invasive methods for its removal, and it can provide information necessary for preventive contamination control.

With a focus on determining the viability of fungi, a few techniques are currently used, but ongoing and intense research aims to develop new, time-saving, easier, non-destructive, and non-invasive methods. In this regard, combining spectroscopic techniques and statistical data processing seems promising to fulfil these requirements.

The main goal of the research was to study the spectral properties of vital and devitalised filamentous fungi – *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Trichoderma atroviride* inoculated on the substrate in two phases – conidia and mycelium. UV-Vis-NIR and NIR Fibre Optics Reflection Spectroscopy (FORS), FTIR spectroscopy and Raman spectroscopy, each combined with the Principal Component Analysis (PCA), were set to determine whether the spectra of vital and devitalised forms of studied samples differ.

Based on the obtained results, it is possible to state that UV-Vis-NIR and NIR spectra of vital and devitalised forms of filamentous fungi generally differ, at least in one studied spectral region. FTIR spectroscopy seems to be a less sensitive method, and, in most cases, PCA could not clearly distinguish the spectra of vital and devitalised fungi. Portable Raman spectrometers were unable to detect the signal of fungi conidia. On the other hand, the spectra of viable conidia were obtained using the Raman spectrometer with microscope DXR™3.

This research received a financial contribution from the STU Grant scheme for Support of Young Researchers, the Slovak Research and Development Agency under the contract APVV, grant No. 20-0410, and the Scientific Grant Agency VEGA, grant No. 1/0602/19.

Keywords: **Filamentous fungi, Viability, Spectroscopy, Principal Component Analysis**

S34

COPPER(II) AND ZINC(II) COMPLEXES OF REDUCED SCHIFF BASES: SYNTHESIS, STRUCTURE DETERMINATION AND BIOLOGICAL ACTIVITY

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In recent years, Schiff bases and their metal complexes has been gaining more attention because of their simple preparation, wide range of biological activities, unique chemical properties and structure arrangements. Copper and zinc complexes with Schiff bases are well known for their antimicrobial, anticancer or antiradical activity. In our study, we present the preparation of four Schiff bases obtained by condensation of cyclohexane-1,2-diamine and fluorinated benzaldehydes, followed by reduction with $NaBH_4$. Reduced form of the prepared bases was used in coordination with metal salts to produce the respective complexes. The structures of the original and reduced Schiff bases as well as their metal complexes were characterised by single-crystal X-ray analysis, ^{1}H and ^{13}C-NMR, IR spectroscopy and elemental analysis. The antimicrobial activities of reduced Schiff bases and their metal complexes were evaluated in vitro against *E. coli, S. aureus,* and *C. albicans*. Some of the compounds exhibited very good antimicrobial activity. Metal complexes showed significantly higher activity compared to corresponding free ligands. This observation confirms the fact that complexation with a metal ion enhances biological activity of ligands. All compounds were evaluated for urease inhibition against jack bean urease. Antiurease activity was observed in all copper complexes.

Keywords: antimicrobial activity, metal complexes, Schiff bases, zinc, copper

S35

BIOLOGICALLY ACTIVE DECAVANADATES

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The decavanadate anion, HxV10O28(6−x)−, is the main species in vanadate solutions at concentrations above 1 mM and in the pH range 2–6. This anion itself has many biological effects, which result mainly from its ability to interact with biomacromolecules (proteins or enzymes). The coordination compounds incorporating HxV10O28(6−x)− are scarce and the conditions of their formation is unclear. Herein, we deal with a systematic study of the formation and isolation of HxV10O28(6−x)− complexes under variable conditions. Five new substances were prepared and characterized: (Hnad)2{[Co(H2O)3(nad)2]2[μ-V10O28]}·6H2O I, {[Co(H2O)4(iso-nad)2]3}V10O28·4H2O II, {[Co(H2O)4]2[Co(H2O)2(μ-pza)2][μ-V10O28]}·4H2O III, {[Co(H2O)4(μ-pza)]3V10O28·4H2O IV, (NH4)2{[Ni(H2O)4(2-hep)]2}V10O28·2H2O V, nad = nicotinamide, iso-nad = isonicotinamide, pza = pyrazinamide, 2-hep = 2-hydroxyethylpyridine. Compounds I and III are decavanadate complexes, compounds II, IV and V are complex salts with V10O286− anion. 51V NMR spectroscopy confirmed that substances I and III are stable in aqueous solutions. Although no interactions with the model proteins thaumatin, lysozyme and proteinase K was observed, in the reaction of catalytic oxidation of water, substance I achieved up to 9-fold efficiency compared to uncoordinated HxV10O28(6−x)−, producing 143.37 nmol O2, demonstrating a high cooperative effect of the decavanadate and Co(II) center. In addition, compound I is less toxic to A549 cell lines by 40% (0.05 mM) and HeLa by 26% (0.1 mM).

Keywords: decavanadate, cobalt, cytotoxicity, water oxidation catalysis, proteins.

S36

DETERMINATION OF STEROIDAL SAPONINES IN *TRIBULUS TERRESTRIS* FOOD SUPPLEMENTS BY LC-MS/MS METHOD.

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Tribulus terrestris L. (TT) is a plant used in traditional folk medicine, and sport nutrition to improve health and performance. Its positive effects on the body are mainly due to its steroidal saponin content. The content and composition of saponins vary depending on the area from which they originate. The aim of the work was to develop an LC-MS/MS method for the determination of the main steroid saponins in dietary supplements containing TT. The saponins were determined in seven dietary supplements intended for athletes. The main steroidal saponins: protodioscin, protogracillin, diosgenin, gitogenin, hecogenin, ruscogenin, and tigogenin have been specified. Protodioscin, a typical steroid saponin of the Bulgarian chemotype was found only in one sample, although its contents were labeled on several food supplements.

The other samples of tested food supplements declared a standardized extract of TT, but the determined content of saponins did not correspond to the stated data on the product. The results showed that the composition of tested food supplements did not correspond to the label, even the yohimbine (used to be prescribed as a treatment of erectile dysfunction) was detected in one product instead of steroid saponins from TT Based on the results of the analysis, it is appropriate to alert the consumer's attention to the importance of ensuring the correct choice of a nutritional supplement and its quality.

Keywords: LC-MS/MS analysis, *Tribulus terrestris L.*, food supplements, steroid saponins

POSTERS

P1

EFFECT OF SILVER IONS ON ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF EXTRACTS OBTAINED FROM *OPHIOCORDYCEPS SINENSIS*

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Mushrooms from the genus *Cordyceps* are characterized by a wide range of biological effects due to the diverse amount of substances contained in them and are used as an important source of bioactive compounds. In China, they have been used as a medicinal preparation of traditional Chinese medicine for centuries. They represent an important source of bioactive substances that are used in the treatment of various diseases. The presence of various chemical components makes them interesting also from the point of view of searching for new master structures. There are also a number of professional works dealing with the ability of substances isolated from the extracts of these mushrooms to form complexes with various metals. It is believed that the polysaccharides, which are the main component of the extracts, are involved in the complexation with metals. This improves and deepens their biological effects.

The work deals with antioxidant and antimicrobial activity. The possibility of the formation of complexes of extracts with silver ions was monitored. Extracts alone as well as extracts with added $AgNO_3$ showed antioxidant activity. Among *Ophiocordyceps sinensis* extracts without $AgNO_3$ addition, the highest antioxidant activity was shown by the sample extracted by reflux, which was cultivated on chickpea (2R), with an IC_{50} value of 6.11 $mg.ml^{-1}$. The sample obtained by reflux extraction cultured on corn (1R) had the lowest activity, with an IC_{50} value of 9.34 $mg.ml^{-1}$. The antioxidant activity was reduced by the addition of silver nitrate in some samples, on the contrary, it was increased or even manifested in some, even though the sample without $AgNO_3$ did not show it. After adding $AgNO_3$ to the extracts obtained by maceration, the highest antioxidant activity was recorded in the sample cultivated on chickpea (2M), with an IC_{50} value of 4.99 $mg.ml^{-1}$, and the lowest activity was recorded in the 2R sample, which had an IC_{50} value of 10.08 $mg.ml^{-1}$. *Ophiocordyceps sinensis* extracts showed antimicrobial activity only after adding $AgNO_3$, pure extracts did not show any effectiveness against the tested pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*). Antibacterial activity was proven only against a strain of gram-negative bacteria.

Keywords: *Ophiocordyceps sinensis*, antioxidant activity, antimicrobial activity

P2

SYNTHESIS AND STUDY OF BIOLOGICAL PROPERTIES OF NEW CARBAMATES WITH A MODIFIED BASIC FRAGMENT IN THE ARYLOXYAMINOPROPANOL CHAIN

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Drugs with a carbamate functional group are an important part of many drugs and prodrugs. Today, this functional group is part of many approved drugs that act as chemotherapeutics (mitomycin C, irinotecan), cholinesterase inhibitors in the treatment of neurodegenerative diseases (rivastigmine, neostigmine, physostigmine, pyridostigmine), human immunodeficiency virus (ritonavir, amprenavir), anticonvulsants (felbamate, retigabine), muscle relaxants (methocarbamol, metoxalone). Carbamate functional group is also part of prodrugs with different therapeutic applications (irinotecan, bambuterol, gabapentin enacarbil, capecitabine). Propranolol was the first clinically approved β-blocker introduced by James Black in 1964, and his discovery is considered a turning point in the treatment of angina pectoris and is one of the most significant points in pharmacology of the twentieth century. Since then, more than 20 new β-blockers have been patented. β-Blockers as are a specific type of drugs that are indicated for various diseases: angina pectoris, cardiac arrhythmias, atrial fibrillation, heart failure, hypertension, glaucoma, hyperthyroidism and other. Currently, the design and synthesis of drugs within the framework of disease therapy are focused on the preparation of new drugs that could influence several biological systems at the same time. Using this design, two or more pharmacophores are combined with each other within a single molecule, while this new molecule, a new drug, should have properties that are characteristic of both starting pharmacophores. These new multipotent compounds are referred to as "multi-target-directed ligands" (MTDLs).

The work deals with the synthesis of new carbamate derivatives with an aryloxyaminopropanol fragment in the molecule. By modifying the basic part of the aryloxyaminopropanol chain to benzylpiperidine and substituted benzylpiperazines, a series of new derivatives was prepared. The ether functional group was also replaced with an ester functional group in the aminopropanol chain. Selected compounds were tested for their antimicrobial and anticholinesterase activity, and their effect on β-adrenoreceptors.

Keywords: synthesis, carbamates, aryloxyaminopropanol, piperidine, piperazine

P3

SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITY OF NEWLY DESIGNED HYDROXAMATES AS POTENTIAL HDAC INHIBITORS

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Histone deacetylases (HDACs) are metalloenzymes involved in the regulation of fundamental cellular processes, such as cell cycle progression, differentiation, and tumorigenesis. The abnormal function of HDACs can induce severe human diseases, including cancer, pulmonary disease, and neurodegenerative disorders. Histone deacetylase inhibitors (HDACi) represent a relatively new generation of drugs with the ability to affect the expression of specific genes, which can repair controlled proliferation or damage apoptotic processes in tumor cells. HDAC inhibitors have considerable anticancer activity forming a complex with the Zn^{2+} ion in the catalytic pocket of enzymes.

Based on the common three-motif pharmacophore model of HDACi, we have designed a series of hydroxamate derivates. Compounds with variously substituted anilids as a capping group and hydroxamic acids as a zinc-binding group were synthesized. The antiproliferative activity of the series was investigated in the monocytic leukemia cell line THP-1 and evaluated by WTS-1 analysis. The first registered HDACi, Vorinostat®, was used as a positive control for the detection. The most important part of the study was to verify the ability of the hydroxamates to inhibit the enzymatic activity of HDAC class I and II (HDAC1 – HDAC10). Based on the previous data demonstrating the antiproliferative activity of new hydroxamic acids in THP-1 cells, for the assessment of HDAC inhibition, we selected compounds, whose antiproliferative effect after 72 h of incubation was quantitatively comparable to the effect of Vorinostat®. A series of tests confirmed that the synthesized hydroxamic acids have antiproliferative activity, an effect on the cell cycle progression, and induction of apoptosis.

The most potent inhibitors are compounds that contain methyl or bromine substituent at the para position at the aromatic ring with IC_{50} less than 1,6 μM.

Keywords: hydroxamic acid, histone deacetylase inhibitors, anticancer agents

P4

SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITIES OF COPPER(II) COMPLEXES OF LIGANDS DERIVED FROM 4-METHOXYSALICYLALDEHYDE AND β-ALANINE AND γ-AMINOBUTANOIC ACID

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In this study, a series of Cu(II) complexes of Schiff base and reduced Schiff base ligands were prepared. The Schiff bases were prepared from 4-methoxysalicylaldehyde and two short linear amino acids – β-alanine and γ-aminobutanoic acid. The Schiff bases were reduced to prepare secondary amine compounds. Both Schiff bases and their reduced analogues were used in complexation reactions using copper(II) acetate, chloride, or nitrate. The prepared 4 ligands and 8 complexes were characterized by elemental analysis and spectral methods (^{1}H and ^{13}C NMR, IR). Two of the prepared complexes were suitable for X-ray crystallographic structure determination.
DNA cleavage assay based on partial or full cleavage of plasmid DNA was used to determine antineoplastic activities of the prepared complexes. Agarose gel electrophoresis was used to separate the cleavage products. The complexes were able to cleave pDNA at 1 – 5 mM concentrations.
Cytotoxicity of the prepared complexes was studied using the Resazurin (7-hydroxy-3*H*-phenoxazin-3-one 10-oxide) model. This test is used as an oxidation-reduction indicator in cell viability assays for yeasts and mammalian cells and cytotoxicity prediction assay for cancer cells. Prepared complexes were able to affect cell survivability in 1 – 5 mM concentration within 3 hours. The prepared complexes were used in antiradical (SOD-mimetic) activity INT assay, where their superoxide anion-radical scavenging abilities were determined in 20 – 55% range, compared to the agent INT **(**2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride**)** at sub-millimolar concentrations.

This project was supported by the research grant VEGA 1/0145/20.

Keywords: copper(II) complexes, Schiff bases, SOD-mimetic activity, cytotoxic activity, DNA cleavage

P5

A STUDY OF THE MICELLIZATION OF CARBISOCAINIUM CHLORIDE IN AQUEOUS SOLUTION USING THE OPTICAL DENSITY METHOD

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The critical micellar concentration (CMC) of carbisocainium chloride in an aqueous solution was determined by measuring the optical density on 96-well microtitre plates. The measurement was carried out using the Epoch 2 Microplate Reader in distilled water at temperatures T = 294,15 – 313,15 K. CMC values were determined by the dependence of the optical density (*OD*) on the concentration (*c*) of carbisocainium chloride. The thermodynamic parameters of micellization, molar Gibbs energy ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$), were calculated according to the pseudophase separation model and subsequently analyzed. In the measured temperature range, the contribution of enthalpy increased with increasing temperature, and the contribution of entropy, on the contrary, decreased. It was also found that temperature does not affect $\Delta G°$ and at the same time, the effect of temperature on $\Delta H°$ was more significant. This means that as the temperature increases, the micellization process becomes more exothermic. Finally, the enthalpy-entropy compensation was also determined, which showed a linear course. The value of the compensation temperature (*Tc*) was 304,16 ± 1,51 K.

Keywords: critical micelle concentration, thermodynamics, optical density

P6

ANTIPROLIFERATIVE METAL COMPLEXES OF THE FLUORINATED CURCUMIN DERIVATIVE: 1,7-BIS(4-FLUOROPHENYL)HEPTA-1,6-DIENE-3,5-DIONE

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Curcumin and its derivatives have found in the last decades multiple applications in various fields of our daily life. This natural antioxidant compound can be found not only in the food industry and cosmetics but also in the pharmaceutical industry as a potential remedy for multiple diseases. However, curcumin and its derivatives have an inconvenient, poor bioavailability and fast metabolism, which induces the formation of several metabolites and so forth the poor bioactivity in the human body. Therefore, the derivatization of curcuminoids or the coordination to metal centers constitutes a solution for keeping the molecules of aromatic β-dicarbonyl compounds not decomposed. In this way, the curcuminoids are capable to reach biological targets and develop their antioxidant, anti-inflammatory, immunomodulatory, anticancer, etc. activities.

Our work was focused on the preparation and structural characterization of transition metal complexes of a mono-fluorinated curcumin derivative. Palladium and ruthenium complexes have been synthesized and biologically tested *in vitro* on human cancer cell lines and normal healthy cells, using the MTT assay. Palladium complexes were tested on human colorectal adenocarcinomas DLD-1 and RKO, and normal colorectal cells CCD-18. The ruthenium complexes were tested on adenocarcinoma human alveolar basal epithelial cells A549, human liver cancer cells HepG2, and lung fibroblast normal cell line HEL299. The biological activity was expressed by the determination of cytotoxicity (IC_{50} values) of synthesized complexes towards the mentioned cancer cell lines. Both categories of complexes show a significant cytotoxicity, over the values shown by classical chemotherapeutic drug CisPt. Moreover, in the case of palladium complexes was proved a fold higher activity as the free ligand displayed in the same biological assay. These preliminary results, open a perspective for us to search further for optimization of the structure and activity of curcumin derivatives.

This work was supported by the research grant VEGA 1/0145/20.

Keywords: fluorinated curcuminoid, palladium complexes, ruthenium complexes, cytotoxicity, antiproliferative activity

P8B

THROMBOLYSIS *IN VIVO*

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This innovative experimental work focuses on the development of targeted thrombolytics combinations for the diagnosis and therapy of cerebral infarction, their preparation and testing in a rat animal model. The effect of the addition of plasminogen to the standard recombinant alteplase in the treatment of cerebral infarction was tested. A rat model of systemic embolism was used for this purpose. The design of the model is set to simulate as much as possible the course of thrombolysis in the human organism and the implementation of preclinical research into clinical practice is realistic.

This work was supported by the Ministry of Health of the Czech Republic, grant nr. NU21-08-00510.

Keywords: thromboembolism, micro-fluoroscopy, ischemic stroke, limb ischemia, alteplase

P9

DNA CLEAVAGE AND CYTOTOXIC ACTIVITY OF COPPER (II) COMPLEXES BASED ON REDUCED SCHIFF BASES DERIVED FROM SALICYLALDEHYDE AND AMINO ACIDS.

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Metal complexes, which under physiological conditions have antioxidant activity and have the ability to bind and cleave the DNA chain are important for their use as antineoplastic drugs. We synthesized ligands derived from short chain amino acids and from salicylaldehyde. The prepared ligands of the type of reduced Schiff bases were subsequently used for the preparation of copper(II) complexes. The aim of the study was testing of copper(II) complexes *in vitro*, for their capability of cleaving DNA structure. Their cytotoxic activity was also confirmed on *S. cerevisiae* by the resazurin redox method which is based on preserved healthy mitochondrial function.

This study was supported by the Grant VEGA 1/0145/20.

Keywords: copper(II) complexes, anticancer drugs, DNA cleavage activity, cytotoxicity

P11

ASSESSMENT OF LIPOPHILICITY OF NEWLY SYNTHESIZED CARBONIC ANHYDRASE INHIBITORS USING REVERSED-PHASE HPLC AND SCHRÖDINGER COMPUTATIONAL PLATFORM

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A series of 24 potential carbonic anhydrase inhibitors useful for glaucoma therapy has been synthesized. The structure of the studied molecules contains a sulfonamide skeleton typical for carbonic anhydrase inhibitors (brinzolamide, dorzolamide) and also an aryloxymaninopropanol chain present in antiglaucoma active beta blockers (timolol, betaxolol). The lipophilicity of newly synthesized substances was assessed using the RP-HPLC method and QikProp modul of Schrödinger computational platform. Chromatographic measurements were performed on a Dionex UltiMate 3000 Series UHPLC System using a Symmetry® C18 5 µm, 4,6 x 250 mm column. 10 µl of a methanolic solution of the substance with an approximate concentration of 0,1 mg/ml was injected. The flow rate of the mobile phase was 1 ml/min. The column temperature was maintained at 40 °C. A wavelength close to the absorption maximum of the studied substances (224 nm) was chosen for detection. The analysis was performed in five or six methanol/water mobile phases with volume ratios of 90:10, 85:15, 80:20, 75:25, 70:30, 65:35 (*V*/*V*). The measurement of each substance in each mobile phase was performed three times. Methanolic solution of potassium iodide was a dead time marker. Based on the retention times of studied substances (t_R) and dead time (t_0), the logarithms of the capacity factors $\log k = \log((t_R - t_0)/t_0)$ were calculated. The linear dependence of the $\log k$ values on the methanol content in the mobile phase was extrapolated to zero methanol content in the mobile phase. The $\log k_w$ value was thus obtained, which is used as a lipophilicity parameter corresponding to the aqueous environment. Subsequently, all experimentally determined $\log k_w$ values were compared with partition coefficients ($\log P$) obtained by computational method, and the relationship between lipophilicity and the structure of the studied substances was evaluated.

Keywords: lipophilicity, RP-HPLC, Schrödinger platform, carbonic anhydrase inhibitors

P12

NEW FLUOROPHORES FOR THE DETECTION OF GLYCANS

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Changes in the composition of glycans correlate with the progression of many diseases, therefore they are investigated as disease markers. On the other hand, their analysis is complicated because they do not contain a chromophore or charges that would enable their electrophoretic separation. Our goal was to develop a new fluorescent marker covalently bound to glycans providing fast labelling kinetics, high quantum yield, increased detection sensitivity using MS, and at the same time, carrying a charge in the structure, which will enable the studied glycans to be separated electrophoretically.

The basic skeleton of this fluorophore is based on pyrrole and indolizine. The synthesis was based on two building blocks, synthon A and synthon B, which were subsequently connected. The plan was to prepare variants of the fluorophore carrying electron-donating or electron-accepting groups. These substitutions allow for variability in the absorption maximum wavelength and, thus, fluorescence wavelength. The molecule also holds a trimethylammonium functional group responsible for electrophoretic mobility and a propanoate chain, which is necessary to attach the fluorophore to the glycan.

The prepared substances will also be characterized in cooperation with the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic. The usability of the newly developed fluorophores will be demonstrated by profiling glycoproteins associated with breast cancer.

This work was supported by the Grant Agency of the Czech Republic [grant number 22-00236S].

Keywords: fluorophore, pyrrole, glycan

P13

Cu(II) COMPLEXES OF QUINAZOLINONE DONOR LIGANDS AS POTENTIAL ANTICANCER AGENTS

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Transition metals play a fundamental role in the chemistry of life. In trace amounts, they enable a number of selective catalytic conversions necessary to sustain biological processes. Excessive intake of these elements can cause various toxicological effects including carcinogenesis. However, it is this toxicological potential that forms the basis of transition metal-based anticancer therapy. Metal-based drugs are gaining more and more importance in modern medicine, especially in the field of oncology. The well-known platinum-based compounds are widely used in the cancer treatment. However, high toxicity and significant side effects are often observed. Current efforts are therefore directed towards metal-based compounds with lower toxicity and novel mechanisms of action.

In the search for new anticancer agents, three Cu(II) complexes based on quinazolinone Schiff bases were synthesized. The antiproliferative activity of *O,N,O*-quinazolinone donor ligands and their Cu(II) complexes towards human cancer cell lines (Caki-1, HepG2, HT-29) was examined. Experimental data revealed that studied compounds possess substantial biological activity. Free quinazolinone ligands showed higher antioxidant effect and DNA-protective ability in comparison with their Cu(II) complexes. On the other side Cu(II) complexes exhibited significant anticancer activity in all tested cell lines. These findings confirm the considerable impact of complexation on bioactivity, and suggest that Cu(II) complexes represent model structures for the development of promising anticancer metallodrugs.

This work was financially supported by Slovak scientific grant agency, VEGA 2/0071/22 and 1/0429/21.

Keywords: Cu(II) complexes, quinazolinone ligands, cytotoxicity, cancer cell lines

P14

PHYTOCHEMICAL ANALYSIS OF THE WATER-ETHANOL ROOT EXTRACT OF *DIPSACUS FULLONUM* L.

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Dipsacus fullonum L., a flowering plant from the family Caprifoliaceae, is used in traditional folk medicine, among others, as a supportive treatment for Lyme disease. Its antioxidant and antibacterial effects were confirmed experimentally in vitro. The aim of this work was to identify secondary metabolites extracted in solution ethanol/water in a ratio of 70/30 (V/V), prepared via ultrasonication, from the root of *D. fullonum L.* growing in Slovakia. LC-MS was used for secondary metabolites identification, and HPLC-DAD for their subsequent quantification by the method of external standards. Iridoid structures identified in the extract were: derivatives of loganic acid and secoiridoids cantleyoside and sylvestroside III and IV, respectively, which are mutual isomers. Anyway, by this method, it was impossible to identify which of these two isomers is present in the analysed extract. In addition, the extract contained a derivative of sylvestroside III/IV - possibly sylvestroside III/IV dimethyl acetal. Other identified secondary metabolites are derivatives of caffeic acid: two hexosides of caffeic acid, three isomers of caffeoylquinic acid, and three isomers of caffeoylquinic acid, the exact structure of which could not be outrightly confirmed by this method. By quantification, we found that the most represented substances in the prepared extract were the bis-iridoids cantleyoside and sylvestroside III/IV. Of the polyphenols, the most represented molecules were dicaffeoylquinic acid isomers. Identified iridoids and dicaffeoylquinic acid derivatives are suggested as the antioxidant and antimicrobial active compounds in *D. fullonum* root.

This work was supported by grants VEGA 1/0284/20, APVV-19-0056, and APVV-15-0123.

Keywords: *Dipsacus fullonum*, LC-MS, sylvestroside, cantleyoside

P17

GREEN TEA AND PEPPERMINT IN MIXTURE SYNERGISTICALLY ENHANCE THEIR ANTIOXIDANT CAPACITY

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Especially in the case of medicinal plants, analysis of their individual constituents does not provide a satisfactory explanation of their effectiveness. In mixtures, such as medicinal plants or their extracts, the substances finally interact among themselves, which can lead to an increase in their efficacy through synergy, where the final response is higher than the expected one, based on summation of the partial effects. Green tea and peppermint tea are favourite beverages commonly prepared as water infusion from Camellia sinensis Kuntze and Mentha × piperita L. leaves. In addition, they are a rich source of polyphenols, natural compounds with great antioxidant capacity, which can be used in food stabilization or in processes, where quenching of free radicals are desired. In this work we have studied an antioxidant activity of green tea and peppermint lyophilizates in combinations. Diverse mixtures were prepared before and after lyophilization in various extract ratios. In addition we arranged the equimolar mixtures of polyphenols present in the peppermint tea: rosmarinic acid and in green tea: epigallocatechin gallate and quercetin, respectively. Antioxidant activity of single lyophilizates and compounds and their mixtures as well were measured using in vitro DPPH radical quenching assay and in the DCF cell-based antioxidant assay. The quantification of interaction as a synergism or antagonism was done by the general Median effect equation according to Chou. Interaction analysis has shown mainly synergy in lyophilizates and compounds mixtures in both DPPH assay as well as in the DCF cell-based antioxidant assay. Synergy among polyphenols from lyophilizates can partly explain the interactions of the lyophilizates on chemical basement. In conclusion, our study confirmed the old practice of combining the medicinal plants into herbal tea mixtures, where they reach higher effects, than is the assumed.

This work was supported by grants VEGA 1/0284/20, VEGA 1/0226/22, APVV-19-0056.

Keywords: synergy, green tea, peppermint, antioxidant, polyphenol

P18

PHOTOCHEMICAL TRANSFORMATIONS OF PYRAZOLONE-TYPE SUBSTRATES

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In recent years, photochemical transformations have gained popularity in organic synthesis. Since most organic molecules cannot absorb visible light, most photochemical reactions require the use of an external photocatalyst, metal-based photocatalysts being the most prevalent. Because of their expensive nature and occasionally complex preparation, it is difficult to achieve large-scale syntheses in the industrial setting. Additionally, metal-based photocatalyst represent a big environmental and economic issue, making the development of novel visible-light transformations in the absence of an external photocatalyst an important challenge.

This study presents a method for the visible-light transformation of pyrazolone-type substrates, without the need for an external photocatalyst. These systems are of important biological value because of their common use as peptidomimetics. The irradiation of the selected compounds under different reaction conditions affords a wide array of final products. Among them products containing 4- and 7-membered rings respectively are achieved. Such scaffolds are challenging to synthesize using the traditional chemistry but are exceptionally desirable in medicinal chemistry due to their unique structural and biological properties. Our method is compatible with various functional groups and does not require an external photocatalyst, which makes it a cost-effective and environmentally friendly solution for obtaining these potentially valuable products.

Keywords: photochemical transformations, visible-light, bond cleavage, pyrazolone-type compounds

P19

SCHIFF BASES AS POTENTIAL THERAPEUTIC AND ANTICANCER AGENTS

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Basic thiosemicarbazone and semicarbazone derivatives of acetophenone with imine functional group belong to the group commonly called Schiff bases. Therapeutics and compounds belonging to this group are versatile pharmacophores with a significant capability of forming chelates with various metal ions. Such metal complexes play an important role in therapeutics due to their remarkable broad-spectrum of biological activities.

Due to the above-mentioned complex formation, many Schiff bases appear as an important intermediate in a number of enzymatic reactions. One of the possible target enzyme is a neutral zinc-binding metalloenzyme aminopeptidase N (AP-N), also called membrane alanyl aminopeptidase. Potential inhibitors of this omnipresent enzyme may offer effective and broad-spectrum therapy.

Through three-step synthesis it is possible to obtain three arene substitution isomers of basic thiosemicarbazone and semicarbazone derivatives of acetophenone. The initial step of the synthesis is the chloroacetylation of aminoacetophenone, followed by substitution with a secondary amine. Symmetrical secondary amines and heterocyclic amines with saturated heterocyclic skeleton were used for substitution. Synthesized compounds with the best half maximal inhibitory concentration against the enzyme AP-N underwent testing for inhibition of cell proliferation on the three different cell lines. A simple QSAR model describing the dependence between the inhibitory activity expressed as IC_{50} and the descriptors derived from the chemical structure was established.

Keywords: Schiff bases, basic acetophenone derivative, aminopeptidase N, metalloenzyme inhibitor

P21

THE EFFECT OF 5-FLUOROURACIL AND LACTOBACILLI TREATMENT ON THE MODEL OF THE INTESTINAL BARRIER *IN VITRO*

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Caco-2 cell line, a type 1 human intestinal epithelial cell, exhibits spontaneous differentiation and polarization, forming distinct surfaces and structures. We used this cell line as a model to study intestinal mucositis induced by the anticancer and antimetabolic drug 5-fluorouracil (5-FU). We monitored changes in the relative gene expression of enterocyte differentiation markers aminopeptidase (ANPEP), tight junction proteins claudin-1 and occludin (CLDN1, OCLN), and citrate synthase as a marker of intermediary metabolism. We also tested the potential of modulating mucositis through co-cultivation with *Limosilactobacillus reuteri* E.

Caco-2 cells were cultured at 37 °C, 5% CO_2 atmosphere in RPMI medium. Subsequently, cells ($3.85x10^5$ cells) were transferred onto 12-well hanging inserts. The experiment lasted for 15 days, and samples were divided into five groups:

The "time 0" group was cultured for 24 hours. Subsequently, total RNA was isolated from the samples and transcribed into cDNA.

On the 14th day, 5-FU (100 μmol/l) was added to induce mucositis in two groups (5-FU and LRE+5-FU). Lactobacilli were added to the LRE and LRE+5-FU groups on the 15th day for six hours. As control samples were used cultures without treatment. Total RNAs were isolated, transcribed into cDNA, and used for gene expression analysis.

The differentiation of Caco-2 cells into enterocytes was confirmed by increasing trans-epithelial electrical resistance (more than 10-fold between day 0 and 15) and increasing expressions of occludin (12-fold), claudin-1 (64-fold), and aminopeptidase (23-fold).

The addition of 5-FU resulted in decreased gene expression of tight junction proteins compared to the control group (CLDN1 by 96%, OCLN by 60%). Similarly, ANPEP and citrate synthase showed reductions of 90% and 70%, respectively. Administration of lactobacilli improved three parameters, with expression levels of CLDN1 reaching 40% of the control, ANPEP at 25%, and citrate synthase at 60%.

This study was supported by grants FaF/22/2023 and VEGA1/0429/21.

Keywords: caco-2, 5-Fluorouracil, *Limosilactobacillus reuteri* E, intestinal mucositis, tight junctions proteins

P22

TOWARDS SUSTAINABLE CHEMISTRY: RECOVERABLE CATALYSTS FOR DEUTERIUM-LABELED ORGANIC COMPOUNDS

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Deuterium (D)-labelled organic compounds find widespread applications in many areas including drugs and their analysis.[1,2] To synthesise D-labelled compounds, catalysts are commonly used due to their regio- and stereo-selectivity, mild reaction conditions, and broad substrate compatibility.[3] However, the reliance on costly catalysts containing precious metals such as Ir, Pd, and Pt poses sustainability challenges while the recovery of homogeneous small-molecule catalysts from reaction mixtures is costly and difficult.

Our ongoing research focuses on developing immobilised recoverable and recyclable catalysts to synthesise D-labelled organic compounds. These basically consist of existing catalysts covalently attached to macromolecules such as polymers which can easily be recovered from reaction mixtures. Utilising immobilised catalysts can offer several advantages, such as minimising metal contamination in products / waste streams and facilitating the efficient recovery and reusability of catalysts.[4] These benefits ultimately contribute to cost reduction in synthesis. The transition to recyclable catalysts represents a significant step towards achieving Circular Chemistry, an environmentally sustainable approach that emphasises the cyclic reuse of catalysts.[5]

I will present preliminary results from research done in our labs, highlighting significant results and potential implications of the work.

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Keywords: H/D exchange, recyclable, circular chemistry, catalysis, isotopic labelling

P23

1,2,3-TRIAZOLIUM SALTS: THE PATH TO MESOIONIC N-HETEROCYCLIC OLEFINS

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1,3,4-Trisubstituted-1*H*-1,2,3-triazolium salts, which upon deprotonation give access to mesoionic N-heterocyclic carbenes (MIC), are a widely investigated class of compounds. Because of MICs unique electronic properties, they quickly found success in the field of (organo)catalysis. Similarly, 1,3,4,5-tetrasubstituted-1*H*-1,2,3-triazolium salts upon deprotonation yield mesoionic N-heterocyclic olefins (mNHOs)[1], which raised our interest as a new class of carbon-based ligands. mNHOs were first described by Hansmann in 2020[1] and so far, limited examples have been reported. They are stronger donors than N-heterocyclic carbene ligands but form weaker metal-ligand bonds.[1] To date only three rhodium complexes with different mNHOs have been described.[1] Herein we present the synthesis of 1,3,4,5-tetrasubstituted-1*H*-1,2,3-triazolium salts and their corresponding mNHOs. A series of differently substituted 1,2,3-triazolium salts was designed to explore the coordinating ability of mNHOs to transition metals and the effects of different substituents on coordination as well as properties of potential mNHO-transition metal complexes.

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Keywords: triazolium salts, mesoionic N-heterocyclic olefins, coordination

P25

STUDY OF THE PROTECTIVE EFFECT OF MONOPHOSPHORYL LIPID A ON UVC IRRADIATED THP-1 MONOCYTES

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The experiment aims to observe the potential protective effect of monophosphoryl lipid A (MLA - vaccine adjuvant, enhancer of the immune response) at a 1.0 µg/ml concentration on cells exposed to stressors. We observed the effect of MLA on non-irradiated and irradiated samples of THP-1 cells (source: human leukaemia monocytic cell line). The basis of the experiment was to study the effect of different doses of radiation on the vitality and biological activities of the THP-1 cells at values of 64, 318, 636, and 954 J/m^2. Due to the expected weakened defensive capabilities of THP-1 cells corresponding to samples after exposure to UVC in the doses, we selected a dose of UVC radiation of 64 J/m^2 to induce stress in THP-1 cells in the main part of the experiment. The effect of bio-active MLA was followed on samples without UVC irradiation and samples exposed to UVC after one-hour pre-incubation. Thereafter, the samples were incubated for 18 more hours in the atmosphere of CO_2. The effect of MLA compounds on reductase activity, production of superoxide radicals, nonspecific immune response in case of phagocytosis and the changes in the activity of cell antioxidant defence enzyme - catalase were observed. Based on the results, finally, we can evaluate that MLA as a well-known TLR4 receptor agonist can protect the biological activities in cells irradiated with UVC radiation at a dose of 64 J/m^2.

The study was supported by VEGA 1/0429/21.

Keywords: MLA, UVC radiation, stressor, THP-1 cells, biological activities

P26

CHARACTERIZATION OF GENES RELATED TO PROBIOTIC PROPERTIES OF *LIMOSILACTOBACILLUS REUTERI* E BY *IN SILICO* ANALYSIS

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Nowadays whole genome sequencing become more and more incorporated into routine analysis, identification, and characterization of bacteria. Next generation sequencing methods, based on massive parallel sequencing of amplified DNA fragments become conventional. On the base of whole genome sequence can be predicted the health beneficial properties usable in selection of potential probiotic strains. Lactobacilli belong to the most studied probiotic bacteria. The promising potentially probiotic strain *Limosilactobacillus reuteri* E was subjected to whole genome sequencing by the Illumina Next seq 2x150 bp method (genome length 1,902.828 bp) and selected for *in silico* analysis. We focused on the *pdu-cob-cbi-hem* and exopolysaccharide (Eps) gene clusters. For *in silico* analysis the web tools Rapid Annotation using Subsystem Technology (RAST), Bacterial and Viral Bioinformatic Resource Center (BV-BRC), and Basic Local Alignment Search Tool (BLAST) were used.

Using RAST 31% of *L. reuteri* E genes were subjected to function and divided to subsystems. The most represented gene function covers metabolism of amino acids (106), carbohydrates (104), and proteins (103). Gene products of the *pdu-cob-cbi-hem* cluster are involved in the synthesis of the potent antimicrobial agent reuterin. This cluster was located on contig 5 (under accession JAHQZV010000005) and assume *L. reuteri* E to produce reuterin.

Genes responsible for exopolysaccharide synthesis, attaching of monosaccharide units and their linking together by glycosylic bounds were identified in *L. reuteri* E. They were located on contigs 47 (exopolysaccharide biosynthesis glycosyltransferase EpsF (EC 2.4.1.-) and 57 (tyrosine-protein kinase EpsD (EC 2.7.10.2); tyrosine-protein kinase transmembrane modulator EpsC; and cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2). Usually, they are closely attached to the bacterial surface or are released into surrounding environment. They are important for immunomodulatory, antitumour and antioxidative activity.

This work was supported by VEGA 1/0429/21.

Keywords: whole genome sequence, probiotics, lactobacilli, reuterin, exopolysaccharides

P28

EFFECT OF AIR OXIDATION ON THE PHASE TRANSITION OF PULMONARY SURFACTANT

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The pulmonary surfactant is a mixture of lipids and proteins that spreads into a film at the air-lung interface. Its main function is to reduce surface tension at the air-liquid interface in the alveoli. This reduces the work of breathing and prevents alveolar collapse at expiration. Oxidative processes in the lungs, which are highly increased during inflammatory processes or by exposure to some air pollutants, can cause inactivation of pulmonary surfactant function. The effect of oxidation on the function of pulmonary surfactant can be attributed to oxidative alterations of proteins and to the peroxidation and hydrolysis of phospholipids.

In this work, we used differential scanning calorimetry (DSC) to study the effect of air oxidation of commercially available porcine pulmonary surfactant Curosuf (PSur) on its phase transition temperature (T_m) based on the length of exposure of the PSur to the air. During the oxidation process, we kept PSur samples incubated at 37°C and continuously stirred for time periods of 1-14 days. The unoxidized Psur measured right after the opening of the original package had phase transition temperature T_m= 27.44 °C. The first significant changes appeared after 3 days from the start of oxidation, when we observed, in addition to the unoxidized fraction, a separated fraction presumably consisted of oxidized lipids with a slightly higher phase transition temperature (T_m=29.36 °C). As the oxidation time increased, the proportion of oxidized lipids increased, as did the phase transition temperature of the oxidized fraction. After 14 days of oxidation, the PSur sample contained only 1 fraction of lipids with the large shift of T_m to 44.5 °C, and we assume that all unsaturated lipids have been already oxidized. The phase transition after 14 days also increased significantly increased to 23.4 J/g, while for the first 7 days of oxidation, the enthalpy remained relatively unchanged at 18 J/g. Our experiment proved that oxidation of pulmonary surfactant significantly affects its phase transition. In our experiments, the oxidation of lipids took a relatively long time, but this process can be accelerated by the presence of free radicals that are present *in vivo* during inflammation.

This work was supported by grants VEGA 1/0223/20, APVV 17-0250 and JINR project 04-4-1142-2021/2025

Keywords: pulmonary surfactant, oxidation, differential scanning calorimetry

P29

CREATION AND OPTIMIZATION OF THE *IN VITRO* MODEL FOR STUDYING INFLAMMATION-DRIVEN CHANGES OF LIPID METABOLISM IN HEPATIC CELL LINE

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Besides direct immunological function, inflammation is associated with complex changes in organism such as quantitative and qualitative alternations in lipid metabolism (decreased HDL, LDL levels, pro-inflammatory remodelling of HDL, disrupted cholesterol efflux, increased LDL oxidation, etc.). These alternations may explain increased cardiovascular morbidity in patients with chronic inflammatory diseases. The research in this area is important, however, there are only a few reliable models for studying inflammatory changes in lipid metabolism recently. Therefore, we decided to create and analyze *in vitro* model of inflammation-driven changes in hepatic cell line HepG2 induced by conditioned media (CM) from THP-1 monocytes exposed to different inflammatory stimuli: lipopolysaccharide (LPS) for 8 hours, Phorbol-12-myristate-13-acetate (PMA) for 24 hours or their combination (PMA for 24 hours, LPS 4 hours). After stimulation, the medium in THP-1 cells was replaced with serum-free RPMI, which was collected after 24 hours and added to HepG2 cells. The effect of CM from differently stimulated THP-1, variable exposure times, and CM dilutions on mRNA expression in HepG2 was tested by quantitative real-time PCR. According to our results, the most suitable was the stimulation of HepG2 cells with CM from THP-1 treated with a combination of PMA and LPS diluted in a ratio 1:3. While 4-hours long exposure of HepG2 to CM influenced mainly mRNA expression of inflammatory genes and some transcription factors (increased IL-β, NF-κB, SAA, decreased PPARα expression), the longer exposures (20/24 hours) were associated mainly with changes in lipid metabolism-associated genes (relative decrease of ApoAI, PON1, ABCA1, apoC3 expressions when compared to CM-unexposed controls at the same time points).

The study was supported by UK/162/2023 and VEGA 1/0429/21.

Keywords: inflammation, lipid metabolism, HepG2

P32

SIMPLE AND SENSITIVE ANALYSIS OF CLENBUTEROL IN URINE MATRICES BY UHPLC–MS/MS METHOD WITH ONLINE-SPE SAMPLE PREPARATION

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Clenbuterol is one of the most misused anabolic agents in professional sports. Therefore, the monitoring of clenbuterol in body fluids such as human urine is related to the development of rapid, selective and sensitive analytical methods that produce reliable results. In this work, these requirements were met by a two-dimensional separation method based on online solid-phase extraction coupled with ultra-high performance liquid chromatography–tandem mass spectrometry (SPE–UHPLC–MS/MS). The developed method provides favorable performance parameters, and it is characterized by minimum manual steps (only dilution and the addition of an internal standard) in the sample preparation. A limit of quantification (LOQ) of 0.1 ng/mL, excellent linearity (0.9999), remarkable precision (1.26% to 8.99%) and high accuracy (93.1% to 98.7%) were achieved. From a practical point of view, the analytical performance of the validated SPE–UHPLC–MS/MS method was demonstrated on blinded spiked urine samples from ten healthy volunteers. The estimated concentrations of clenbuterol were in accordance with their corresponding nominal values, as supported by the precision and accuracy data (relative standard deviation ≤5.4%, relative error ≤11%). The fulfilment of the World Anti-Doping Agency's screening and confirmation criteria indicates that the proposed method is suitable for implementation in routine use in toxicologic and antidoping laboratories. Due to its high orthogonality and separation efficiency, the SPE–UHPLC–MS/MS method should also be easily adapted to the separation of structurally related compounds (such as clenbuterol metabolites). Thus, future antidoping applications could also include monitoring of clenbuterol metabolites, providing a longer detection widow.

Keywords: clenbuterol, ultra-high performance liquid chromatography, tandem mass spectrometry, online SPE extraction, antidoping analysis

P33

SYNTHESIS OF ANTIBACTERIAL CINNAMAMIDES

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Nature is an inspiring and rich source of compounds with biological activity. Most of the drugs approved for clinical use in the last 30 years have been small molecules either directly of natural origin or structural analogues of natural products. Many plant secondary metabolites show biological activity and serve as templates for research and development of synthetic analogues. One such example is cinnamic acid and its derivatives, such as coumaric, ferulic, caffeic, or sinapic acid, which can be isolated from many plant sources. Cinnamic acid and its derivatives have shown interesting antimicrobial, antiproliferative, antiparasitic, neurological and anti-inflammatory activity.

Substituted *N*-phenyl amides of 3,4-dichlorocinnamic acid have been the subject of earlier studies. Some derivatives have shown excellent antimicrobial and antimalarial activity. The newly synthesized compounds that are the subject of this study are direct structural analogues differing in phenyl substitution. 40 derivatives with multiple substitution of both electron-donating and electron-accepting groups were synthesized and characterized, and the antimicrobial activity against Gram-positive bacteria of the genus *Staphylococcus* was determined. Some derivatives shown excellent activity comparable to the clinically used antibiotics ampicillin and ciprofloxacin.

The work was supported by project MUNI/A/1210/2022.

Keywords: cinnamamide, synthesis, antibacterial activity

P37

SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF QUATERNARY AMMONIUM HOMOCHIRAL SULFONAMIDES

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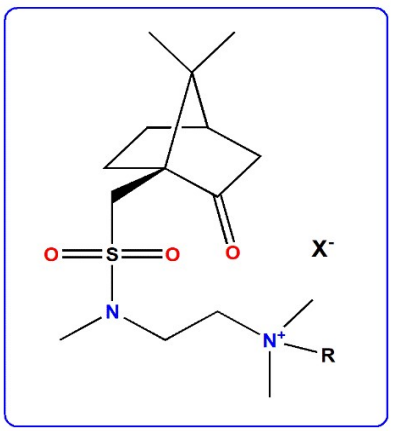
The strong bactericidal activity of quaternary ammonium salts (QUATs) with long alkyl chains have been known from 1915 and studied further on a broad range of microorganisms such as bacteria (both G+ and G-) and fungi, certain viruses and even anticancer agents. Development of resistance in microorganisms towards disinfectants or antibiotics brings the necessity to supply recently applied antimicrobial agents by new, potent and safe ones and thus search for new and effective molecules goes on. The well-known antibacterial effect of essential oils containing bicyclical camphor or borneol brought us to the idea to design and synthesize QUATs bearing hydrophobic camphor derived sulfonamides, with biologically active contra anions hoping that incorporation of more important antimicrobial active structures in one compound will improve their bioactivity. The introduction of an ester group into the molecule contributes to better biodegradability of such compounds. A group of homochiral quaternary ammonium sulfonamides bearing hydrophobic camphor derived moieties were synthesized and characterized. The described synthetic procedure is quick and efficient. The novel quaternary ammonium bromides, cinnamates and gallates were tested as antimicrobial and antifungal agents.



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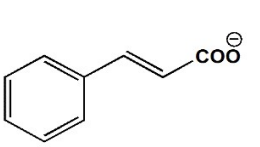

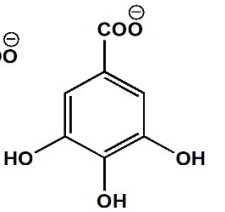

R = $-C_{10}H_{21}$, $-C_{12}H_{25}$, $-C_{14}H_{29}$, $-C_{16}H_{33}$

$-CH_2COOC_{10}H_{21}$, $-CH_2COOC_{12}H_{25}$
$-CH_2COOC_{14}H_{29}$, $-CH_2COOC_{16}H_{33}$

Key words: quaternary ammonium salts, antimicrobial, camphor sulfonamide