UNIVERSITAS CAROLINA PRAGENSIS

FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

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ABSTRACTS

11th POSTGRADUATE AND POSTDOC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ, CHARLES UNIVERSITY, HRADEC KRÁLOVÉ, 27–28 JANUARY 2021

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

SUPRAMOLECULAR INTERACTION OF TETRAPYRAZINO-PORPHYRAZINES

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Tetrapyrazinoporphyrazines (TPyzPzs), members of the phthalocyanine family, are synthetic planar macromolecules. TPyzPzs have remarkable spectral properties due to their system of aromatic double bonds. These macromolecules can absorb light between 300–700 nm, and afterward, they could release the absorbed energy by emitting fluorescence. Alkylamino substituted TPyzPzs are an interesting subtype of TPyzPzs, changing their spectral properties due to supramolecular interaction. The supramolecular interaction lays in stacking two molecules to each other – J-dimer formation (Fig. 1). The mono-

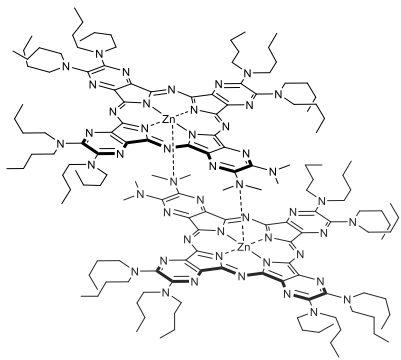

Fig. 1. J-type of aggregate

meric alkylamino TPyzPzs have only weak fluorescence because the fast relaxing process quenches it.[1] On the other hand, dimeric alkylamino TPyzPzs have good fluorescence with a significant bathmotropic shift. Aggregates could be dissolved by the addition of coordination solvent (*e.g.*, pyridine, *N*-methylimidazole), which caused changes in absorption spectra (Fig. 2).[2] This project studied the background for possible further application of alkylamino TPyzPzs.

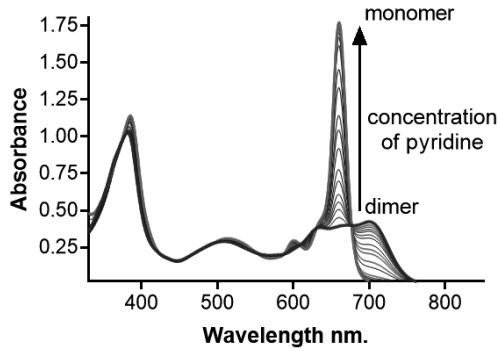

Fig. 2. Changes in the absorption spectra

The study was supported by the Czech Science Foundation (Project No. 17-19094S), by the Grant Agency of Charles University (Project No. 1168217) and from the project of Specific Academic Research (SVV 260 547).

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ANIONIC VERSUS CATIONIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY: WHAT A DIFFERENCE THE CHARGE MAKES

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Phthalocyanines (Pcs) and their aza-analogues represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area over 600 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. The literature reports on cationic and anionic Pcs for photodynamic therapy suggest systematically significant differences in their photodynamic activity.

This project was focused on the series of anionic and cationic zinc(II) Pcs and investigated their photophysical, physicochemical, binding and biological properties with the aim of finding the parameters and/or factors that may contribute to the substantial difference in photodynamic activity between Pcs bearing opposite charges on peripheral substituents. Four different sets of compounds were introduced into the study, namely anionic hydrophilic, cationic hydrophilic, anionic amphiphilic and cationic amphiphilic to compare both the influence of the charge type and its distribution on the macrocycle core. All Pcs were tested on photodynamic activity *in vitro* on HeLa cells with different activity for anionic Pcs (EC_{50} ~ 0.3–10 μM) and cationic Pcs (EC_{50} ~ 3–50 nM). The effect of pH, binding to serum proteins, interaction with biomembranes, subcellular localization and relocalization after irradiation were disclosed to be the main factors responsible for lower photoactivity of anionic Pcs.[1]

The work was supported by the Czech Science Foundation (Project No. 19-14758Y), by the Charles University Project PRIMUS/20/SCI/013 and from the project of Specific Academic Research (SVV 260 547).

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PREPARATION OF NEW MELTING TEMPERATURE MODIFIERS

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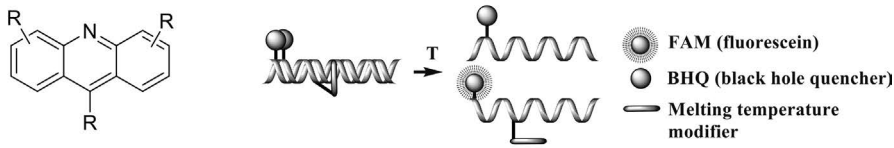
Melting temperature difference (ΔTm) between complementary and mismatched duplex has a crucial role for discrimination of point mutations or single nucleotide polymorphisms. The ΔTm is decreasing with the length of oligodeoxynucleotide. From this point of view, shorter oligodeoxynucleotide probes are advantageous due to higher ΔTm compared with longer probes. On the other hand, their low melting temperature is the main disadvantage. Melting temperature modifiers are used for elimination of the disadvantage. There are three types of modifiers that can be used for thermal stabilisation of oligodeoxynucleotide duplexes: polyamines[1], minor groove binders[2] and intercalators.[3] In our work we focused on preparation of new acridine derivatives. They were prepared by modified published procedures. Our acridine derivatives were tested in solution and compared to the polyamine (spermine) and well known MGB Hoechst 33258. Majority of acridine derivatives showed comparable or better activity in solution than spermine or Hoechst 33258. Two most promising acridine derivatives were selected and covalently attached to the oligodeoxynucleotide probe by click chemistry. Probes were tested at PCR conditions.



The study was supported by the Grant Agency of Charles University (Project No. 994218) and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS OF AMINOADAMANTANE PHTHALOCYANINES FOR SUPRAMOLECULAR COMPLEXATION WITH CUCURBITURIL

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Phthalocyanines (Pc) are macrocyclic compounds which are used *e.g.* as photosensitizers in photodynamic therapy in the treatment of tumor diseases. They have tendency to make aggregates and then lose their desired photophysical and photochemical properties. Creating supramolecular complexes with cucurbituril (CB, macromolecules composed of methylene bridged glycoluril oligomers[1]) should improve those properties. One of the strongest supramolecular interactions reported is between CB[7] and 1-aminoadamantane.[2] Five phthalonitrile precursors were prepared and LK-3 with CB[7] was studied by X-ray crystallography and orientation of aminoadamantyl moiety was confirmed to be buried fully in CB[7] cavity. Five zinc Pcs were prepared by cyclotetramerization reaction using zinc acetate and pyridine or lithium in butanol. First measurements of absorption spectra showed improved monomerization of Pcs in water after addition of CB[7].

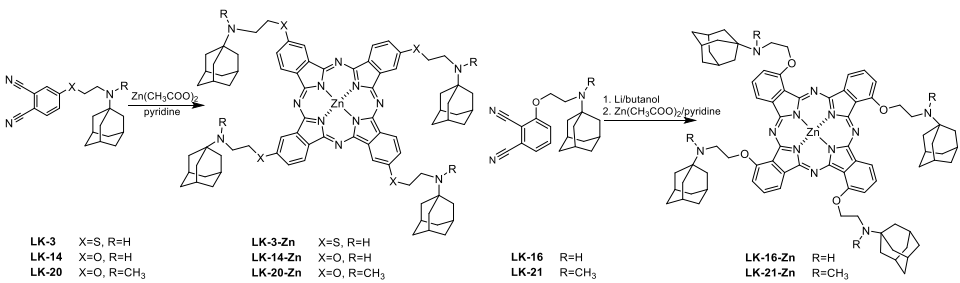

Fig. 1. Structures of the studied compounds

The study was supported by the Czech Science Foundation (Project No. 20-09212S) and from the project of Specific Academic Research (SVV 260 547).

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EFFICIENCY OF A CLICK REACTION IN OLIGODEOXYNUCLEOTIDE PROBES PREPARATION

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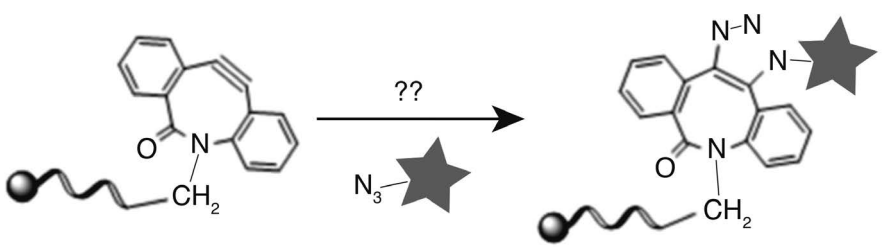
Oligodeoxynucleotide (ODN) probes are synthetized sequences of nucleic acids modified with molecule label such as fluorophores, quenchers or drugs. Labels determine their main use as an imaging and detection tool in biochemistry and medicinal fields. Typically, ODN probes are synthesized and labelled on solid phase in oligo synthesizers. One approach of ODN labelling is "click" reaction through Huisgen's cycloaddition.[1] Even though this reaction has exceptional reaction kinetics there is significant yield fluctulation in practice. Considering financial aspects of such a synthesis, we decided to examine the influence of different conditions on the efficiency of click reaction. We labeled, deprotected, and purified through HPLC method over 150 probes. Three types of molecules (azaphtalocyanine, BODIPY, and acridine derivative) were used for the labelling in five diferent concentrations (10 μM to 100 mM). Three positions in the strand of a 24-base identical sequence were tested (2, 13, and 24, counted from 3′-end of oligonucleotide, where it is bound to the solid phase). An influence of solid phase support was observed using two commercially most frequently used types (controlled pore glass, polystyrene) and their various porosity.

Experiments prooved that hydrophilic/-phobic compatibility of solid phase support and the label has the crucial impact on the concentration of label needed for fully labelled ODN probe preparation.

The study was supported from the project of Specific Academic Research (SVV 260 547).



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NEAR INFRARED ABSORBING BODIPY DYES FOR PHOTODYNAMIC THERAPY

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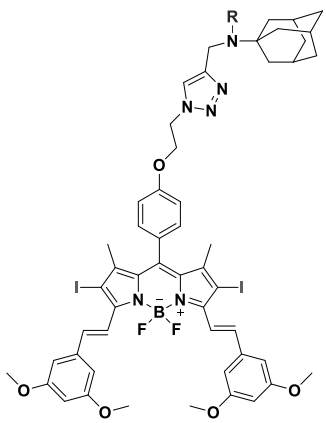
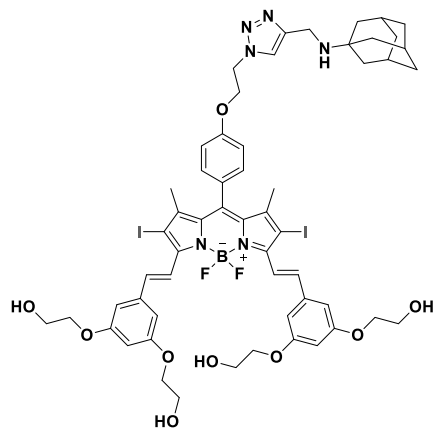
Photodynamic Therapy (PDT) is arguably one of the most effective technique in cancer treatment. PDT involves three main components, *i.e*. photosensitizer, light and oxygen which generates reactive oxygen species (ROS) and helps to kill the cancer cells.[1] In recent times, supramolecular ensembles formed through non-covalent host-guest interactions have attracted great attention for the development of photosensitizers.[1,2] In this regard, we are focusing on the synthesis of NIR activable BODIPY dyes as guest for supramolecular ensembles with cucurbiturils as host for PDT. We have synthesized NIR activable BODIPY dyes (BPS-1a, BPS-1b and BPS-2) linked with adamantylamino unit and preliminary photophysical experiments were also carried out.

The study was supported by EFSA-CDN (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 547).



BPS-1a (R = H)
BPS-1b (R = CH_3)



BPS-2

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VIRTUAL SCREENING OF TGR5 SMALL-MOLECULE: AGONIST OR ANTAGONIST?

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TGR5 plays important roles in bile acids pathways and is involved in various disease mechanisms.[1] Activation of TGR5 impacts the metabolism of bile acids, blood glucose, energy expenditure and anti-inflammatory effects.[1] Although it has a positive impact on some diseases related to metabolism syndrome, activation of TGR5 has pleiotropic effects that trigger side effects such as increased risk of inappropriate gallbladder filling and increased proliferation of polycystic liver disease (PLD).[2,3] The spacious TGR5 binding cavity becomes a challenge in finding selective and potential ligands. It causes TGR5 ligands, both the steroidal and non-steroidal groups, to have different sites of interaction, although there can be overlapping involving several residues.[4] Various studies were conducted to find TGR5 modulators, both agonists and antagonists. Known TGR5 ligands from a created database will be used to generate pharmacophores hypothesis, agonist and antagonist. Ligand-based virtual screening will be conducted using the pharmacophore hypothesis against ZINC, ChEMBL, and PUBCHEM databases. Furthermore, structure-based virtual screening will be conducted with the results of screened potential ligands against TGR5. At the end of the study, it is expected that selective and potent TGR5 modulators can be developed as lead compounds for drugs targeting TGR5.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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ELECTRON POOR DENDRALENES – REACTIVITY OUTLOOK

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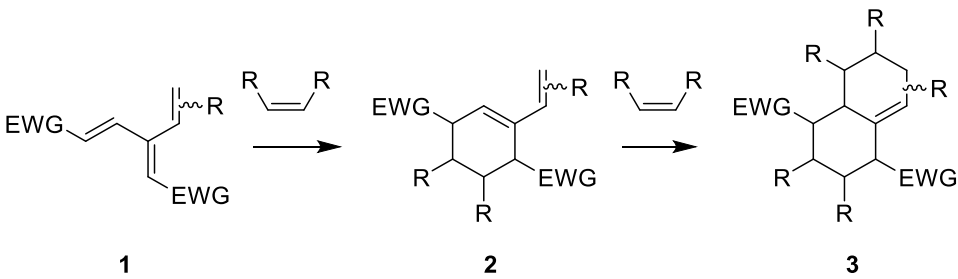
Diene-transmissive Diels-Alder (DTDA) sequence offers a step-efficient way for construction of complex polycyclic structures, even in one flask with different dienophiles.[1] These products could be then used for preparation of various natural compounds.

Dendralenes, a cross-conjugated polyenes, can perform especially well in DTDA, because, in theory, they can provide up to [X]−1 of Diels-Alder cycloadditions, where [X] is a number of cross-conjugated double bonds.

Our [3]dendralenes **1** (Scheme 1), substituted with electron-withdrawing groups (EWG), show high selectivity in cycloaddition partners, providing high yielding DTDA, further supported by calculation of free energies.



Scheme 1. DTDA of dendralenes

The study was supported by EFSA-CDN (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS OF ELECTRONICALLY TUNED [3]DENDRALENES AND THEIR REACTIVITY IN DIELS-ALDER SEQUENCES

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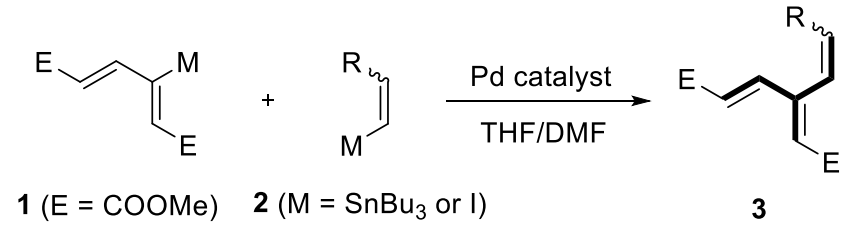
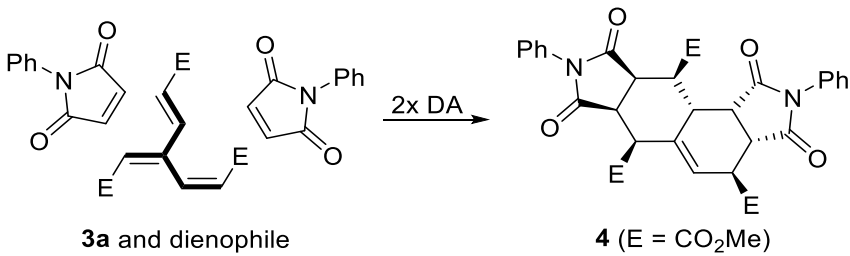
Dendralenes are acyclic, cross-conjugated oligoenes with an interesting, as yet unexplored reactivity. Their dissonant character makes them highly attractive for synthetic applications.[1] We have focused on the synthesis of variously substituted electron-poor [3] dendralenes, containing electron withdrawing groups (*e.g.*, carboxylic group), or a combination of electron withdrawing and donating groups. The synthesis is based on readily available Z-metallodienes **1**, which are subjected to Migita-Stille coupling[2] yielding the desired final products **3** (Scheme 1). We also attempted to study the reactivity of model compound **3a** in reactions with various dienophiles (Scheme 2).



Scheme 1. Migita-Stille coupling



Scheme 2. Example of Diels-Alder reaction

The study was supported by the Czech Science Foundation (Project No. 18-17868S), by the Grant Agency of Charles University (Project No. 1348119) and from the project of Specific Academic Research (SVV 260 547).

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TOTAL SYNTHESIS OF BIOLOGICALLY ACTIVE ISOCOUMARINS – SESCANDELIN B AND ITS DERIVATIVES

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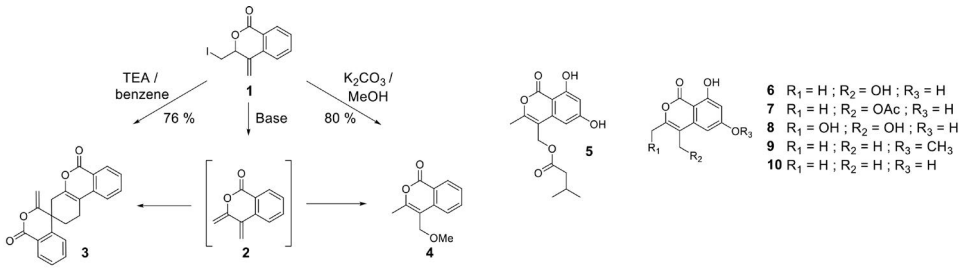
Elimination reaction of iodide (Scheme 1) in the presence of organic or inorganic base gives reactive, unisolable diene **2**. Based on the conditions, this intermediate immediately undergoes auto Diels-Alder reaction to give **3** (*e.g.*, TEA in benzene), or is transformed to isocoumarin derivative **4** (in the presence of K_2CO_3 in methanol). Compound **4** shares structural similarity with a number of biologically active compounds such as penicimarin E (**7**) and F (**8**), polygonolide (**9**), sescandelin B (**6**) (and their derivatives **5** and **10**) with antidiabetic[2] or antiinflammatory activity.[3] Total syntheses of these isocoumarins are currently being undertaken.



Scheme 1. Synthesis of the title compounds

The study was supported by the Czech Science Foundation (Project No. 18-17868S), by the Grant Agency of Charles University (Project No. 205007) and from the project of Specific Academic Research (SVV 260 547).

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MICHAEL AND ANTI-MICHAEL ADDITIONS TO [3]DENDRALENES

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Dendralenes are acyclic cross-conjugated polyenes with an interesting, as yet unexamined reactivity and high potential for further synthetic applications.[1] Our research group developed a novel synthesis of variously substituted electron-poor [3]dendralenes with a distinct dissonant character, and discovered an unexpected behaviour of these compounds upon nucleophilic attack.

In view of the preliminary results and assuming that elimination of the dissonant nature was the driving force for these transformations, we further focused on various versions of Michael additions to dendralenic structures. In some cases, anti-Michael additions also occurred. Using mild conditions and different combinations of nucleophiles and dendralenes, cyclizations and/or simple additions have been observed.

This study was supported by the Czech Science Foundation (Project No. 18-17868S), by the Grant Agency of Charles University (Project No. 1348119) and from the project of Specific Academic Research (SVV 260 547).

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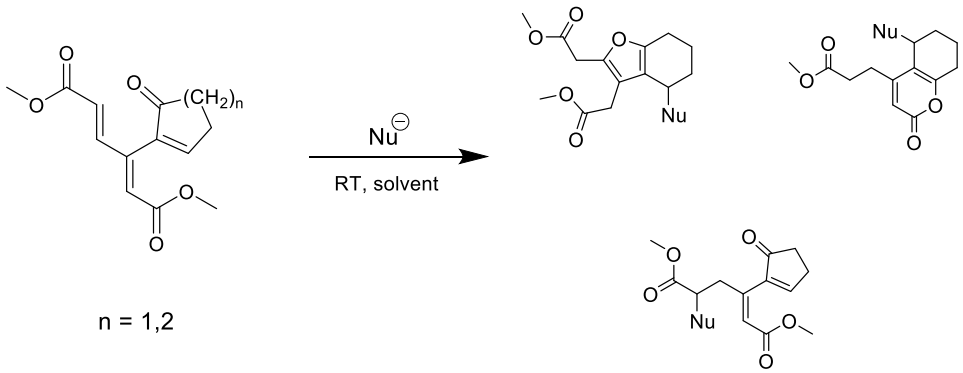

Scheme 1. Additions of nucleophiles to [3]dendralenes

STUDY OF 2,5-DISUBSTITUTED 1,3,4-OXADIAZOLES AS POTENTIAL ANTITUBERCULOTICS

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Nowadays, small heterocyclic molecules (*e.g.*, oxadiazoles, tetrazole, triazoles) have been studied as potential antituberculotics. Compounds with this structural motif target *M. tuberculosis* (Mtb.), including resistant strains.[1] We have prepared a series of 2,5-disubstituted 1,3,4-oxadiazoles with very promising activity against several mycobacterial strains. Asymmetric 1,2-diacylhydrazides were prepared by the reaction of monosubstituted commercially available or in-house prepared (acyl) hydrazine with a appropriate acyl chloride in the presence of a base using anhydrous tetrahydrofuran (THF) as the solvent. 2,5-Disubstituted oxadiazoles were obtained directly by dehydrative cyclization of 1,2-diacylhydrazides (Scheme 1). In general, the syntheses gave satisfactory yields. The compounds were evaluated for their *in vitro* antimycobacterial activity against drug-susceptible Mtb H37Rv and nontuberculous mycobacteria (NTM, *M. avium, M. kansasii*). Determining the activity against multidrug-resistant Mtb. as well as selectivity index are under investigation. No activity against Gram-positive and Gram-negative bacteria as well as fungal pathogens was identified.

The study was supported by the Czech Science Foundation (Project. No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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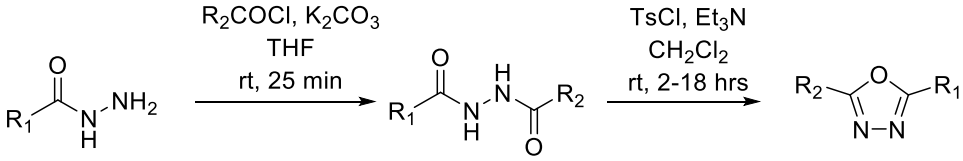

Scheme 1. Synthesis of the title compounds

SYNTHESIS OF OMEGA-HYDROXYLATED CERAMIDES USING OLEFINATION REACTIONS

ONDREJČEKOVÁ, V., OPÁLKA, L., VÁVROVÁ, K.

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Omega-hydroxylated ceramides (O-Cer) belong to a subclass of Cer with ultralong *N*-acyl chains. Together with other groups of Cer, free fatty acids and cholesterol, these lipids create multilamellar structures in stratum corneum, which are responsible for skin barrier function. O-Cer occur in two forms: free form or bound to the surface of corneocytes where they form a corneocyte lipid envelope (Fig. 1). The detailed role of covalently bound O-Cer remains unclear.

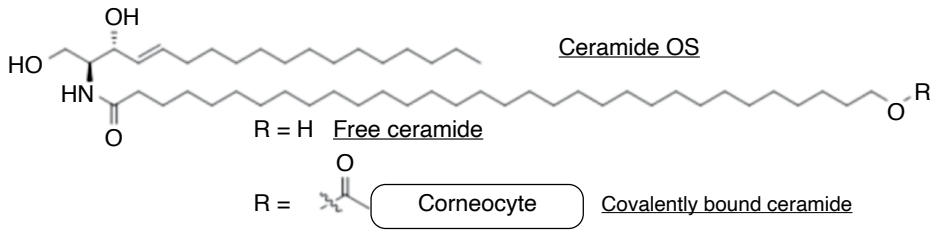

Fig. 1. Structure of free O-Cer and O-Cer covalently bound to the corneocyte

The main objective of this project was to optimize the synthetic procedure towards three subclasses of O-Cer, specifically Cer OS, OP and OdS.

Complete synthesis of O-Cer has not been reported yet. In this project, we modified a previously published procedure of ultralong Cer synthesis where we focused on an improvement of the most complicated steps of this synthesis. Protected 16-hydroxyhexadecanal as a crucial component for olefination reactions was prepared in four steps from hexadecanolide with high yield. This aldehyde was then connected with various heterocyclic sulfones using olefination reactions, such as Julia and Julia-Kocienski reactions to obtain a protected 32-hydroxydotriacontenoic acid, which is an O-Cer precursor. This change in reaction procedure led to a significant improvement in the reaction yield and to a decrease in costs of starting materials.

The study was supported by the Czech Science Foundation (Project No. 19-09135J), by the Grant Agency of Charles University (Project No. 1194119) and from the project of Specific Academic Research (SVV 260 547).

LIPID-DECORATED DENDRIMERS FOR STUDYING THE BEHAVIOR OF CORNEOCYTE LIPID ENVELOPE

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Skin is the biggest organ in the human body and protects it from excessive water loss while hampers the entrance of undesired substances, allergens and microbes. Skin's outermost layer, stratum corneum (SC), holds the principal skin barrier and consists of flattened dead cells, known as corneocytes, embedded in a hydrophobic lipidic matrix. Covalently attached to corneocyte's surface are ceramides forming the so-called "corneocyte lipid envelope" (CLE).[1] Most biophysical SC models are taking into consideration only the lipidic matrix. In our approach, we are focusing on the development of corneocyte mimicking compounds to create a more complex SC model that would incorporate hydrophilic corneocyte mimicking entities with/without CLE in the lipidic matrix to study the putative scaffolding role of CLE. For this purpose, we plan to decorate fourth generation PAMAM dendrimers with ceramides. For the development of an effective synthetic protocol, PAMAM dendrimers and ceramides were not used directly but were replaced by in-house synthesized dendrimers and long-chain aliphatic alcohols with a terminal triple bond, respectively. A copper-catalyzed azide-alkyne cycloaddition protocol was developed to couple the modified dendrimers with the ceramide mimicking compound. The synthetic protocol has already been established and will be applied to conjugate the PAMAM dendrimers with the ceramides.

The study was supported by the Czech Science Foundation (Project No. 19-09135J) and from the project of Specific Academic Research (SVV 260 547).

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-AMINOPYRAZINE-2-CARBOXAMIDE DERIVATIVES AS POTENTIAL ANTIMICROBIALS

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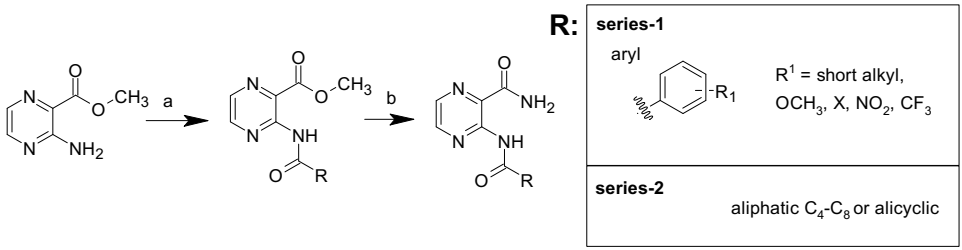
Tuberculosis (TB) remains among the WHO top 10 causes of death despite available treatments[1] and BCG vaccine. As part of our ongoing research on pyrazinamide (a first-line antitubercular) derivatives, we report the design and synthesis of novel 3-aminopyrazine-2-carboxamide derivatives along with their biological evaluation. Almost 50 compounds were prepared according to Scheme 1 and evaluated for their *in vitro* activity against various strains of mycobacteria and other strains of pathogenic bacteria and fungi. The active compounds were only from series-1 (R is a substituted phenyl). The most active compounds we selective towards inhibition of Mtb H37Ra and Mtb H37Rv (over other mycobacterial strains) and exerted MIC (Minimum Inhibitory Concentration) ranging from 1.98 to 7.81 μg mL^{-1}. The final compounds were also studied for cytotoxicity on HepG2 cell line followed by SAR. Title compounds will also be studied as potential inhibitors of (human) prolyl-tRNA synthetase based on their structural similarities to confirmed inhibitors reported in the literature.[2]



Scheme 1. Synthesis of final compounds
a: acyl chlorides/pyridine/Ar medium, b: 2M ammonia in EtOH

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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INSECT MODEL, *GALLERIA MELLONELLA*, AS NEW POWERFUL TOOL FOR THE DRUG DISCOVERY RESEARCH

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The final and often crucial phase of drug discovery research involves the using of appropriate animal models. Mice and rats are the most frequently chosen models for this purpose. However, in taking into account 3Rs (Replacement, Reduction, and Refinement) criteria, which are considered to be a framework for conducting high-quality science in the academic sector, it is desirable to look for a suitable alternative approach.[1]

Galleria mellonella represents an insect animal model enjoying increasing popularity in research communities. This animal model is employed in studies focused on mechanisms of microbial pathogenesis and virulence, in discrimination between *in vivo* high and non/low-toxicity, or in the study of *in vivo* efficacy of candidate anti-infective drugs. This model's undeniable pros are that ethical approval is not needed, financial costs are significantly lower than the mouse or rat model, and no special lab equipment is required.[2,3]

We are currently experienced in this animal model rearing and its use for *in vivo* toxicity testing. Optimization of the methodical approaches for *in vivo* candidate anti-infective drug efficacy testing and *in vivo* microbial biofilm formation is now in the process.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 549).

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STUDY OF BOOSTER EFFECT OF COMPOUNDS IN COMBINATION WITH STANDARD DRUGS USED IN THERAPY OF TUBERCULOSIS

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Tuberculosis is still one of the most threatening health problems all over the world. Although the absolute numbers of new cases are decreasing, problem has arisen with resistant strains of *Mycobacterium tuberculosis*, which is the causative agent of tuberculosis. These cases are hard to cure and it is necessary to use second line drugs. This treatment is expensive, time consuming and the risk of side effects is high.

Treatment of tuberculosis is always based on combination of at least 2 to 4 drugs according to therapy regimen. Using combinations can be very useful as well as tricky. Combinations can improve the effect and lower the risk of resistance development. On the other hand, these combinations mean higher risk of interactions that can result in antagonistic effects of these molecules. Outcome of those factors is failure of therapy that can lead to significant spreading of infection and higher mortality rate.

Synthesis of novel compounds is one of the most important steps for tuberculosis eradication. But the chance to find molecule with novel mechanism of action, for which mycobacteria will not be able to evolve resistance, is quite low. So, one opportunity to slow down the spreading is to study interactions between drugs used for treatment and new molecules.

One of the methods to study these interactions is called checkerboard assay. It is based on studying combinations of two compounds in various concentrations affecting the result. There are 4 possible interactions expressed as FIC (Fractional Inhibitory Concentration), namely Antagonism, Indifference, Additivity, Synergism. Last three named categories are optimal for practice, but synergism is the most desired. Methodology for antimycobacterial checkerboard assay was optimized in this work.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y), by Research programme Development and Study of Drugs (Progress Q42) and from the project of Specific Academic Research (SVV 260 547).

TOWARDS TO *IN VITRO* ANTIBIOFILM ACTIVITY SCREENING – INTRODUCTION OF APPROPRIATE METHODICAL APPROACH FOR STAPHYLOCOCCAL BIOFILM FORMATION

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Staphylococcus aureus (SA) and *Staphylococcus epidermidis* (SE) are the most common pathogens from the genus Staphylococcus, causing biofilm-associated infections. Bacteria in biofilms are difficult to eradicate due to their resistance and serve as a reservoir for recurring persistent infections.[1] A variety of protocols for *in vitro* drug activity testing against staphylococcal biofilms has been introduced. However, there are often fundamental differences. In our preliminary study, we developed optimal conditions for staphylococcal biofilm formation on plastic pegs in order to set a methodology for the evaluation of the antibiofilm activity of candidate molecules. The convenience of the plastic pegs lies in their removability from the lid for easy access to multiple equivalent biofilms, and in possibility of *in situ* detection and quantification by confocal laser microscopy. For the purpose of enhancement in staphylococcal biofilm formation, the impact of peg surface modification with 3 different coating materials was studied as well. An increase of biofilm biomass was evaluated by crystal violet staining method.[2] The basic

precondition for obtaining relevant and reproducible data regarding antibiofilm activity is the formation of robust biofilms with typical attributes such as the presence of a biofilm matrix. In our study, *in vitro* conditions revealed that we fully met the preconditions for the SA and methicillin-resistant SA strains. In conclusion, we demonstrated statistically significant enhancement of biofilm formation in all studied staphylococcal strains, including either strong biofilm producer phenotype (SA, methicillin-resistant SA) and weak biofilm producer phenotype (SE).

The study was supported by the Czech Science Foundation (Project No. 20-19638Y), and from the project of Specific Academic Research (SVV 260 549).

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DERIVATIVES OF QUINOXALINE-2-CARBOXAMIDES AS POTENTIAL ANTIMYCOBACTERIALS

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Despite the established treatments, tuberculosis remains an alarming threat to public health according to WHO.[1] Novel agents are needed to overcome the increasing rates of resistance and perhaps achieve eradication. As part of our long-term research on pyrazine derivatives, we prepared a series of *N*-substituted quinoxaline-2-carboxamides (Fig. 1) and evaluated their *in vitro* antitubercular activity. Several quinoxaline derivatives were found in the literature to possess antitubercular activity.[2] Quinoxaline-2-carboxylic acid was activated by oxalyl chloride and reacted with different anilines or benzylamines in the presence of pyridine at room temperature, overnight with stirring, and obtained crudes were then purified with flash chromatography. In addition to activity assessment, final compounds were screened for their *in vitro* cytotoxicity on HepG2 liver cancer cell lines. *In vitro* activity against Mtb H37Ra (represented by MIC) ranged between 3.91–500 μg mL^{-1}, with most compounds having moderate to good activities (MIC < 15.625 μg mL^{-1}).

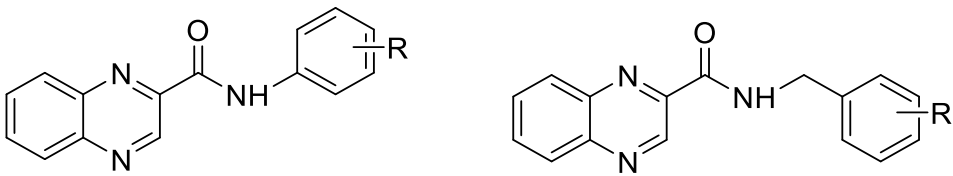

Fig. 1. Structures of the studied compounds

This work was supported by EFSA-CDN (Reg. No. CZ.02.1.01/0.0/0.0/16_019/00008 41) co-funded by ERDF, by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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PYRAZINE-2-CARBOHYDRAZIDE DERIVATIVES AS POTENTIAL ANTIITUBERCULARS

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Tuberculosis, an infectious disease, is a major problem when it comes to number of casualties and growing antimicrobial resistance.[1] It is treated by first-line drugs (*e.g*., pyrazinamide), but new agents are needed.

In 2014, Rodrigues *et al*.[2] tested a series of pyrazine-2-carbohydrazide derivatives as potential anticancer class. Compounds were tested on three different tumor cell lines and their growth inhibition was not satisfactory to develop such compounds as new anticancer agents. This work inspired us to the development our title series and evaluate their antimicrobial activity. Current series is based on pyrazine-2-carbohydrazide that is bound to different heterocycles by imine bond (example of compound: *N*-benzylidenepyrazine-2-carbohydrazide) that will be further derivatized into disubstituted compounds (example *N*-benzoyl-N′-benzylidenepyrazine-2-carbohydrazide). Compounds were tested for biological activity against selected strains of Mycobacterium, eight fungi strains and eight bacterial strains. The minimum inhibitory concentration (MIC) for tested mycobacterial strains was determined for all tested compounds beside isoniazid, ciprofloxacin and rifampicin as reference standard drugs. Results of the biological testing and structure activity relationships are discussed in the presentation.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRAZINE-BASED INHIBITORS OF MYCOBACTERIAL METHIONINE AMINOPEPTIDASE

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Tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), is the number one cause of deaths due to a single infectious agent worldwide.

In this project, we present the synthesis and biological evaluation of new potential pyrazine-based inhibitors (Fig. 1) of the prominent drug target mycobacterial methionine aminopeptidase 1 (MtMetAP1). The activity of all compounds was evaluated against the isoform MtMetAP1a.

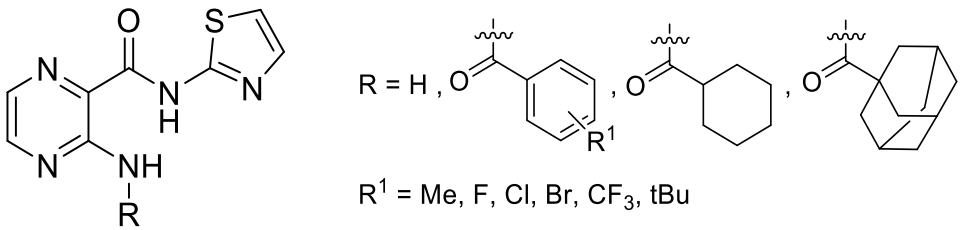

Fig. 1. General structure of the synthesized compounds

Overall, high inhibition of the isolated enzyme was observed. However, as described previously[1], the activity was strongly dependent on the used metal cofactor. The highest activity was seen in the presence of Ni(II) (IC_{50} = 0.6 μM, 2Br substitution), the lowest with Co(II). Several compounds also showed mediocre *in vitro* potency against Mtb (MIC = 15.625 μg mL^{-1}). Unfortunately, no direct correlation with the activity against the isolated enzyme was seen. Despite the high structural similarities of bacterial and fungal MetAP to mycobacterial MtMetAP1, final compounds did not exert any antibacterial nor antifungal activity.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y), and from the project of Specific Academic Research (SVV 260 547).

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COMPUTATIONAL *DE NOVO* DESIGN OF INHIBITORS OF HUMAN PROLYL-tRNA SYNTHETASE

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This work is about searching for new inhibitors of human prolyl-tRNA synthetase (hsProRS) with use of Computer-Aided Drug Design. The idea of such an inhibitor is based on previously reported inhibitors of hsProRS[1] and our previous simulations.[2] In this phase of our research, we are trying to identify new promising ligands to hsProRS with a Fragment Growing tool in Molecular Operating Environment (Chemical Computing Group, Canada). The main purpose of these simulations is the *de novo* design of new and potentially unexpected ligands. The unimportant parts of the original Adachi inhibitor were deleted and replaced with different fragments to achieve higher affinity to the receptor (see Fig. 1 – deleted parts behind the dashed line).

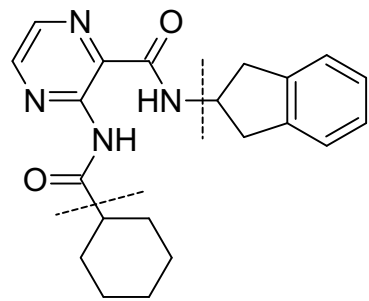

Fig. 1. General structure of the studied compounds

Suggested ligands were evaluated according to the availability of the fragments from common suppliers and their price. The best synthesizable ligands will be prepared and tested for cytotoxicity on human cell lines (HepG2). Additionally, based on structural similarity between human, mycobacterial and bacterial orthologues of ProRS, prepared compounds will be tested for *in vitro* antimicrobial activity.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS SECTION

BIOGUIDED ISOLATION OF ALKALOIDS FROM *PAPAVER RHOEAS* BY PREPARATIVE CHROMATOGRAPHY

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Papaver rhoeas is a common plant in many regions around the world. It is regarded as a source of many bioactive compounds with beneficial health effects. In folk medicine, *P. rhoeas* is often used for the treatment of various diseases and is known for centuries for its pharmacological properties.[1] Extracts of *P. rhoeas* are studied for their soothing abilities in anxiety-related digestive problems, and are studied as a potent antitussive, antispasmodic, antigenotoxic, antimutagenic, anticarcinogenic as well as bactericide agents.[2] Recent studies showed interesting activities of its extract, that prevents neurodegenerative diseases such as Alzheimer's. Previous phytochemical investigation has revealed the presence of various alkaloids.[1,3] As a part of our on-going screening of plant extracts for biological activities, the alkaloidal extract from aerial parts of *P. rhoeas* was studied. After fractionation with flash chromatography, obtained fractions were examined for their butyrylcholine esterase and acetylcholine esterase inhibitory activities, as well as their cytotoxicity. Fractions with highest biological activities underwent further separation using preparative chromatography, to isolate the most biologically active compounds. After purification, the fractions were analyzed by HPLC-MS and GC-MS to elucidate their chemical composition.

The study was supported by the Research program Development and Study of Drugs (Progres Q42)) and from the project of Specific Academic Research (SVV 260 548).

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ALKALOIDS OF *ZEPHYRANTHES CITRINA* (AMARYLLIDACEAE) AND THEIR IMPLICATION TO ALZHEIMER'S DISEASE: ISOLATION, STRUCTURAL ELUCIDATION AND BIOLOGICAL ACTIVITY

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Zephyranthes is a genus of bulbous perennial plants belonging to Amaryllidaceae family. Phytochemical screening of these plants revealed diverse group of compounds, especially Amaryllidaceae alkaloids, which are responsible for the most of biological activities.[1]

Twenty known Amaryllidaceae alkaloids of various structural types and seven undescribed alkaloids have been isolated from fresh bulbs of *Zephyranthes citrina*. The chemical structures of the isolated alkaloids were elucidated by a combination of MS, HRMS, 1D and 2D NMR, and CD spectroscopic techniques, and by comparison with literature data. Compounds isolated in a sufficient quantity were evaluated for their *in vitro* acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and prolyl oligopeptidase (POP) inhibition activities. Significant human AChE/BuChE (*h*AChE/*h*BuChE) inhibitory activity was demonstrated by the novel alkaloid of narcikachnine-type named narcieliine, with values of IC_{50} 18.7 ± 2.3 μM and 1.34 ± 0.31 μM, respectively. This compound is also predicted to cross the blood-brain barrier (BBB) through passive diffusion. The *in vitro* data were further supported by *in silico* studies of narcieliine in the active site of *h*AChE/*h*BuChE.

The study was supported by the Grant Agency of Charles University (Project No. 178518) and from the project of Specific Academic Research (SVV 260 548).

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ALKALOIDS FROM *GEISSOSPERMUM VELLOSII* AND THEIR BIOLOGICAL ACTIVITY

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The genus *Geissospermum* (Apocynaceae) are Amazonian trees native to Brazil, commonly found in the eastern region. Native tribes use aqueous and ethanolic extracts of bark for various diseases, *e.g.*, malaria, cancer, and bacterial infections. The species of *Geissospermum vellosii* is a rich source of indole and β-carboline type of alkaloids. There are few phytochemical studies of this genus. However, almost none of the studies is dealing with the isolation of alkaloids. The biological activity of extracts from *G. vellosii* is broad and copies the native use of decoction. The preliminary screening study for determining cholinesterase inhibition of extract from the bark of *G. vellosii* showed interesting inhibition activity against huBuChE $IC_{50} = 0.37 \pm 0.05$ μg mL^{-1}, and at least 12 alkaloids were identified by GC/MS and TLC. Primary ethanolic extract was prepared from 40 kg of dried crushed bark. The alkaloidal extract was prepared with different solvents (diethyl ether and chloroform) depending on the polarity. The purified diethyl ether extract (53 g) was separated using column chromatography to give 16 fractions. After purification and crystallization, five compounds have been isolated so far. The inhibitory activity against recombinant human AChE and BuChE, GSK-3β of isolated alkaloids and their blood-brain barrier penetration was determined.

The study was supported by the Research program Development and Study of Drugs (Progres Q42) and from the project of Specific Academic Research (SVV 260 548).

STRUCTURE ELUCIDATION OF AMARYLLIDACEAE ALKALOIDS

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NMR spectroscopy is an indispensable technique used in the structure elucidation of alkaloids. 1D NMR experiments give an idea about the structural moiety, but 2D NMR experiments are measured to complete the structure if there is no reference NMR data for the analyzed compound. Due to many stereocentres in molecules of most alkaloids, determining the relative configuration is possible employing the nuclear Overhauser effect spectroscopy experiment and, in some cases, the values of coupling constants in the ^{1}H NMR spectrum. The comparison of chiroptical data of analogous compounds can elucidate the absolute configuration. However, the only conclusive method for the absolute configuration is the single-crystal X-ray diffraction or an enantioselective total synthesis of the compound.

Thus, these approaches have been used for the structure identification of Amaryllidaceae alkaloids in this project. Structures of new alkaloids have been determined among well-known molecules. Furthermore, the phenomenon of atropisomerism has been identified for unusual new structure unities by dynamic NMR analysis.[1,2] All presented alkaloids were isolated at the Department of Pharmaceutical Botany.

The study was supported by the Czech Science Foundation (Project No. 18-17868S) and from the project of Specific Academic Research (SVV 260 547).

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AMBELLINE DERIVATIVES AS SELECTIVE INHIBITORS OF LIVER STAGE MALARIA *IN VITRO*

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Malaria is a severe parasitic protozoal infection of global importance caused by unicellular protozoa from *Plasmodium* genus. According to the WHO, around 40% of the global population live in threatened areas. The most important factors related to malaria treatment are prevention, early diagnosis and appropriate and effective medication. That brings us to a serious problem – development of drug resistance in *Plasmodium* spp. which is the crucial incentive for new potential drugs research.[1] 51% of all drugs and 65% of small-molecule drugs approved between 1981–2014 are in certain connection with natural compounds.[2] This proves, that natural compounds and their derivatives are still an important source where new potential drugs can be sought. In the treatment of malaria it was quinine at first and most recently it is sesquiterpene lactone artemisinine and its semisynthetic derivatives.

One of the interesting groups of bioactive compounds are Amaryllidaceae alkaloids (AA). Over 70 AA and their semisynthetic derivatives were screened *in vitro* due to their activity against the liver stage malaria caused by *Plasmodium berghei* sporozoites. The most promising activities against the *P. berghei* liver stage were shown by aromatic derivatives of ambelline. Compound LC-104 with $IC_{50} = 0.048 \pm 0.014$ μM was considered to be the most active one (primaquine $IC_{50} = 5.74 \pm 0.86$ μM). Considering the inactivity against the blood stage of *P. falciparum*, this compound seems to be an interesting selective inhibitor of malaria liver stage.

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INFLUENCE OF DEHYDROFLAVONOLIGNANS FROM SILYMARIN ON METAL-BASED FENTON REACTION

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Silymarin, the extract from milk thistle (*Silybum marianum* (L.) Gaertn. (Asteraceae)) fruits, contains a variety of flavonolignans and displays antioxidant, anti-inflammatory, immunomodulatory and hepatoprotective properties.[1] In the EU, it is approved for the adjuvant therapy of alcoholic liver disease and is also consumed in various food supplements. Its flavonolignans can interact with transition metals as was demonstrated in our previous study with 2,3-dehydrosilybin (DHS).[2] DHS both chelated and reduced copper and iron ions. Since it is difficult to assess the effect of a compound having both metal reducing and chelating properties on the Fenton chemistry, DHS and 2,3-dehydrosilychristin (DHSCH) were tested on metal-based hydroxyl radical generation *in vitro* analysed by HPLC with coulometric detection at four (patho)physiologically relevant pH conditions ranging from 4.5 to 7.5. None of both dehydroflavonolignans was able to block the iron-based Fenton reaction. The effect was always prooxidative or neutral. In the case of the copper-based Fenton reaction, the effect of DHSCH was also prooxidative or neutral while the effect of DHS was dependent on conditions.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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SCREENING OF INHIBITORY ACTIVITY AGAINST CHOLINESTERASES OF VARIOUS SPECIES OF THE GENUS *FICUS*

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Alkaloids are important group of biologically active secondary metabolites. Many of them inhibit human cholinesterases and therefore have potential to be used in the treatment of Alzheimer's disease (AD). For example, Amaryllidaceae alkaloid galanthamine is used in therapy of AD.[1]

AD is one of the most frequent causes of dementia in the world. AD consist of many cognitive and neuropsychiatric manifestations. Enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) play important role in the progression of this disease.[1]

The genus *Ficus* includes arboreous and evergreen plants belonging to the family Moraceae, that is distributed widely throughout the tropical and subtropical regions. It is represented by about 725 species. Plants from the genus *Ficus* contain among other alkaloids. These alkaloids display various structures and some of them have interesting biological activities such as anti-inflammatory, antitumor, antifungal, antibacterial and antimalarial.[2]

Within the phytochemical study, 19 extracts from leaves and stems of 14 species of the genus *Ficus* were prepared. Dry material was ground and then extracted by boiling in ethanol. Ethanolic extracts were purified by liquid-liquid extraction (ether, ethyl acetate, chloroform). All extracts were tested on the ability to inhibit human cholinesterases. In testing hBuChE inhibition potency has been demonstrated by the extract AL-700C ($74.82 \pm 2.78\%$ at concentration 50 $\mu g\ mL^{-1}$).

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NEW AMARYLLIDACEAE ALKALOIDS FROM *NARCISSUS PSEUDONARCISSUS* cv. CARLTON AS INSPIRATION FOR THE DEVELOPMENT OF NEW DRUGS FOR ALZHEIMER'S DISEASE

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Plant alkaloids are one of the most interesting groups of secondary metabolites and are present also in the plants of the family Amaryllidaceae. To date, nearly 600 Amaryllidaceae alkaloids (AA) have been isolated.[1] *Narcissus pseudonarcissus* cv. Carlton (NPC) is an Amaryllidaceae species, exclusively used for the commercial extraction of galanthamine as a drug for the treatment of mild to moderate stages of Alzheimer's disease (AD). The

treatment of AD is only symptomatic including therapies with acetylcholinesterase (AChE) inhibitors. Butyrylcholinesterase (BuChE) is another cholinesterase (ChE), whose activity increases to 40–90% in the later stage of AD brain.[1] So far, thirteen known and four novel AA have been isolated from the alkaloidal extract of NPC and have been screened for AChE, BuChE, and prolyloligopeptidase (POP) inhibition activity. Three new compounds named carltonine A, B, and C demonstrate significant inhibition potential towards *h*BuChE (IC_{50} = 910 nM, 31 nM, and 14.84 μM, respectively).[1] Carltonine A also showed moderate POP inhibition activity (IC_{50} = 143 μM).[1] The novel compound narciabduliine inhibits both *h*AChE (IC_{50} = 3.29 μM) and *h*BuChE (IC_{50} = 3.44 μM). The next part of the current study was the preparation of synthetic derivatives structurally inspired by carltonines and screening of their biological activities connected with AD.

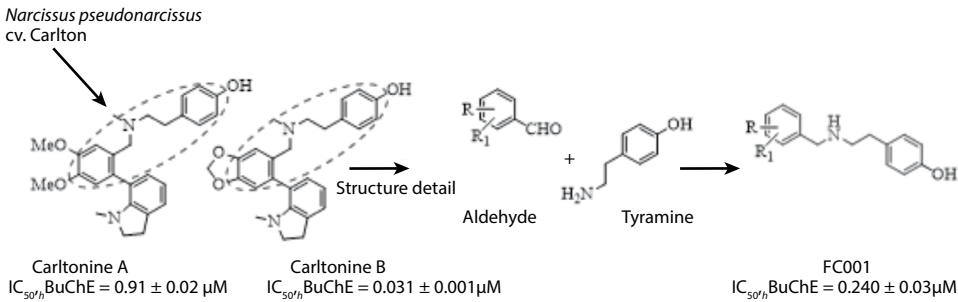

Fig. 1. Synthesis of structural analogues of carltonins

The project was supported from the project of Specific Academic Research (SVV 260 548).

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AMARYLLIDACEAE ALKALOIDS OF NORBELLADINE-TYPE AS INSPIRATION FOR DEVELOPMENT OF SELECTIVE BUTYRYLCHOLINESTERASE INHIBITORS

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Alzheimer's disease is a serious and irreversible progressive neurodegenerative disorder, that will reach a prevalence of more than 100 million by 2050 due to the aging of

population.[1] Pathological changes in the affected brain include: intracellular neurofibrillary tangles, extracellular amyloid plaques, increased oxidative stress, cholinergic disfunction, and others. Cholinergic neurotransmission is terminated by acetylcholine hydrolysis regulated by two enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). With the progression of disease the activity of AChE decreases, while that of BuChE increases.[2] The alkaloids carltonin A and B demonstrated exceptional selective inhibition potential towards the enzyme BuChE in tens of nanomoles (IC_{50} = 0.031 ± 0.001μM). Unfortunately, these alkaloids are present in plant material only in trace amounts. The aim of this work is a preparation of synthetic compounds inspired by alkaloids of the belladine-type with subsequent structure-biological activity relationship study.

The work was supported from the project of Specific Academic Research (SVV 260 548).

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PHYTOCHEMICAL INVESTIGATION OF THE *DICRANOSTIGMA FRANCHETIANUM* (PRAIN) FEDDE (PAPAVERACEAE) HERB: PRELIMINARY STUDY

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Dicranostigma franchetianum (Prain) Fedde, syn. *Chelidonium franchetianum* Prain (Papaveraceae) is one of the representatives of the scanty genus *Dicranostigma* Hook.f. & Thomson. This annual plant grows endemically in the Himalayas and western China and is used as an ornamental plant in Europe. Although it is the source of isoquinoline alkaloids (especially isocorydine) and the hitherto little-studied alkaloids (chelilutin, chelirubin and cheilanthifolin), the plant has not been systematically studied until this time. In primary screening of the summary alkaloid extract for cholinesterases inhibition, the inhibitory potency was high (*h*AChE/*h*BChE, IC_{50} = 1.67 ± 0.11/3.85 ± 0.31 μg mL^{-1}) and together at least 25 alkaloids were found in the extract. The primary ethanol extract was prepared from 11.8 kg of dry herb (Garden of Medicinal Plants, Faculty of Pharmacy in Hradec Králové), from which alkaloid extracts with different solvents with increased polarity (n-hexane, diethyl ether, ethyl acetate, chloroform, chloroform-ethanol) were prepared. The purified diethyl ether extract of the alkaloidal bases (93 g) was separated by flash chromatography on silica to give 12 combined fractions. From these fractions after purification (+)-isocorydine **1**, protopine **2**, allocryptopine **3**, berberine **4**, chelerythrine **5**, sanguinarine **6**) according to the TLC comparison have been obtained so far. Their inhibi-

tory activity on *h*AChE and *h*BChE was determined by using recombinant human brain cholinesterases with the following results: *h*AChE/*h*BChE (IC_{50}, mM) (**1**: > 1000/657.1 ± 15.5, **2**: 230.0 ± 21.0/208.9 ± 17.7, **3**: 250.0 ± 25.2, **4**: 0.71 ± 0.10/30.7 ± 3.5, **5**,**6**: not determined so far).

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SEMISYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology and data indicate an exponential rise in the number of cases of this disease. The well-known Amaryllidaceae alkaloid (AA) galanthamine is a marketed drug for AD therapy under the commercial name Reminyl© (galanthamine hydrobromide).

Studies also pointed out various pharmacological properties of semisynthetic derivatives of some AA, such as alkaloid haemanthamine (HMT), which is widely distributed through Amaryllidaceae plants. Based on our previous results, where several HMT derivatives demonstrated promising *h*AChe/*h*BuChe inhibition potency, we continued the preparation of further HMT semisynthetic derivatives.[1]

Several new esters showed interesting inhibition of both studied cholinesterases, thus structure-activity relationship (SAR) was also studied.[2] Newly prepared compounds were identified by 1D-, 2D-NMR and ESI-MS methods. The most potent compounds were studied in more detail (*e.g.*, type of inhibition, docking studies, logBB *etc*.).

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IN VITRO ANTIAGGREGATION POTENTIAL OF SELECTED ISOQUINOLINE ALKALOIDS

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The platelet is an anucleate cell circulating in the bloodstream. The normal platelet function is to regulate haemostasis but in a pathological state, such as endothelial dysfunction, enhanced platelet aggregation can lead to certain cardiovascular diseases such as stroke, acute myocardial infarction *etc*.[1] Some plant secondary metabolites, alkaloids in particular, are promising candidates in the development of antiplatelet drugs.[2] So far, fourteen isoquinoline alkaloids have been screened for inhibition of platelet aggregation induced by arachidonic acid and collagen and compared with the standard drug acetylsalicylic acid. The screening was performed by impedance aggregometry using whole human blood. Papaverine and bulbocapnine demonstrated inhibition at the concentration of 80 μM. Their mechanisms of action could be an influence on arachidonic acid cascade including cyclooxygenase-1, thromboxane A_2 synthase, and antagonism at thromboxane A_2 receptors. The antiaggregation activity of papaverine and bulbocapnine seems to be related to antagonism at thromboxane receptors. In addition to antiplatelet activity, the prothrombin time and activated partial thromboplastin time of all alkaloids have been determined and none of the alkaloids showed anticoagulant effects in human plasma.

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SEMI-SYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID AMBELLINE AND THEIR CYTOTOXIC POTENTIAL

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Amaryllidaceae alkaloid ambelline, belonging to the crinane-type group, lacks any significant biological activity. However, its analogues prepared by the derivatization of C_{11}-hydroxyl group possess various interesting properties (*e.g.*, inhibitory activity of cholinesterases, antimalarial activity). As a continuation of our earlier work and extension of the prepared derivatives scale, eleven novel aromatic esters were developed (**LC-108**, **LC-180**, **LC-181**, **LC-177**, **LC-131**, **LC-189**, **LC-179**, **LC-119**, **LC-110**, **LC-106**, **LC-178**). To characterize their biological activity spectrum, MTT assays were performed to determine their cytotoxic potential. To predict the structure-activity relationship for further research, substances described in our previous work were also included in this cytotoxicity study.[1] Molecules with the most pronounced cytotoxic activity contain a methyl group (**LC-108**), methoxy group (**LC-180**, **LC-176**, **LC-104**, **LC-181**), ethoxy group (**LC-182**), or disubstitution with different functional groups (**LC-106**) on C11.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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INDOLE ALKALOIDS FROM *VINCA MINOR* L. AND THEIR BIOLOGICAL ACTIVITY

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Vinca minor L. (*Apocynaceae*), an evergreen trailing subshrub common in Western and Southern Europe, is a rich monoterpene indole alkaloid source. So far, we have isolated 23 alkaloids from aerial parts of *V. minor*, 12 of them were reported in this species for the first time. Two alkaloid structures are entirely new. The names cosimonine and vincaminorudeine are proposed, and relative configuration determined. In an ongoing study into the discovery of new anti-neurodegenerative drugs from natural sources, we have tested our isolated compounds to inhibit human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BuChE). None of the alkaloids were active against *h*AChE, however, most structures showed interesting inhibition of *h*BuChE with $IC_{50} < 100$ μM. In the later and severe stage of Alzheimer's disease (AD), the expression of AChE is reduced, but the expression BuChE is increased and takes over its role. BuChE is also involved in other pathological AD processes, such as the production of neurofibrillary tangles

and the formation of β-amyloid plaques. The active substances were also evaluated for the probability of a blood-brain barrier penetration (logBB) by *in silico* computational method. The cytotoxicity of isolated compounds was assessed on the panel of 10 tumorous cell lines. Except for eburnamonine, none of the tested alkaloids showed significant cytotoxic activity.

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AROMATIC DERIVATIVES OF HAEMANTHAMINE-TYPE ALKALOID VITTATINE AS POTENTIAL LIGANDS FOR ALZHEIMER'S DISEASE

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Recent research on *Amaryllidaceae* plant family reports the isolation of more than 600 different Amaryllidaceae alkaloids (AA) with different structure types possessing wide range of biological activities. Among the most important biological activities of AA belong activities associated with Alzheimer's disease (AD). Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology and data indicate an exponential rise in the number of cases of this disease. Galanthamine is the one of the most important AA used for treatment of AD and commercially available under the name Reminyl® (galanthamine hydrobromide).

Based on our previous published work of *Hippeastrum* x *hybridum* cv. Ferrari 18 different alkaloids have been isolated so far and they were identified by MS, HRMS and 1D- and 2D-NMR techniques. All alkaloids were tested for their activities associated with AD (*h*AChE, *h*BuChE and POP) and their ability to inhibit the growth of several cancer cell lines. Moreover, the isolated amount and the structure of vittatine allowed us to develop and synthesize thirteen new aromatic esters of the haemanthamine-type alkaloid vittatine. All the semisynthetic derivatives were studied for their inhibitory potential against both *h*AChE and *h*BuChE. The potential candidates were selected to measure the ability to penetrate the blood-brain barrier (BB) and availability to the CNS.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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SEPARATION OF THE CAROTENOID MYXOXANTHOPHYLL FROM *SYNECHOCYSTIS SALINA* BY HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY AND EVALUATION OF ITS IMMUNE-STIMULATING PROPERTIES

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Cyanobacterium *Synechocystis salina* is well-known for biosynthesizing different types of pigments including chlorophyll, carotenoids and phycobiliproteins, many of which have attracted the attention of the industries and researchers due to their varied bio-functionality and applications. One of its synthesized carotenoid pigments is myxoxanthophyll (Fig. 1), a glycosylated monocyclic carotenoid that is rarely found in nature. This yellow pigment has been shown to exhibit antioxidant and anti-hyperglycemic activities.[1,2] Moreover, its potential in the prophylactic and/or therapeutic treatment of undesirable conditions due to oxidative processes has also been disclosed.[3] To the best of our knowledge, this pigment has not yet been offered commercially, and its bio-functional properties of interest in the food, nutraceutical and cosmetic sectors have not been extensively studied. In this study, myxoxanthophyll was isolated from *Synechocystis salina* by high performance countercurrent chromatography (HPCCC) and its identity was confirmed using high-performance liquid chromatography connected to a high-resolution tandem mass spectrometry detector with electrospray ionization source (HPLC-ESI-HRMS/MS). In addition, the potential activation effect on immune cells in response to myxoxanthophyll treatment was investigated, measuring cell surface CD69 expression using flow cytometry. An increase in the number of granulocytes was observed after myxoxanthophyll treatment. The presented data may support the potential use of myxoxanthophyll for strengthening the immune system against bacterial and fungal infections.

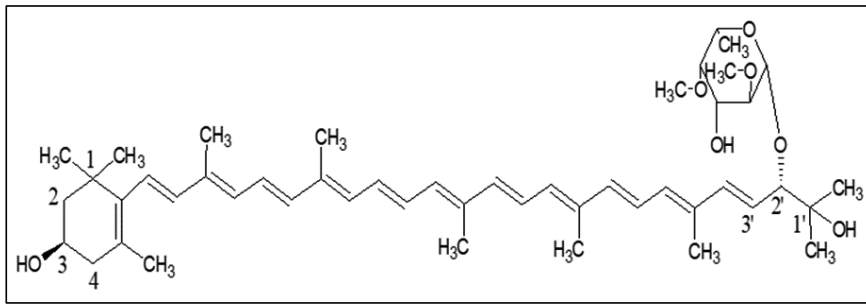

Fig. 1. Chemical structure of myxoxanthophyll

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DERIVATIVES OF MONTANINE-TYPE ALKALOIDS AND THEIR IMPLICATION TO ALZHEIMER'S DISEASE: SYNTHESIS, BIOLOGICAL ACTIVITY, DOCKING STUDY

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The Amaryllidaceae plant family is one of the most important alkaloid containing plant families with biological properties such as antitumor, antimicrobial, antimalarial, and significant antineurodegenerative activities. Among all Amaryllidaceae alkaloids, montanine-type alkaloids are characterized by 5,11-methanomorphanthridine ring system and known for their potential antiproliferative, antibacterial, antimalarial, antirheumatic and anticholinesterase activities.[1] The effect of montanine as an inhibitor of acetylcholinesterase has been reported in a dose-dependent pattern with more than 50% inhibition of enzyme at 1 mM concentration, introducing montanine as a moderate inhibitor of acetylcholinesterase.[2]

In this study, twenty-eight new derivatives of montanine-type alkaloids were synthesized and evaluated for their ability to inhibit human recombinant acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BuChE). Among all, 3 derivatives of 3-*O*-methylpancracine showed significant selective inhibitory potency of *h*AChE (IC_{50} values of 1.6 ± 0.1 μM, 3.1 ± 0.2 μM, and 4.3 ± 0.5 μM). Derivatization of 3-*O*-methylpancracine using 1-piperidinecarbonyl chloride resulted to the most potent *h*BuChE inhibitor with IC_{50} of 1.73 ± 0.05 μM. The same acyl changes on C4 of montanine led to reduction of inhibitory activity on both enzymes.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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PHARMACEUTICAL TECHNOLOGY SECTION

THE ROLE OF pH IN THE FORMATION OF SKIN LIPID BARRIER

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The skin's pH has the peculiar characteristic of being near-neutral (pH ~ 7.4) in deeper layers of the epidermis, while it becomes acidic (pH ~ 5.5) on the surface of the stratum corneum (SC).[1] As it has been previously suggested, the skin's "acid mantle" contributes to the protection against pathogens, the activation of enzymes for skin function, the inhibition of enzymes perturbing SC integrity, and finally, the impedance of the pro-inflammatory cytokines release.[2] This study suggests a new role for the skin's pH, closely related to the formation of the unique multilamellar structure of the lipidic matrix in the SC which is essential for the excellent skin barrier function. Isolated human SC lipids model membranes were treated with buffer pH 5.5 or pH 7.4. X-ray diffraction showed that acidic pH treated lipids formed long periodicity phase (d = 13.53–13.72 nm), short periodicity phase (d = 5.56–5.60 nm), traces of cholesterol phase and for lateral packing orthorhombic conformation (two reflections at 4.1 and 3.7 nm). On the other hand, pH 7.4 treated lipids showed poorly resolved diffractograms with traces of long periodicity phase and a prevalent unknown peak at q = 1.35 nm^{-1}. The functional consequence of the pH-driven change in lipid microstructure was an increased transepidermal water loss at pH 7.4 (27 ± 5.0 g m^{-2} h) compared to pH 5.5 (21 ± 5.2 g m^{-2} h) in either model lipid membranes or isolated SC. These results are consistent with the diminished barrier function in newborn babies, elderly or patients with atopic dermatitis, where SC acidification is incomplete.

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THE USE OF BREAK ENERGY, AN AVALANCHING PARAMETER, FOR POWDER FLOWABILITY PREDICTION

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Pharmaceutical particulate materials are composed of relatively widely size-distributed particles. As flowability of a powder is not an absolute physical property, several measurement techniques are usually needed to describe flow behaviour. Although the conventional pharmacopoeial methods are generally easy to perform, some of them provide inappropriate results in case of more cohesive powders.[1] Accordingly, shear cell testing as well as testing of avalanching behaviour in a rotating drum have become useful methods especially in the case when the conventional methods are less successful.

In this work, the break energy BE (mJ kg^{-1}), a less known avalanching parameter representing the energy needed for an avalanche to start, was investigated. Using 23 pharmaceutical excipients including binary mixtures, the relationship between the BE and powder cohesion estimated by an annular shear cell (*i.e.* shear scan) was studied. The results revealed a good linear correlation between the BE and cohesion with the coefficient of determination $R^2 = 0.823$. Even though a similar correlation was detected also between cohesion and the traditional parameter of avalanche energy, the latter parameter represents the energy produced by an avalanche. Considering theoretical definition of BE as the energy needed to break the interparticle forces, BE represents a better measure of powder cohesion and ability to flow than other avalanche parameters, which will be interesting to study with further materials.

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PLGA NANOSPHERES AS TOOL FOR MACROPHAGES TARGETING

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Macrophages play an important role in maintaining homeostasis of the organism. They influence the progression of various diseases, including chronic inflammatory or immune system disorders.[1] Therefore, macrophages-specific drug delivery systems seem to be interesting therapeutic tool for wide variety of diseases.[2] In this project we investigated several variants of PLGA (poly(lactic-co-glycolic acid) nanospheres (NSs) for their capability of macrophages-specific drug delivery. We used four structurally different PLGA NSs using both nanoprecipitation and emulsification solvent evaporation methods.[3] Prepared formulations were tested in mouse bone marrow-derived macrophages, in mouse AML-12 normal hepatocytes, and in human HepG2 hepatocyte-derived tumor cells. We examined cytotoxicity by MTS assay, inflammatory response of cells by determination of proinflammatory cytokines IL-1β and TNF-α and cell uptake of our NSs by means of fluorescence measurement of loaded fluorescent dye Rhodamine B. We found that nanospheres larger than 100 nm prepared by nanoprecipitation significantly enhanced the distribution of fluorescent dye into macrophages compared to Rhodamine solution, but not into AML-12 cells. No effects of nanospheres on cellular viability was observed. Additionally, no significant proinflammatory effect after nanospheres phagocyting by macrophages was detected.

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EXISTENCE OF A LIPID PHASE TRANSITION CLOSE TO THE PHYSIOLOGICAL TEMPERATURE IN HUMAN SKIN AND ITS POSSIBLE ROLE IN THE PERMEABILITY BARRIER

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Stratum corneum (SC) lipids form major barrier against water loss within the skin of mammals, making their terrestrial life possible. These barrier lipids undergo a transition from a very tight orthorhombic to a slightly looser hexagonal arrangement at approximately 38 °C, which is only a shade above the physiological temperature.[1] The purpose of this lipid transition is unknown. We proposed a *heat-shock lipids* hypothesis: loosening of the lipid arrangement may allow increased water loss, thus enhancing the cooling of the organism at elevated temperatures. The aim of this work was to characterize the human SC permeability for water and model permeants around this lipid transition.

Permeabilities of an *ex vivo* human SC for water, indomethacin as a model permeant and fluorescent inulin as a model macromolecule were studied at 10–50 °C (or 28–50 °C for model permeants). Water loss increased with temperature, however, Arrhenius plot did not show any change in activation energy. In contrast, the activation energy for indomethacin permeability decreased above 40 °C. The permeation experiment with a model macromolecule also gave promising results. Thus, the phase transition of SC lipids just above the physiological temperature is not important for water loss but apparently enables translocation of larger compounds, such as signaling or defensive molecules, in response to heat stress or inflammation.

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DEVELOPMENT OF NANOFORMULATIONS FOR DELIVERY OF BILE ACIDS

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In the 21st century, nonalcoholic fatty liver disease and nonalcoholic steatohepatitis are one of the leading causes of deaths worldwide.[1] Farnesoid X receptor (FXR) is a nuclear receptor expressed mainly in hepatic and intestine cells. FXR is a key regulator of bile acid, inflammatory, fibrotic, and metabolic pathways. Its activation can suppress the inflammation and fibrosis in the liver. Bile acids are endogenous ligands of FXR and play an important role in maintaining hepatic homeostasis. In the case of systemic administration, bile acids have numerous side effects. To reduce side effects and to enhance site specific action, development of hepatic-targeted delivery system is needed. Nanoparticles were prepared using nanoprecipitation method. Size, polydispersity and zeta-potential were measured using the Zetasizer (Malvern Nano ZS, United Kingdom). Cholic acid concentration was measured using the modified spectrophotometrical method. In this study, we optimized the preparation of PLGA nanoparticles, studied a relationship between PLGA type and concentration, Pluronic F127 concentration and PLGA nanoparticles properties such as size, polydispersity and zeta potential. Moreover, lipid-PLGA hybrid formulations were prepared.[2] They were studied in terms of the relationship between different material ratios and nanoparticles properties. Besides that, we developed a fast, robust and reproducible assay for the bile acids. Initially the classic method with sucrose was chosen. Then the assay was optimized in order to achieve sufficient sensitivity. Based on the obtained data, the best conditions were chosen and a calibration curve using blank nanoparticles samples spiked with model bile acid was made. The assay was validated.

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CRITICAL PORE DIAMETER OF FAST CRYSTALLIZING DRUGS NANOCONFINED IN MESOPOROUS SILICA

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Poorly water-soluble drugs are especially difficult to formulate if they are fast crystallizers.[1] Amorphous systems are an obvious challenge for such compounds, and mesoporous silica bear much promise here because of their different stabilizing principle compared to other solid dispersions. The mesoporous silica-based systems generally profit from their ability to significantly inhibit the crystallization process by drug confinement in carrier pores, particularly if the pore dimensions are comparable to the critical nuclei dimension.[2] Therefore, the aim of this study was to investigate the impact of the calculated critical pore diameter of three model drugs (haloperidol, carbamazepine and benzamide) on stabilization of their amorphous form confined in four commercially available mesoporous silica carriers (Parteck® SLC 500, Neusilin® US2, Syloid® XDP 3050 and Aeroperl® 300 Pharma). The absence of crystallinity was periodically studied over three months storage by X-ray powder diffraction. A superior stability was observed for the formulation containing benzamide having rather large values of critical pore diameter of 29.5 nm, particularly when compared to haloperidol with critical pore diameter of 8.4 nm. These findings confirm the importance of estimating the critical pore diameter for nanoconfinement of especially fast crystallizing drugs.

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IMPROVING OF DRUG SOLUBILITY USING SPRAY DRYING PROCESS

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Spray drying is a modern method in which the liquid feeding material (solution, emulsion, suspension) is converted into dry solid particles. Resulting particles of a pharmaceutical excipient or drug have generally improved properties, *e.g.*, flowability. Recently, spray drying has beeen investigated also for improving solubility of poorly soluble drugs. The properties of spray dried particles are directly affected by properties of the used feed (concentration, viscosity, dispersion type) as well as by the process parameters (*e.g.*, inlet air temperature, drying gas flow rate, nozzle diameter, and feed flow rate). In this work, the influence of the solution concentration and drying temperature on the properties of spray-dried lactose particles were investigated using a nozzle with a diameter of 1.4 mm. The lactose solutions of concentration 15% and 20% were spray dried at temperature range 170–210 °C and 150–210 °C, respectively. Obtained particles were evaluated by optical microscope for geometric properties and by differential scanning calorimetry (DSC) for thermal characteristics.

Unlike the initial material (D-lactose monohydrate), the produced particles were spherical and without surface irregularities, having particle size mostly within the interval 2.5–5 μm. At both concentrations, the largest particles were formed at higher drying temperatures. The temperature of the glass transition, crystallization, dehydration and melting point were detected in the thermograms after 6 months of evaluation showing the structural changes in products. The glass transition indicating the amorphous form of lactose was observed in particles produced from 15% solution while no glass transition was observed in case of the 20% solution. Instead, the crystallization occurred, which may indicate lower stability of the material. Based on the results, higher inlet temperature (190–210 °C) is recommended for spray-drying of lactose. The dependence of particle size on solution concentration has not been proved in this experiment.

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APPLICATION OF ADVANCED DESIGN OF EXPERIMENTS TECHNIQUES FOR IMPROVEMENT OF SOLUBILITY OF POORLY SOLUBLE SUBSTANCES

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Scientific research in pharmaceutical technology is based on a large number of laboratory experiments due to a multitude of influencing factors. The most popular types of design used in pharmaceutical technology are factorial and fractional factorial designs for factors with usually two levels.[1] In this work, our goal was to modify current designs of experiments in order to find more sophisticated model applicable for drug development, in particular with reference to the number of levels. The solubility of active substances in water is a fundamental property that plays a key role in bioavailability of the drug product. Unfortunately, more than 70% of new active pharmaceutical substances are either slightly soluble or practically insoluble in water.[2] The process of drug solubility improvement increasing the surface area of the particles by micronisation was chosen as a model as it requires a large number of materials (carriers) and experiments with more than two levels of variables.[3] For our experiments we used an advanced design of experiments that is one of the modifications of the central composite design (CCD) to improve the micronisation process of pharmaceutical carriers for production of interactive mixtures with drugs.

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INTERACTION OF PAMAM DENDRIMERS WITH SKIN BARRIER LIPIDS STUDIED BY MONOLAYERS

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Polyaminoamide (PAMAM) dendrimers have been used to date as effective skin deposition or permeation enhancers for topical delivery. Thanks to their characteristic hyperbranched structure, they can either encapsulate or surface interact with an active substance and deliver it to/*via* the skin.[1] However, the mechanism of action for this process remains unknown. It was previously suggested that PAMAM dendrimers could interact with intercellular skin barrier lipids by fluidization or lipid extraction.[2]

The aim of this study is to probe PAMAM dendrimers' effect on a model monolayer consisting of human *stratum corneum* lipids. This approach is believed to reveal additional information about the interactions between the tested nanostructures with the

skin barrier lipids. Our preliminary data have shown that there is a direct dependence of monolayer formation on the number of terminal amino groups of each dendrimer. More specifically, different generations (G2, G3 and G4) of PAMAM dendrimers were studied in different concentrations. Interestingly, when G4 PAMAM dendrimer was used in different concentrations (2.5, 5 or 10 μM), the organization of lipids to monolayer was affected most by the lowest dendrimer concentration. Additional experiments are in progress to evaluate the level of interaction between the different PAMAM generations and the skin barrier lipids.

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EFFECT OF POLYMER COMBINATIONS ON THE DISSOLUTION PROFILES OF A MODEL DRUG IN BIORELEVANT MEDIA

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Controlled release drug delivery systems are used for optimizing therapeutic efficacy, with the view of maximizing bioavailability and reducing side effects. The most widely used strategy for controlling the release of drugs involves the use of hydrophilic swellable polymers as the release-retardant material, in the form of matrix systems. In this work, the effect of combining two polymers for the purpose of tailoring the drug release characteristics of matrix systems was studied. Theophylline-loaded matrix tablets using guar gum, hydroxypropyl methylcellulose (HPMC) and their combinations in different ratios without any additional excipients (*e.g.*, fillers, lubricants) were prepared by direct compression. Dissolution studies were performed for 24 hours in three biorelevant media – Fasted state Simulated Gastric, Intestinal and Colon Fluids (FaSSGF, FaSSIF and FaSSCoF) of pH 1.6, 6.5 and 7.8, respectively. The results indicated desired sustained release profiles of all formulations with variation in drug release with respect to the proportion of each polymer. Moreover, it was observed that the pH-independent release may be achieved by combining the polymers. These formulations which are essentially pH-independent could lead to a more predictable dissolution profile, which is desirable in the treatment of ailments where there is a constant fluctuation in the GIT pH (*e.g.*, inflammatory bowel diseases). It is expected that varying the ratios of used polymers would optimize the mucoadhesion potential as well. This aspect will be explored in future studies.

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SELF-EMULSIFYING DRUG DELIVERY SYSTEMS ENABLE ORAL OLIGONUCLEOTIDE DELIVERY

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Oligonucleotide-based drugs represent a highly specific therapeutic approach. However, oral delivery of these drugs is hampered by their low stability and poor permeability. Therefore, we formulate an oligonucleotide (OND) into a well-established oral self-emulsifying drug delivery system (SEDDS).[1] Hydrophilic OND was effectively complexed by hydrophobic ion paring with a cationic lipid, either dimethyldioctadecylammonium bromide (DDAB) or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP).[2] Resulting hydrophobic complexes enabled loading into a lipid-based SEDDS containing intestinal permeation enhancers. Negatively charged Citrem SEDDS and neutral Standard SEDDS were tested in terms of OND protection against nucleases and permeability *in vitro* across intestinal Caco-2 cell monolayer. Negative surface charge of Citrem SEDDS was found to interfere with OND complexes and thus diminished its protective effect against nuclease to 16%. Simultaneously, negative charge hinders interactions with negative cell surface resulting in low permeation. Without negative surface charge, neutral Standard SEDDS protected 58% of OND. Due to enhanced interactions with cells, the permeation of OND in Standard SEDDS more than doubled. An appropriate *in vivo* model could shed more light on performance of these promising novel formulations.

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SURFACE ENERGY ANALYSIS OF BINARY MELOXICAM POWDER MIXTURES USING INVERSE GAS CHROMATOGRAPHY

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Nowadays, improving a dissolution rate of poorly water-soluble drugs is one of the major challenges for pharmaceutical research. The aim of this work was to analyze surface energy based on inverse gas chromatography to determine surface properties as a precondition for interactive powder mixtures formation.

The interactive powder mixtures of meloxicam (MX, BCS class II) and chitosan (CH) were prepared in different ratios using physical mixing and co-milling. The dispersive and specific components of the surface energy were determined using non-polar (hexane, heptane, octane, nonane) and polar (dichloromethane, ethyl acetate, chloroform, toluene, ethanol, acetone, 1,4-dioxane) probes, respectively. The fractional sample surface coverage (5%) was constant and carrier gas (helium) with the flow rate of 10 mL min^{-1} was used.

The results showed the higher dispersive γ^D = 46.5 mJ m^{-2} and lower specific γ^{SP} = 2.9 mJ m^{-2} component of MX having an acidic nature, while a lower dispersive γ^D = 40.5 mJ m^{-2} and higher specific γ^{SP} = 4.5 mJ m^{-2} component was detected for CH with a basic nature.

The cohesion forces of the drug particles were in balance with drug-carrier adhesion forces (expressed as the w_{adh}/w_{coh} ratio that was close to one) indicating the successful production of an interactive mixture and its stability. The total surface energy was found to increase after milling but a massive surface amorphization of drug was not observed, which is beneficial for drug stability.

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MICROEMULSIONS FOR DERMAL DELIVERY OF IMIQUIMOD

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Imiquimod (IMQ) is a topically-applied imidazoquinoline used for the treatment of several skin diseases, like actinic keratosis and basal cell carcinoma.[1] Traditional formulations restrict IMQ's efficiency for dermal delivery because of the drug's poor solubility and low cutaneous permeability.[1] The aim of this work was the development and evaluation of an IMQ containing, skin-friendly microemulsion system intented for topical delivery. A pseudo-ternary phase diagram was constructed using a mixture of phospholipids in ethanol as a surfactant system and oleic acid as the oil phase. The physicochemical properties of selected formulations were determined in terms of particle size, conductivity, and rheology. The IMQ skin uptake was evaluated by *ex vivo* permeation experiment in full-thickness human skin. Two of the prepared formulations were able to deliver the active substance more efficiently than the commercial formulation. In addition, the barrier function of microemulsion treated skin recovered faster than that of the control, as was shown from transepidermal water loss experiments. Infrared spectroscopy showed the possible penetration of the microemulsion components into the skin at a molecular level. In conclusion, the presented findings demonstrate that phospholipid microemulsions could become a promising alternative in the topical administration of IMQ.

The study was supported by the Czech Science Foundation (Project No. 19-09600S) and from the project of Specific Academic Research (SVV 260 547).

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PHARMACEUTICAL ANALYSIS AND BIOANALYTICAL CHEMISTRY SECTION

OPEN DATABASE SEARCH FOR ILLUMINATING THE "DARK MATTER" OF BOTTOM-UP PROTEOMICS

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Despite the advancements in proteomic instrumentation, a significant portion of MS/MS spectra from bottom-up analyses still evade identification, even though the spectra quality is sufficient. This "dark matter" of proteomics usually compromises biologically relevant components such as uncommon and rare posttranslational modifications, protein sequence polymorphisms, *etc*.[1] A large fraction of unassigned MS/MS spectra is also a consequence of unexpected protein cleavage sites or peptide modifications during sample handling.

We have recently scrutinized the adverse effects of the combination of elevated column temperature, extended in-column residence time, and low pH of the mobile phase

on tryptic peptides integrity during LC-MS proteomic analyses. To describe possible peptide modifications in an unbiased fashion, we took advantage of an open database search of MS/MS spectra followed by multivariate statistical analysis. In this way, we revealed that peptides bound to the stationary phase can undergo various artificial modifications. Moreover, we serendipically discovered that peptides can be artificially formylated before LC-MS analyses due to dissolving them in a solution containing a mere 0.1% formic acid. We utilized the gained experience in the open-database search to clarify what changes to a model protein induces an irradiated phthalocyanine for photodynamic therapy.

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PERSPECTIVES OF USE OF PROPIONIC ACID TO INCREASE THE SENSITIVITY OF PROTEOMIC BOTTOM-UP LC-MS METHODS

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Proteomic LC-MS analyses can identify and quantify proteins and peptides and characterize their modifications, interactions between proteinous and other molecules, *etc*.[1] Proteomic samples are usually of limited quantity, and they consist of an enormous number of analytes present in small concentrations below the level of appropriate MS detection by typical analytical flow LC-ESI-MS. Although nanocolumns with an inner diameter of 0.075 mm have been successfully implemented to proteomics to introduce peptides via nano-electrospray ionization to a mass spectrometer with the right intensity, they still fail to provide sufficient sensitivity for the most demanding analyses, such as in single-cell proteomics. Currently, several strategies are considered to increase sensitivity in proteomic LC-MS analyses. The modification of the mobile phase composition represents the most straightforward one.

We have recently demonstrated that replacing 0.1% formic acid in the mobile phase by 1% propionic acid significantly increased peak intensities of five standard peptides. Thus, we trust that replacing 0.1% formic acid in the mobile phase with an optimized concentration of weaker propionic acid will increase the MS sensitivity in real-life LC-MS proteomic analyses.

This study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 548).

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EVALUATING THE EFFECT OF LOWER FORMIC ACID CONCENTRATION IN MOBILE PHASE FOR PROTEMIC LC-MS ANALYSIS

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Ideal peak shapes of peptides in proteomic LC-MS analyses have been traditionally obtained when using mobile phases of high ionic strength, but in turn, suppress electrospray ionization. Recent advancements in the stationary phases have introduced the Charged Surface Hybrid (CSH) technology.[1] Reversed stationary phase with CSH technology produces peak shapes with a mobile phase of lower ionic strength equivalent to those when a higher ionic strength mobile phase is used.

Under the scope of the study, we will evaluate the effect of reduced formic acid concentration in the mobile phase on peak symmetry, MS sensitivity, the extent of peptide identification, and the rate of artificial modification. The data on peak characteristics obtained from the advanced CSH column at the varied formic acid concentration (0.1% to 0.01%) were compared with an equivalent reversed-phase column, which is widely used to analyze proteomic samples. A well-characterized set of peptides with varied properties and charge states were included. Because of the surface charged property of the CSH column, much improved peak shapes were obtained at a lower concentration of formic acid when compared to the equivalent reversed-phase column.

This study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003 /0000465) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 548).

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HPLC-FLD/DAD DETERMINATION OF SALIVARY IMMUNE SYSTEM ACTIVATION MARKERS, URIC ACID AND CREATININE IN CLINICAL RESEARCH

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Neopterin, kynurenine and tryptophan are useful and important markers for monitoring the activation of the immune system that accompanies number of diseases such as infection, autoimmune and various malignant diseases. Uric acid (UA), the major urinary nitrogen-containing compound, is the product of purine metabolism in the human body and important antioxidant with albumin and ascorbate in saliva. The main advantages of saliva as a diagnostic biological material are simple, safe, and non-invasive collection. The new chromatographic method using monolithic stationary phase for determination of neopterin, kynurenine, tryptophan, creatinine, and UA in human saliva has been developed and will be presented. Neopterin and tryptophan were detected by fluorescence detection and creatinine, kynurenine, and UA by diode array detection. Filtration as a simple and suitable sample treatment procedure for human saliva was used. This novel HPLC-FLD/DAD method in combination with simple and fast sample preparation procedure was used for testing 56 real saliva samples from patients with cancer (breast, ovarian, colorectal, and renal cancer) and 14 saliva samples from patients with periodontal diseases to monitor early inflammatory response.

The study was supported by Ministry of Health of the Czech Republic – conceptual development of research organization (UHHK, 00179906), by the Czech Health Research Council (Project. No. NV18-03-00130) and from the project of Specific Academic Research (SVV 260 548).

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DETERMINATION OF PLATINUM DRUGS IN HUMAN PLASMA AFTER HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY

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Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) is a therapeutic method that maximally removes the tumour followed by intraoperative administration of a warmed chemotherapeutic agent. The approach aims are to maximize the anticancer effect and lower chemotherapy's systemic side effects. Intraperitoneal application of chemotherapeutics leads to their decreased levels in the bloodstream.[1] Therefore we need to develop more sensitive methods, which allow us to detect the lower concentration to monitor therapeutic levels and toxicity.

An HPLC-PDA method for the determination and quantification of cisplatin or oxaliplatin in the human plasma will be presented. The aim of the method development was to achieve a simple, fast, and sensitive method that could be applied in standardly equiped routine laboratories. As the derivatization agent diethyldithiocarbamate (DDTC) was used.

The separation was carried out in 4.5 minutes using the C18 core-shell column (100 × 4.6 mm, 2.7 µm) combined with F5 column guard. The mobile phase consisted of acetonitrile and water, and the target analyte was detected at 254 nm. The method is compensated by application of palladium chloride as the internal standard. The lower limit of quantification for platinum complexes was 20 ng mL^{-1}.

The study was supported by Ministry of Health of the Czech Republic – conceptual development of research organization (UHHK, 00179906), by the Czech Health Research Council (Project. No. NV18-03-00130) and from the project of Specific Academic Research (SVV 260 548).

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DEVELOPMENT OF UHPLC METHOD FOR ANALYSIS OF VITAMIN K IN SERUM

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Determination of vitamin K, which plays an important role in the human organism, is a challenge in many aspects of bioanalysis. Although vitamin K is not currently routinely analysed, information on its concentration in the human body is crucial, mainly in terms of possible manifestations related to its deficiency. In the analysis of this vitamin, it is a common problem to detect its low concentrations. It is present only in nanomolar concentrations in human biofluids. In addition to the low concentration, there are also obstacles such as its high photosensitivity, the matrix interference, and its adhesion to plastic and glass surfaces. The use of appropriate sample preparation in combination with chromatographic analysis is a solution for enabling the determination of particular forms of vitamin K.[1]

The presented UHPLC method using fluorescence detection was developed for determination of four vitamin K homologues (K1, MK4, MK7, MK9) in serum. The chromatographic separation was achieved on KinetexTM C18 column (1.7 µm, 100 × 3 mm) using gradient elution with methanol/propanol (phase B) and H_2O (phase A). The flow rate was 0.5 mL min^{-1} within a total run time of 13 min. Current results of the method development will be presented.

The study was supported by Ministry of Health of the Czech Republic – conceptual development of research organization (UHHK, 00179906) and from the project of Specific Academic Research (SVV 260 548).

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OPTIMIZATION OF CAPILLARY ELECTROPHORESIS AND SUPERCRITICAL FLUID CHROMATOGRAPHY METHODS FOR THE SEPARATION OF SILYMARIN

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Silybum marianum has been used since ancient times for the treatment of a variety of disorders. The main active constituent of the plant is silymarin, a mixture of the structurally similar flavonolignans silybin A and B, isosilybin A and B, silychristin, silydianin, and the flavonoid taxifolin. The method optimization processes for the silymarin separation using capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) are covered in this study.

The CE method was optimized in terms of concentration and pH of borate buffer, type and concentration of cyclodextrin, organic modifier content, and capillary length. Base-line separation of all flavonolignans was achieved with a background electrolyte containing 100 mM boric acid of pH 9.0, 5 mM heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and 10% (v/v) MeOH in a 80.5/72 cm (50 µm id) fused silica capillary, at an applied voltage of 25 kV with UV detection at 220 and 320 nm.

The SFC method was optimized on two coupled-column systems. Experimental conditions optimized during SFC method development were modifier type and content, flow rate, additives type and concentration, backpressure, column temperature and sample solvent. Baseline separation of all compounds was not achieved. The best performing method was on a Lux Amylose-1 + Lux Cellulose-3 system, with the following chromatographic conditions: 40% MeOH with 0.1% (v/v) trifluoroacetic acid, flow rate 2.8 mL min^{-1} backpressure 125 bar and column temperature 30 °C. The resolution of the critical peak pairs silychristin and silydianin, and isosilybin B and A was 0.85 and 0.70, respectively.

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NEW GRAPHENE BASED SORBENT FOR SOLID PHASE EXTRACTION

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Graphene (G) is two-dimensional sp2 single-atom-thick carbon sheet with hexagonal structure. High specific area (theoretical value 2630 m^2 g^{-1}) and the affinity to carbon

ring structures via π-π stacking interactions make G and graphene oxide (GO) promising candidates for application in analytical chemistry.[1] Several studies used GO or G for preparation of the new sorbents for SPE or SPME, and materials with improved properties were presented.[2–4]

The aim of our project is to try to prepare a new alternative sorbent based on graphene. The work is focused on the modification of the titanium oxide particles (ZirChrom-SAX) by graphene. That will be followed by the study of sorbent retention on the mixture of model analytes (*e.g.*, ibuprofen, lidocaine, propylparaben, metoprolol) with different acid-base properties. Such kind of material is presented for the first time.

Obtained results will be compared with the properties of unmodified particles and GO or G in free form. The quality of particle coating together with retention performance will be discussed.

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DIRECT ELECTROMEMBRANE EXTRACTION OF ANTHRACYCLINES FROM TISSUE SAMPLES

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Tissue analysis presents bioanalytical challenge compared to analysis of liquid biological samples. Firstly, homogenization prior sample treatment is required to ensure both representative sample and effective sample extraction. Further difficulty is determination of analyte extractability, as the spiked tissue sample or even spiked homogenate do not exactly mimic drug binding and distribution in intact tissue. Electromembrane extraction (EME) is a hybrid microextraction technique laying between liquid-liquid extraction (LLE) and electrophoresis. The extraction of charged analytes is performed from the aqueous sample through the water immiscible organic supported liquid membrane (SLM) to the acceptor solution. The driving force of the extraction is an electrical potential, which is applied across the SLM. The aim of this study was to optimize the EME for isolation of anthracyclines (ANT) from tissues (liver, heart and skeletal mus-

cle). Prior the extraction, the collected tissues were shocked frozen and pulverized under the liquid nitrogen using the mortar and pestle method. Calibration samples were spiked directly to the pulverized tissue therefore the binding process of the drugs was not disrupted by the presence of solvent which occur in commonly used homogenate. The EME was performed without further homogenization step. The optimized extraction followed by UHPLC-MS/MS method was validated. In addition, the level of phospholipids in the EME extracts were examined. Finally, the reliability of the method was evaluated by analysis of real tissue samples (n = 6) taken after administration of DAU (3 mg kg^{-1}, *i.v.*) to rabbits. The extraction was done by both, optimized EME and conventional LLE with homogenization (Bio-vortex mixer) prior extraction. The differences between the concentrations determined using EME or LLE were lower than 10%. EME was proved to be simple, reliable, effective and repeatable microextraction technique for isolation of ANT from tissues.

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CHROMATOGRAPHIC DETERMINATION OF ACETYLCHOLINESTERASE REACTIVATORS: K869 OXIME PHARMACOKINETIC PROFILE

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Oxime reactivators of acetylcholinesterase (AChE) represent an integral part of standard antidote treatment of organophosphate poisoning. Oxime K869 is a novel bis-quaternary non-symmetric pyridinium aldoxime with two pyridinium rings connected by a tetramethylene bridge where two chlorines modify the pyridinium ring bearing the oxime moiety. Based on *in vitro* assays, K869 is a potent AChE and butyrylcholinesterase (BChE) reactivator. For the investigation of the basic pharmacokinetic properties of K869 after its intramuscular application, new HPLC-UV and LC-MS/MS methods were developed and validated for its determination in rat body fluids and tissues. In this study, the SPE procedure for sample pretreatment was optimized as an alternative to routine protein precipitation widely used in oxime pharmacokinetics studies. K869 oxime is quickly absorbed into the central compartment reaching its maximum in plasma (39 ± 4 μg mL^{-1}) between 15 and 20 minutes. The majority of K869 was eliminated by kidney via urine when compared with biliary excretion. However, only a limited amount of K869 (65 ± 4 ng g^{-1} of brain tissue) was found in the brain 30 minutes after oxime administration. Regarding the brain/plasma ratio calculated (less than 1%), the penetration of K869 into the brain did not exceed conventionally used oximes.

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CHIRAL SEPARATIONS OF BORON CLUSTER COMPOUNDS IN SFC

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Boron clusters are artificial three-dimensional structures, which exhibit unique physicochemical properties. Carboranes as a subgroup of boron cluster compounds were derived by substituting BH units for CH units. The similar steric volume occupied by a rotating phenyl ring and an icosahedral carborane cage has led to the extensive research of carborane moieties as new pharmacophores. The carborane moiety has already substituted phenyl groups of some conventional pharmaceuticals, *e.g.*, tamoxifen, penicillin, lidocaine, celecoxib, *etc*. and their activity has been tested.

Chirality of carboranes is caused by introducing endo-/exoskeletal substituents, which impairs the symmetry of the cage. Hence, it is vital to evaluate analytical methods for chiral separation of boron clusters concerning their potential use in pharmacy.

Although the successful chiral separations of neutral, zwitterionic, and recently anionic carboranes were achieved by HPLC, no chiral separations of these species have been carried out in SFC so far. In pharmaceutical industry, SFC is generally accepted as the most widely used and most versatile technique for chiral separations thanks to the more straightforward and faster chiral method development and faster chiral separation methods than in HPLC. Hence, we employed SFC to evaluate the chiral separability of the carboranes. Firstly, the chiral screening method was performed on six polysaccharide-based columns. Secondly, the successful enantioseparations achieved in chiral screening were subsequently optimized to gain the baseline separation of the carboranes in the shortest possible time.

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UHPLC-MS IN PHARMACOKINETIC STUDIES OF ANTHRACYCLINES

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Cardiotoxicity, unfortunately, is very often side effect of many drugs. This is also true for frequently used cytostatics – anthracyclines (ANT). GK-667 was developed as a prodrug of ICRF-193, a novel cardioprotective agent against antracycline mediated toxicity. However, the impact of GK-667 on the ANT pharmacokinetics (FK) would be a certain obstacle for its further development. The aim of this project was to develop and validate the UHPLC-MS bioanalytical method for simultaneous determination of daunorubicin (DAU) and its metabolite daunorubicionol (DOL) in plasma and myocardium and apply the method for the analysis of samples taken from FK study with DAU in rabbits. For these purposes, UHPLC instrumentation coupled with a triple quadrupole mass spectrometer with AJS ESI^+ ion source was used (Agilent 1290 Infinity II LC System, Agilent 6470 LC/TQ/MS System). The analysis was achieved on Phenomenex Kinetex C18 column (100 × 2.1 mm, 1.7 μm) protected with a guard column. A mixture of acetonitrile and formic acid (0.0025%) as the mobile phase in a gradient mode was applied. Liquid-liquid extraction with a mixure of chloroform and methanol (4 : 1, v/v) was developed as the most convenient extraction method for both plasma and heart tissue. Method for the analysis of plasma samples was validated according to EMA "Bioanalytical method validation guideline". Concentration profiles of DAU and DOL were assayed in plasma taken after administation of DAU (3 mg kg^{-1} i.v.) alone and with GK-667 to rabbits. These analyses proved no effect of GK-667 administation on the anthracycline's pharmacokinetic profile in plasma. The method validation of ANT determination in myocardium is currently being proceeded for further *in vivo* pharmacokinetic and toxicity studies.

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HPLC-UV-MS STUDY ON THE STABILITY OF PROTEASOME INHIBITOR IXAZOMIB

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Inhibitors of proteasome became a new era of drugs in treating multiple myeloma. Ixazomib is highly selective, reversible and the only orally active proteasome inhibitor approved for clinical use in EU in 2016. The aim of this project was to 1) develop and validate a first stability indicating analytical method for ixazomib, 2) apply it for investigation of degradation kinetic of the drug under stress test conditions and 3) identify main degradation products. The forced degradation of ixazomib was evaluated using the UHPLC-PDA system (Nexera, Shimadzu), identification of degradation products was done on the orbitrap mass spectrometer with ESI ion source (Q-Exactive™, Thermo Scientific). Following chromatographic columns were tested during method development: Zorbax Bonus-RP (100 × 3.0 mm, 1.8 µm, Agilent), Kinetex C18 and Kinetex F5 (both 100 × 2.1 mm, 1.7 µm, Phenomenex) and Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 µm, Waters). The best separation was achieved on the last column and its length was finally shortened to 50 mm to reduce the time of the analysis. A mixture of ammonium formate, acetonitrile and methanol in a gradient mode was used as a mobile phase. The method was validated according to ICH guidelines in a concentration range 2.5–100 µmol L^{-1}, accuracy and precision was assessed in 3 concentrations and 3 replicates each, intermediate precision was measured the following day. Ixazomib was exposed to acidic, alkaline, oxidative, neutral and photolytic stress conditions. Ixazomib was prone to decomposition under oxidative conditions, on the contrary it degraded most slowly under neutral thermal conditions. Main degradation products were identified and they resulted from the splitting of a peptidic bond. In this study, the UHPLC-UV stability indicating method and identification of degration products of ixazomib were described for the first time.

The study was supported from the project of Specific Academic Research (SVV 260 547).

LC-MS/MS STUDY OF FIRST PHASE *IN VITRO* BIOTRANSFORMATION OF NEW PROMISING TACRINE DERIVATIVES

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Alzheimer's disease is a neurodegenerative disorder causing decline in cognitive functions, gradual loss of self-control, development of disorientation, and afterwards motoric failure. Current symptomatic pharmacotherapy is primarily focused on acetylcholinesterase inhibitors and NMDA (*N*-methyl-D-aspartate) receptor blocking. Tacrine molecule, which is one of the acetylcholinesterase inhibitors, was withdrawn from the market due to the hepatotoxicity of its metabolite 7-hydroxytacrine in 2013.[1] Possible substitution of tacrine molecule may potentially hinder the formation of toxic species. The introduction of methoxy or phenoxy group to position 7 on the 1,2,3,4-tetrahydroacridine moiety led to 7-methoxytacrine (7-Meota) and 7-phenoxytacrine (7-Phota), respectively.[2]

The aim of our work was to determine and compare the emerging metabolites of tacrine, 7-Meota and 7-Phota. Human liver microsomes (HLM) were used as the first phase *in vitro* biotransformation model and HPLC coupled with Q Exactive Plus (MS/MS) was used for characterization of metabolites.

New HPLC-MS method for the separation and identification of tacrine, 7-Meota, and 7-Phota metabolites was developed. The structures of metabolites were experimentally designed from Full-MS and MS/MS spectra and confirmed by MassFrontier (MetWorks).

The results of our study showed that the biotransformation of these three compounds led to their monohydroxy and dihydroxy metabolites. Moreover, several novel *in vitro* metabolites, which had not been reported in the literature so far, were found. The relative proportion of individual metabolites was calculated based on chromatographic data.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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HYPHENATION OF SUPERCRITICAL FLUID CHROMATOGRAPHY AND TRIPLE QUADRUPOLE MASS SPECTROMETRY

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The coupling of supercritical fluid chromatography (SFC) with mass spectrometry (MS) brings many benefits, including high selectivity and sensitivity in the analysis of complex matrices. A dedicated SFC-MS interface facilitate this coupling. The commercially available interfaces use a sheat pump delivering a make-up solvent to the system. Even though SFC-MS has already shown its great potential in many application fields, several aspects of this hyphenation remain uninvestigated including the effect of make-up solvent composition on ionization and MS response. Indeed, the composition of the make-up solvent is usually optimized as a function of the properties of the target analytes. This study aimed to search for the correlations between physicochemical properties of the analytes and the increase and/or decrease in ionization when using different make-up solvents.

A generic UHPSFC method with triple quadrupole mass spectrometry detection was developed. 90 analytes with wide range of physicochemical properties were analyzed using (i) 3 different stationary phases including Viridis HSS C18 SB, Viridis BEH 2-ethylpyridine, and Torus diol, (ii) 2 organic modifiers, *i.e.*, methanol and 10 mmol L^{-1} ammonia in methanol, and (iii) 25 make-up solvents including pure alcohols or methanol combined with commonly used additives at variable molarity. The obtained results were statistically evaluated and several conclusions were drawn: (i) The increasing amount of water in the

make-up solvent had the opposite effect on ionization in positive and negative mode. (ii) Different concentrations of ammonia in the make-up solvent had a higher effect than using pure methanol as the organic modifier. On the other hand, (iii) the increasing concentration of formic acid led to the ionization decrease in both modes using both modifiers.

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ADVANCED POLYMERIC SORBENTS IN ONLINE SOLID PHASE EXTRACTION COUPLED TO HPLC

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Current trends in analytical methods development are targeted at the automation of extraction techniques that can significantly save time and sample handling and increase the throughput of routine control laboratories. To avoid time-consuming and laborious sample-preparation, automated online techniques such as online SPE-HPLC by column switching are preferred. Column-switching chromatographic system hyphenizes extraction and separation step by coupling extraction and separation column. The online SPE-HPLC methods introduce the advantages of full automation and excellent repeatability.[1]

The selection of extraction sorbent affects selectivity, clean-up efficiency, robustness, and extraction capacity. The crucial sorbent properties are stability in organic solvents and under high pressure, low cost, reusability, and high extraction capacity. Apart from conventional commercial reversed phase sorbents, we used prepared original sorbents attempting miniaturization or increase in selectivity. In the presented study, advanced polymeric sorbents, such as nanofibers with high surface to volume ratio[2] and selective molecularly imprinted polymers[3], were applied to extract food and environmental contaminants. Preparation of the sorbents, partial steps of optimization, and their evaluation will be discussed.

The study was supported by the Czech Science Foundation (Project No. 20-19297S) and from the project of Specific Academic Research (SVV 260 548).

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COMPARISON OF IMPURITY PROFILES IN LEVOTHYROXINE TABLETS USING METABOLOMICS BASED UHPLC-HRMS METHOD

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The aim of this study was to identify impurities in several formulations of tablets containing levothyroxine using an UHPLC-HRMS metabolomics-based method and to compare the profiles of known and unknown impurities. These impurities may be related to the reported side-effects of levothyroxine tablets. In particular, the method was focused on (1) the identification of known and unknown impurities (molecular or supramolecular) from the active principle ingredient (API) and from the excipients, such as mannitol, lactose, and stearic acid, (2) to the study of molecular interactions between API and the excipients.

An important part of this study was the comparison of different metabolomics workflows including experimental design, data analysis, feature detection (peak picking), statistical evaluation, and compound identification. Analysis of levothyroxine samples underwent randomization process in order to prevent technical/instrumental biases. Three different experimental designs were compared in terms of sample preparation and UHPLC-HRMS analysis. Data processing involved testing of different settings (mass accuracy, thresholds) as well as testing different software platforms.

The data were acquired in MS full scan mode, both ESI negative and positive mode, and will be used in both targeted and non-targeted data processing. In targeted analysis we confirmed the presence of mannitol stearate in levothyroxine drug formulations containing mannitol as an auxiliary ingredient. It was also possible to identify some impurities, for example levothyroxine-lactose adducts (MW = 1100.7923). On the other hand, the presence of supramolecular levothyroxine-mannitol complex and levothyroxine-mannitol adduct was not confirmed in formulations. In the next step, more unknown impurities will be identified, and non-targeted metabolomics will be applied.

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EXTRACTION OF PHENOLIC COMPOUNDS FROM EUCALYPTUS LEAVES USING SUPERCRITICAL FLUID EXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

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Supercritical fluid is a viable alternative extraction solvent for bioactive compounds from their natural sources such as plants, fruits, and flowers. Carbon dioxide is commonly used as supercritical solvent to extract a wide range of non-polar to mid polar compounds. Different parts of eucalyptus tree including leaves, bark, and stems contain both volatile and non-volatile bioactive compounds that have been widely utilized in pharmaceutical and cosmetic industries. In the first part of the present work, supercritical fluid extraction (SFE) using ethanol as the co-solvent is used for the extraction of different phenolic compounds including phenolic acids and flavonoids from eucalyptus leaves followed by the analysis of the extracted analytes using ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS). In the proposed method, the parameters associated with the UHPLC-MS/MS were investigated and optimized first and then factors affecting SFE process will be assessed as well. The electrospray ionization (ESI) parameters were optimized in both ionization modes. MS/MS was performed on triple quadrupole and selected reaction monitoring (SRM) was developed for quantitative analysis of the target phenolics. The calibration curves were prepared to check the linear concentration range and sensitivity. The obtained SFE extract was analyzed using the developed UHPLC-MS/MS and different phenolic acids (such as protocatechuic acid, gallic acid, caffeic acid, and chlorogenic acid) and flavonoids (including catechin, taxifolin, phloridzin, and quercitrin) were identified among the extracted compounds from eucalyptus leaves. In the second stage, we plan to develop supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS) method which would include the analysis of both volatile and phenolic compounds extracted from eucalyptus leaves.

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THE DEVELOPMENT OF EXTRACTION METHOD USING SUPERCRITICAL FLUIDS

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Recently, growing interest in using green technologies for the extraction of valuable compounds with antioxidant properties has been observed. Extractions utilizing carbon dioxide belong to this group. By applying pressure and mild temperature above the critical point of liquid CO_2 or a mixture of CO_2 and more polar co-solvent (ethanol, methanol, water), a supercritical fluid is formed. Supercritical fluids are characterized by higher diffusivity, lower density, viscosity and surface tension. This approach enables the extraction of plant polyphenols under mild conditions and without the use of toxic solvents. Moreover, extractions are performed in the absence of light and air during extraction. Thus, the risk of degradation reactions is minimized.

In this ongoing study, we aim to develop an extraction method for the extraction of polyphenols from dried apples. Since most of the polyphenols typically present in apples are polar, polar co-solvent is needed. For the evaluation of selected analytes (gallic acid, chlorogenic acid, epicatechin, rutin, phloridzin, phloretin, quercetin, quercitrin, catechin, caffeic acid), the newly developed UHPLC-DAD method was used.

In this contribution, the influence of the main tested parameters on the extraction recovery will be discussed. The optimization of SFE was divided into two main parts. The Modde software was used for the preparation of the experimental design. First, Plackett Burman design was used to identify the most significant factors (pressure, temperature, water content in ethanolic co-solvent, CO_2/co-solvent ratio). Second, the central composite design was applied as the second step. At this point, a narrower range of main factors was further optimized. The fixed values of minor factors were chosen regarding the highest recoveries of target analytes. The study will continue with extraction kinetics and the influence of glass beads size on extraction recovery.

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SEPARATION POTENTIAL OF CHROMATOGRAPHIC METHODS IN THE ANALYSIS OF STRUCTURALLY SIMILAR STEROIDS

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The full chromatographic separation of a complex group of isomeric and isobaric steroids remains a complicated analytical challenge, even though GC-MS/MS and LC-MS/MS techniques are well established in their analysis. The analyzed compounds differ only by minor structural modifications. Moreover, a typical loss of one to three water molecules in the MS spectra leads to the formation of additional isobars. To eliminate the observed interferences and correctly quantify the target analytes, it is necessary to achieve full chromatographic separation.

The present study aims at the development and optimization of fast and sensitive UHPLC-MS/MS and UHPSFC-MS/MS methods for the analysis of 37 steroids. The analyzed compounds belong to the C19 androstenes, C21 pregnanes, and synthetic steroid groups and generate 11 critical pairs/groups of analytes due to their structural similarity. Therefore, the separation potential of both chromatographic methods will be examined in detail and compared.

Comprehensive screening of stationary phases was carried out using generic gradient elution in both chromatographic methods, UHPSFC and UHPLC. A screening of mobile phases and gradient optimization followed in the next step using the stationary phases with the most successful separation of critical pairs. Biological samples of mouse plasma were obtained from the Czech Academy of Sciences, dealing with the project focused on

the investigation of changes in levels of expressed steroids due to chronic stress exposure. Hence, the protein precipitation method was developed for a target matrix. Moreover, the physiological levels of some steroids in the plasma make a quantification method even more challenging. Finally, the PP-UHPLC-MS/MS method was selected for validation and the analysis of biological samples.

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AUTOMATED PROCEDURES BASED ON HOMOGENEOUS LIQUID-LIQUID EXTRACTION AND PROTEIN PRECIPITATION USING LAB-IN-SYRINGE APPROACH

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We report on the development of two new Lab-In-Syringe (LIS)-automated salting-out liquid-liquid extraction (SALLE) methods, one in combination with online solid-phase extraction (SPE), coupled to liquid chromatography (LC). In both cases, SALLE was carried out in the void of the syringe including a magnetic stirring bar for on-demand homogenous mixing and using acetonitrile as an extraction solvent miscible with water. A saturated salt solution of magnesium sulfate and sodium chloride was used to induce phase separation. This methodology is suitable for the extraciton of moderately polar compounds, in our work, sulfonamide chemotherapeutics. The advantage of the procedure is the compatibility of the used solvent with LC. On the other hand, only small preconcentration factor can be achieved as phase separation can be achieved for approximately balanced volumes of organic and aqeuous phase.

To improve both preconcentration factor and extract clean-up, in the first work, the extract was diluted in-syringe with alkaline buffer and the analytes were trapped at pH 10 on an anion-exchange resin cartridge integrated into the LC injection loop, thus achieving a double-stage and orthogonal sample clean-up. Analytes were eluted from the cartridge by the acidic mobile phase in gradient elution mode. Running the separation of the analytes and the two-step preparation of the following sample in parallel allowed reducing the total time of analysis to 13.5 min. The method was applied to spiked urine samples yielding an average recovery value of 102.7 ± 7.4% and limits of detection at low ppb level.

A similar procedure can be used for centrifugation-less deproteination of milk samples. In this case, acetonitrile also acts as a precipitating agent. The addition of salt then separates three phases: an organic extract with preconcentrated analyte, precipitated proteins, and the aqueous sample and salt solution mixture. First optimization results from this study will be presented.

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MAGNETIZATION OF COMMERCIAL HLB PARTICLES FOR AUTOMATIC IN-SYRINGE DISPERSIVE MICRO-SOLID PHASE EXTRACTION OF SURFACE WATER CONTAMINANT

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We present a simple approach to magnetic dispersive solid phase microextraction and its automation for the enrichment of water contaminants. Supel™-Select HLB (hydrophilic modified styrene polymer) beads were magnetized by introducing magnetite nanoparticles (Fe_3O_4) into the sorbent. For this, the beads were soaked with $FeCl_2$ and $FeCl_3$ and then washed with NH_4OH leading to the precipitation of magnetite nanoparticles inside the sorbent as reported elsewhere.[1] The so-functionalized sorbent was used in a dispersive solid phase extraction methodology that was automated using the Lab-In-Syringe technique. The methodology followed a previous study on using magnetic nanoparticles.[2] In short, sample and bead suspension are aspirated into the syringe void and dispersed by the aid of a magnetic stirring bar placed inside the syringe. By automatic activation and deactivation of magnetic stirring process, the analytes were captured by the dispersed sorbent beads and then retained on the stirring bar that allowed discarding the sample solution after the phase separation. Thereafter, the eluent of 60 : 40 (v/v) acetonitrile : ammonium phosphate buffer was used to elute the analytes of interest. This extraction system was coupled online to a liquid chromatography instrument for the determination of model analytes mebendazole, bisphenol A, benzyl 4-hydroxybenzoate, diclofenac and irgasan. Essential parameters such as extraction time, elution time, composition and volume of eluent, as well as phase separation time were optimized. The developed method will be implemented for the analysis of the model contaminants in surface water (lakes and rivers).

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WHAT WE CAN AND CANNOT EXPECT FROM EXTRACTION ON NANOFIBROUS SORBENTS?

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Great interest in innovative sorption materials for solid-phase extraction has brought nanofibrous polymers to the scientific scope. Our research group's knowledge about nanofibrous polymers gained over years, resulted in the study where the nanofibrous extraction mechanism is discussed. The eight polymers were tested as extraction sorbents for acetylsalicylic acid, moxonidine, metoprolol, propranolol, propafenone, diltiazem, atorvastatin, and amiodarone present in 3 matrixes, including an organic solvent, human serum, and bovine serum albumin. The extraction was carried out in an extraction cartridge manually filled with nanofibers and online coupled with a high-performance liquid chromatography system via a six-port switching valve. The obtained extraction recoveries were used for the evaluation of analyte retention on individual sorbents. The main aim of this study was to determine how this retention is affected by analyte physicochemical properties and matrix complexity. This understanding should lead to a more conscious application of nanofibrous sorbents in extraction techniques and simplified method development.

As we found out, the tested polymers provided high extraction efficiency for the compounds with log P exceeding 2. It was also confirmed that lipophilicity is the major driving force in the retention mechanism. Moreover, by direct injection of spiked bovine serum albumin and human serum matrix on nanofibers, some of them confirmed their potential to extract analyte and remove protein macromolecules in one step.

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MODERN SORBENTS IN ONLINE SOLID PHASE EXTRACTION COUPLED TO LIQUID CHROMATOGRAPHY FOR DETERMINATION OF BISPHENOLS IN MILK

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Various advanced sorbents for online extraction and determination of bisphenol A, bisphenol AF, bisphenol C, bisphenol A diglycidyl ether, and bisphenol F diglycidyl ether in bovine milk samples have been compared. The milk is a complex matrix containing proteins and lipids. Our approach to sample preparation presents a new online method including fast extraction using precolumn coupled to liquid chromatography with fluorescence detection. Five types of fibrous sorbents, including polyethylene microfibers, polypropylene microfibers, polycaprolactone microfibers/nanofibers composite, polycaprolactone microfibers/polyvinylidene difluoride nanofibers composite, and polyamide 6, were compared in terms of extraction and clean-up efficiency with commercially available molecularly imprinted polymers for bisphenols and restricted access media sorbent RP-18 ADS. The polymer fibers filled in a cartridge and also commercial sorbents were directly connected to the HPLC system and the clean-up step and the subsequent chromatography separation optimized. The separation was carried out using analytical column YMC-Triart C18 ExRS (150 × 4.6 mm, particle size 3 μm) followed by fluorescence detection (Ex 273 nm, Em 300 nm). Solvents suitable for separation were acetonitrile with water under gradient elution, for extraction 15% methanol, and the total flow rate of 1.0 mL min^{-1} was used. The sorbents with the best results were used for control of bisphenol contamination in milk packed in plastic bottles. The measured results were compared with the migration limit established by the European Union for BPA 0.05 mg kg^{-1} in food contact plastics.

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DETERMINATION OF PHENOLIC PROFILE IN FRUIT TREES DURING VEGETATION PERIOD

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The aim of the study was to determine a spectrum of phenolic compounds and their content in apple and pear tree material – leaves, bark, buds, blossom, and fruit. The methanol extracts were obtained from raw material of different cultivars. The main extracted phenolic compounds from apple (phloridzin, phloretin, chlorogenic acid, and quercetin) and pear (arbutin, rutin, chlorogenic acid and its derivatives) tree material were analyzed by two different validated high performance liquid chromatography methods. Finally, YMC-Triart C18 ExRS 150 × 4.6 mm, particle size 5 μm, pore size 8 nm (apple tree) and ASCENTIS Express RP-Amide 150 × 4.6 mm, particle size 2.7 μm (pear tree) analytical columns were used for analysis. Column temperature was 30 °C and injection volume was 1 μl. The separation was performed with gradient elution at flow rate 1 mL min^{-1}.

The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The detection was carried out with diode array detector. To observe changing phenolic profile, sampling was performed in March, June, August, and November during the years 2019 and 2020. The highest concentration of bioactive compounds was found in leaves followed by buds in spring season in both types of studied trees. In that season, the concentration range was from 164.25 mg g^{-1} to 228.85 mg g^{-1} in 10 apple tree cultivars and from 85.04 mg g^{-1} to 161.69 mg g^{-1} in 10 pear tree cultivars. The main phenolic compounds were phloridzin in apple trees, and arbutin and chlorogenic acid in pear trees. This finding can lead to applying fruit tree material as a renewable resources for food supplements or extracts with beneficial effect to human health.

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ADVANCED CHROMATOGRAPHIC APPROACH IN PHENOLIC COMPOUNDS PROFILING IN ARCHIVE TOKAJ WINES

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The presented experiment is focused on the characteristics of archive Tokaj wines, especially in terms of their benefits to human body in the form of antioxidant activity of phenolic compounds.[1] The samples of archive Tokaj wine come from vineyards of the Slovak part of the Tokaj wine region. The Tokaj wine region is one of the few areas with a production of special wine made from grapes affected by noble rot *Botrytis cinerea* under particular environmental conditions, which leads to the production of natural wines with a unique aroma.[2] More than 60 archive samples were evaluated in term of phenolic substances profile, including hydroxybenzoic and hydroxycinnamic acids, stilbenes and flavan-3-ols. Eighteen phenolic compounds were identified. Individual phenolic substances, which differ significantly in their physico-chemical properties, were detected by ultra high performance liquid chromatography method with diode array detection. Separation and quantification were finally conducted on a polar C18 column with core-shell particles (150 mm × 3.0 mm, particle size 2.6 μm) using a gradient elution mode at a flow rate of 1.0 mL min^{-1} with a mobile phase consisting of acetonitrile and 0.1% phosphoric acid at 50 °C. These separation conditions provided reliable validation result with linearity ($R > 0.9996$), precision (CV < 3.91%), recovery (87.35–118.67%), which are considered acceptable for application in the characterization of these types of matrices.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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A VALIDATED UHPLC METHOD FOR THE DETERMINATION OF CAFFEOYLQUINIC AND DI-CAFFEOYLQUINIC ACIDS IN GREEN COFFEE EXTRACTS USING AN RP-AMIDE FUSED-CORE COLUMN

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The aim of the study was to develop and validate new UHPLC method for determination of chlorogenic acids and their di-substituted derivates in nutraceuticals containing the extract of green coffee beans. The developed and validated UHPLC method was used for determination of chlorogenic acids in nutraceuticals Kilostop (Astina Pharm, a.s.), Zelená káva Extra (Medicura Natural), Maxivitalis Zelená káva (Simply You Pharmaceuticals), Vieste Zelená káva Premium (Volt Retail), Zelená káva bylinný extrakt (Topvet), Zelená káva (Vito Life), and Kyselina chlorogenová (Vito Life). For the analysis, extracts were obtained from the samples of nutraceuticals, using methanol and 5% aqueous formic acid solution (25 : 75, v/v). Extracts were filtrated through 0.22 µl PTFE filters. The analysis, which provided satisfying separation of all derivatives of chlorogenic acid, was performed on the Ascentis Express® RP-Amide (100 × 2.1 mm, particle size 2.7 µm) chromatography column using gradient elution program with mobile phase consisted of mixture of acetonitrile and 5% aqueous solution of formic acid. The separation was performed at flow rate of 0.9 mL min^{-1} and the detection was carried out at wavelength of 325 nm using PDA detector. The column temperature was 30 °C. According to the study, in green coffee bean extract samples, chlorogenic acid was found as major component. This research revealed that green coffee bean extracts are rich source of chlorogenic acids. However, the quality of the tested preparations in term of chlorogenic acid content widely differed according to the producer.

The study was supported from the project of Specific Academic Research (SVV 260 548).

BIOCHEMISTRY, PHARMACOLOGY AND TOXICOLOGY SECTION

A PREDICTIVE CAPABILITY OF 3D PRIMARY HUMAN HEPATOCYTE SPHEROIDS IN DRUG-DRUG INTERACTIONS

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Drug-drug interactions (DDIs) represent a serious medical concern being associated with a treatment failure or toxicity. CYP3A4 is among the most important drug-metabolizing enzymes (DMEs) as it is involved in biotransformation of approximately 50% of all marketed drugs. Addressing a capacity of drug candidates to induce CYP3A4 function in drug development is a prerequisite of therapeutic success and patient safety.

Primary human hepatocytes (PHHs) cultured in 2D monolayer are a gold standard to perform CYP induction studies recommended by regulatory agencies. PHHs, however, undergo dramatic dedifferentiation after seeding losing significantly their metabolic capacity. In contrast, 3D spheroids of PHHs generated on ultra-low adherent plates have been shown to closely mimic physiological phenotype of human liver tissue. Moreover, 3D model can maintain stable expression of DMEs for a few weeks enabling to study a long-term effect of drugs on DME expression pattern.

I my talk, I am going to address a role of 3D spheroids in a screening of CYP3A4 inducers putting emphasize on benefits of 3D PHH system such as a high and stable basal expression of CYP3A4. The limits of *in vitro*-to-*in vivo* translation accuracy of CYP3A4 induction in 2D compared to 3D PHH model will be demonstrated on a case of AZD1208 drug candidate. Our in-house experience with 3D spheroids will be also discussed.

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MURINE CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) LIGAND TCPOBOP INDUCED HEPATOMEGALY IN HUMANIZED CAR MICE IS INDEPENDENT OF CAR ACTIVATION

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TCPOBOP – 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene – is a widely-used prototype constitutive androstane receptor (CAR, Nr1i3) ligand. In mice, TCPOBOP after CAR ac-

tivation decreases glucose, triglyceride serum levels and body weight under hypercaloric stress and CAR is thus being studied as potential therapeutic target for metabolic diseases. However, murine CAR activation promotes hepatocyte proliferation and liver tumors. We studied effect of TCPOBOP in the wild type mice as well as in mice with human CAR, which is not activated by TCPOBOP. After treatment, both genetic background mice models had enlarged livers. RNA-seq liver gene expression analysis showed that TCPOBOP triggered massive proliferative response in wild-type mice, but humanized CAR mice transcriptome was completely different with almost no similarly regulated genes. In humanized CAR mice, TCPOBOP regulated significantly endogenous metabolism of fatty acids. Furthermore, array-based western blotting confirmed TCPOBOP-mediated CAR activation only in wild type mice. Liver histology showed only enlargement of hepatocytes and no proliferation of human CAR mice livers. Thus, our data shows that TCPOBOP regulates endogenous metabolism partially in CAR independent manner with possible metabolite accumulation and hepatomegaly.

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DEVELOPMENT OF LRP2 KNOCKOUT MODEL CELL LINES

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Low-Density Lipoprotein Receptor-Related Protein 2 (LRP2, megalin) is responsible for receptor-mediated endocytosis across the tissue barriers. In the kidney, it plays crucial role in the trafficking of high-molecular weight substances from primary urine into the cytoplasm of proximal tubular cells. LRP2 is responsible for internalization of numerous proteins and peptides, *e.g*. albumin, but it can also mediate transport of such compounds as aminoglycoside antibiotics.[1]

Within this project we focused on development of LRP2 knockout cell lines using CRISPR/Cas9 technique, which would represent convenient models for verification of LRP2-mediated drug transport.

Two cell lines originally expressing high level of LRP2, HK-2 and jeg-3 were used for this purpose. Three sgRNA sequences were designed to target 1) NPMY motif, an important site for regulation of function and trafficking, 2) PPPSP motif, that plays central role in phosphorylation of LRP2, and 3) transmembrane domain (TMD).

The modified cell lines exert lower activity of LRP2. The decreased function was verified experimentally using two different approaches. Firstly, lower level of a fluorescent

substrate FITC-albumin was found in LRP2 knockout cell lines in accumulation studies. Secondly, higher viability of the edited cells was observed after treatment with gentamicin indicating reduced accumulation of the toxin. Both findings suggest lower activity of the LRP2 in the edited cell models.

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PROFILING OF PLACENTAL TRYPTOPHAN METABOLISM IN PRETERM BIRTH

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Spontaneous preterm birth is a serious medical condition affecting around 10% of pregnant women. Multiple etiological mechanisms have been suggested, including prenatal infection/inflammation, vascular disorders, breakdown of maternal-fetal tolerance, and stress. Importantly, preterm birth is strongly associated with a wide range of neurodevelopmental, psychiatric, and behavioral sequelae. In recent years, the placental tryptophan metabolic route has been reported as a plausible mechanistic cause implicated in the fetal brain's developmental programming. Recognizing the hostile fetal environment associated with preterm delivery, we aimed to determine the nature and extent of transcriptional alterations in tryptophan homeostasis in pregnancies complicated with preterm delivery. Comprehensive gene expression analysis of the pathway was performed in a well-characterized clinical cohort of pathological pregnancies (n = 197). This study brings information on the differential placental expression of tryptophan pathway genes in term and preterm pregnancies. Moreover, using multivariate analysis, correlation computations and mediation analysis, we provide a putative relationship between maternal inflammation, tryptophan pathway and pregnancy length in the preterm group.

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NOVEL PERSPECTIVES INTO STEROIDOGENIC PATHWAYS IN THE HUMAN PLACENTA AND PRIMARY HUMAN TROPHOBLAST CELLS

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The placenta is a crucial steroidogenic organ secreting primarily progesterone and estrogens, necessary for pregnancy maintenance and proper fetal growth and development. The human placenta has been deemed incapable of androgen production, rendering it dependent on maternal and fetal sources. In this study, we revisit placental steroidogenesis by focusing on the enzymes involved in the synthesis and metabolism of sex hormones and corticosteroids and their regulation during gestation. We evaluate the placental cell models used to study placental steroidogenic functions and explore the *de novo* synthesis and release of steroids by the primary trophoblast cells isolated from the human term placenta. Collectively, we present novel aspects of steroid homeostasis in the placenta and the developmental regulation occurring during human gestation. Importantly, we provide evidence on placental trophoblast cells' contribution to the prenatal maintenance of steroid synthesis, metabolism, and secretion. Altered levels of steroid hormones are associated with a wide range of pregnancy complications. Thus, understanding the physiological dynamics in steroid biosynthesis, metabolism, and release is critical in deciphering the biological importance of placental steroids during the prenatal period.

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EFFECT OF ANTIDEPRESSANTS ON PLACENTAL SEROTONIN HOMEOSTASIS, IMPORTANCE OF FETAL SEX

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It has been reported that up to 20% of pregnant women are affected by depression and approximately 10% are prescribed antidepressant drugs (ADs). However, safety of this treatment is still unclear, since poor pregnancy outcomes such as organ malformations, neurological disorders and preeclampsia have been reported in pregnant woman using ADs. Nowadays, the most frequently prescribed ADs in pregnancy are selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and noradrenalin reuptake inhibitors (SNRIs). Nevertheless, their potential effects on placental serotonin homeostasis have not been properly investigated to date. For proper fetal development/programming, accurate serotonin levels are of crucial importance, therefore, concentrations of this monoamine must be tightly regulated in the feto-placental unit. Here we investigated the effects of antidepressants on the function of serotonin reuptake transporter (SERT) and organic cation transporter type 3 (OCT3) in human and rat placenta. Experiments with *in situ* perfused rat term placenta and *ex vivo* membrane vesicles isolated from human term placenta demonstrate dose-dependent inhibition of SERT and OCT3 by paroxetine, citalopram, fluoxetine, fluvoxamine, sertraline and venlafaxine on apical and basal membranes. Moreover, our data indicate the role of fetal sex in inhibition of OCT3 in rat placenta, which is independent of OCT3 expression.We propose a new mechanism by which ADs alter serotonin homeostasis in the placenta and which may explain reported poor outcomes after antidepressant use in pregnancy.

The study was funded by the Czech Science Foundation (Project No. 20-13017S), by the Grant Agency of Charles University (Project No. 1464119) and from the project of Specific Academic Research (SVV 260 549).

PHARMACOKINETICS OF ANTI-HIV DRUGS TENOFOVIR DISOPROXIL FUMARATE AND TENOFOVIR ALAFENAMIDE FUMARATE IN THE INTESTINE

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HIV represents a serious chronic incurable disease where lifelong treatment based on drugs combination is required to control virus replication and prevent complications. Tenofovir is a backbone of many antiretroviral therapy combinations and is available in two different prodrug formulations that improve its bioavailability – tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF). Both prodrugs have a different pharmacokinetic profile which may affect their effectiveness and safety. Previous studies suggested TDF is hydrolyzed by gut and plasma esterases, while TAF is hydrolyzed mainly intracellularly by cathepsin A. In this study, we aimed at the investigation of the effect

of the intestinal barrier on the pharmacokinetics of these prodrugs and the testing their interactions on/with the intestinal P-glycoprotein (ABCB1). Using *ex vivo* accumulation assays in rat precision-cut intestinal slices (PCIS) we found that, TDF is rapidly and almost completely hydrolyzed to tenofovir monoester and tenofovir while TAF is hydrolyzed to tenofovir to a lesser extent. In addition, we found that the model inhibitor of ABCB1 CP100356 significantly increased the accumulation of TDF/TAF and its metabolites in PCIS. In conclusion, we have demonstrated that the small intestine plays an important role in TDF and TAF hydrolysis, and absorption of both drugs can be influenced by inhibition of P-glycoprotein.

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ROLE OF ABCB1 TRANSPORTER IN DRUG SENSITIVITY OF ACUTE MYELOID LEUKEMIA

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High mortality of patients diagnosed for acute myeloid leukemia (AML) is, among other reasons, caused by resistance to pharmacotherapy. Especially leukemic cells possessing CD34 marker represent intriguingly sturdy population to standard AML treatment. One of the aspects confering resistance to anticancer therapy is expression of ABC transporters, specifically ABCB1 and partly also ABCG2, efflux transporters well-associated with poor prognosis of AML patients. At diagnosis, FMS-like tyrosine kinase 3 (FLT3) mutation is considered a major negative prognostic factor, hence midostaurin, FLT3 inhibitor, has been introduced as a primary treatment option for adult AML patients with the known FLT3 mutation. Our main goal was to determine our cohort of AML patients for the expression of ABC transporters and evaluate the ABC-related behaviour of leukemic cells to midostaurin-potentiated treatment. Using *ex vivo* isolated peripheral blood monocyte cells (PBMC) from patients *de novo* diagnosed for AML, we detected high *ABCB1* expression levels in $CD34^+$ leukemic cells whereas lower number of transcripts was found for *ABCG2*. This *ABCB1* expression correlated well with midostaurin-mediated increase in intracellular levels of mitoxantrone when applied together suggesting the importance of the efflux mediated mechanism in diminished sensitivity of $CD34^+$ leukemic cells to chemotherapy. The same effect was confirmed *in vitro* using resistant HL-60 ABCB1 cells also showing proapoptotic effect of the drug combination when subjected to various apoptosis detecting assays. Therefore, we can assume that the expression of ABCB1 might be an important factor in AML therapy (including the novel FLT3 inhibitor midostaurin) affecting the therapeutic outcomes.

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RELATIONSHIP BETWEEN SELECTED microRNAs AND ABCB1 IN ACUTE MYELOID LEUKEMIA

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ABCB1 expression has been repeatedly associated to drug resistance development and poor prognosis of patients diagnosed with acute myeloid leukemia (AML). MicroRNAs (miR) are short singlestranded non-coding RNAs that are crucial post-transcriptional regulators of gene expression. Using collection of 30 peripheral blood samples obtained from patients *de novo* diagnosed for AML, we evaluated expression of three microRNAs, *miR-9*, *miR-27a* and *miR-331* known to be expressed in AML, and investigated their relation to *ABCB1* expression and function.

Number of miRs copies in peripheral blood mononuclear cells of AML patients was analyzed using digital droplet PCR. Based on the median copy value we identified subgroups of high/low miRNA expressing patients. Interestingly, patients with high *miR-9* showed significantly lower levels of *ABCB1*. Moreover, we could observe clear correlation between number of miR-9 copies and the effect of novel FLT-3 inhibitor midostaurine on ABCB1-mediated efflux of mitoxantrone from the cells. No significant differences in *ABCB1* expression were observed among the *miR-27a-* and *miR-331-* expressing subgroups.

In conclusion, miR-9 appears to be a promising marker of drug resistant AML phenotype, worthy further investigation.

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EVALUATION OF PERIPHERALLY CROWDED CATIONIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY

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Photodynamic therapy is a highly specific and clinically approved therapeutic procedure for cancer treatment based on the production of cytotoxic reactive oxygen species upon the light activation of an otherwise nontoxic photosensitizer. For this project, the novel peripherally crowded cationic phthalocyanines (Pcs) containing 8 or 16 pyridyl moieties (neutral or quaternized) were synthesized and their photodynamic properties were further evaluated in detail.

Photodynamic activity was demonstrated on several cell lines – malignant (HeLa and MCF-7) and non-malignant (3T3 and EA.hy926). The results of individual *in vitro* experiments have shown very high phototoxicity with EC_{50} up to 47 nM (MCF-7 cells) after irradiation while maintaining desirably low inherent toxicity (in the dark), with TC_{50} > 600 000 nM. The Pcs were localized intracellularly, primarily in the lysosomes. This led to their membrane rupture after activation of Pcs and induced apoptosis with subsequent secondary necrosis. This fact was confirmed by real-time monitoring of Annexin V binding and the loss of cell membrane integrity. In conclusion, this work has demonstrated that a bulky and rigid arrangement of peripheral cationic substituents is very efficient for providing good photophysical properties and high photodynamic activity in the development of novel photosensitizes.

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TOPOISOMERASE II INHIBITORS AND PREVENTION OF ANTRACYCLINE TOXICITY ON CARDIOMYOCYTES

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Anthracyclines (ANTs) remain indispensable in many cancer treatments. Use of all drugs of this class, however, is limited due to risk of severe cardiotoxicity. To date, dexrazoxane (ICRF-187) is still the only cardioprotective agent approved for clinical use. This compound, therefore, represents the main lead in the search for effective cardioprotective agents. The focus regarding its cardioprotective mechanism has shifted from metal chelation to topoisomerase II (TOP2) modulation, which is the aim of this work.

To gain more complex insight into the underlying mechanism, diverse commercially available compounds described in literature as TOP2 inhibitors were screened on primary cultures of neonatal rat cardiomyocytes for ability to prevent damage induced by daunorubicin. Additionally, since there are no data of the compounds being assessed for TOP2

inhibition side by side under the same conditions and toward both TOP2 isoforms, the inhibitors of interest were also assayed for their respective inhibitory potency towards both TOP2 isoforms using decatenation assay. Since other aim of this work is also a search for potential lead cardioprotective agent(s), novel analogues of most promising inhibitors were also studied.

The combined results support the notion that TOP2 is involved in the development of ANT-induced cardiotoxicity but the whole situation appears to be more complex as there is not a simple and direct correlation between inhibitory potency and cardioprotection. More detailed investigation of modes of TOP2 inhibition seems necessary to properly answer this question.

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THE EFFECT OF SELECTIVE TOPOISOMERASE BETA INHIBITOR XK469 ON ANTHRACYCLINE CARDIOTOXICITY

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Anthracycline (ANT) cardiotoxicity represents a considerable limitation of several antineoplastic regimes. As much as the ANT anticancer effect, their cardiotoxicity effect excels in its complexity. During their long history, the scientific opinions shifted from the traditionally discussed iron chelation hypothesis to topoisomerase II (TOP2) inhibition. TOP2 occurs in two isoforms with diverse functions. While TOP2 alpha (TOP2A) is a crucial enzyme for cell division and thereby is expressed in replicating cells, TOP2 beta (TOP2B) is present in all cells including quiescent cells. Currently, TOP2B has been massively discussed as the potential mechanism of ANT cardiotoxicity. Although dexrazoxane (DEX) was approved by the FDA as a safe and efficient cardioprotectant, its clinical use is limited only to specific groups of patients. One of the main reasons for the restriction is the concern of the possible negative effect on the antineoplastic activity of ANT in the clinical setting. This interaction (although only speculative) could be based on the non-specific inhibition of both TOP2 isoforms by DEX. The development of TOP2B-specific inhibitor could provide a possible means to the effective cardioprotection avoiding the effect on the cancer cells. XK469 was described in literature as a TOP2B specific inhibitor initially discovered as an antineoplastic drug that proceeded to the second phase of several clinical trials. Nevertheless, its use for cardioprotection has never been studied. Our pilot data showed that XK469 possesses an ability to prevent cardiomyocytes from ANT-induced cell death, although this effect is complicated with the toxicity towards cardiomyocytes in higher doses and prolonged incubation times. We

also described the XK469 inhibitory activity on individual TOP2 isoforms. Moreover, its effects on ANT-induced apoptosis and DNA damage were determined by caspase assay, comet assay and assessment of phosphorylation of H2AX in both leukemic HL-60 cells and cardiomyocytes.

The study was supported by the Czech Science Foundation (Project No. 18-08169S) and from the project of Specific Academic Research (SVV 260 550).

TEPOTINIB REVERSES MULTIDRUG RESISTANCE BY INHIBITING THE EFFLUX FUNCTION OF ABCB1 AND ABCG2 TRANSPORTERS *IN VITRO*

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ABC (ATP-binding cassette) drug efflux transporters play a crucial part in drug-drug interactions and multidrug resistance (MDR) of cancer cells. Tepotinib, a novel c-MET tyrosine kinase inhibitor, has been recently approved for the treatment of non-small cell lung cancer (NSCLC). This study aims at the investigation of the inhibitory effect of tepotinib on human ABC transporters and evaluation of its role in MDR *in vitro*. First, results of our accumulation experiments showed that tepotinib inhibits ABCB1 and ABCG2 transporters. Second, using MTT assay we found that tepotinib can reverse daunorubicin and mitoxantrone resistance mediated by ABCB1 and ABCG2, respectively. Furthermore, bidirectional transport assays designated tepotinib as ABCB1 substrate with no affinity to either ABCC1 or ABCG2. Interestingly, although ABCB1-mediated tepotinib's efflux was demonstrated in transport experiments, functional overexpression of ABCB1 in several cellular models had no appreciable impact on its antitumor activity. Finally, tepotinib did not change the mRNA levels of *ABCB1*, *ABCG2*, and *ABCC1* in selected cell lines by qRT-PCR assay. To sum up, tepotinib could participate in drug-drug interactions by inhibiting the function of ABCB1 and ABCG2 transporters and potentially reverse the MDR in cancer cells. Nevertheless, follow-up experiments with more sophisticated preclinical models are necessary to verify possible clinical impact of our results.

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THE ESTABLISHMENT OF *EX VIVO* PRIMARY LUNG TUMOR MODELS AND THEIR APPLICATION FOR TESTING OF DRUG COMBINATIONS

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In this study, we aimed to establish *ex vivo* explants derived from non-small cell lung cancer (NSCLC) biopsies in cooperation with clinicians. The NSCLC biopsies from patients were obtained immediately after resection, which was followed by collagenase-assisted liberation of tumor cells from tissues. NSCLC cells were separated from physiological cells and fibroblasts and the epithelial origin of cells was confirmed. Once the primary cultures were established, we detected the protein expression levels of ABC drug efflux transporters (ABCB1, ABCG2, and ABCC1) in them. Moreover, transporters' functional activity was assessed in the samples with detectable expression by employing the accumulation flow-cytometric studies. In addition, in drug combination studies, we demonstrated that tepotinib overcame transporter-mediated resistance to conventional cytostatics in primary explants exhibiting functional activity. Finally, *ABCB1*, *ABCG2* and *ABCC1* gene induction studies showed that tepotinib does not have the potential to affect the multidrug resistance phenotype of NSCLC cells. Our results indicate that tepotinib might be a valuable candidate for the combination therapy of NSCLC tumors expressing drug efflux transporters.

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The study was approved by the University Hospital Ethics Committee (Document No. 202002 S04P).

SILENCING OF SELECTED UDP-GLYCOSYLTRANSFERASE GENES BY RNAi IN *HAEMONCHUS CONTORTUS*

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Drug resistance is a serious problem in many organisms, including parasites. The identification of specific enzymes responsible for drug resistance can lead to a new approach to the treatment. UDP-glycosyltransferases (UGTs), enzymes metabolizing xenobiotics and eobiotics, protect the helminth from the toxic action of anthelmintics by their conversion to inactive glycosides.

In our project, we focused on gastrointestinal nematode, a parasite of small ruminants, *Haemonchus contortus*, and revelation its mechanism of drug resistance. RNA interference (RNAi) was used for the characterization of gene functions in nematode *C. elegans*. Therefore we tested the usability of this method in *Haemonchus contortus*. The silencing of selected UGTs can prove their involvement in the metabolism of anthelmintics.

The silencing of selected UGTs can be mediated through specific double-stranded siRNA (small interfering RNA), which cause degradation of the corresponding mRNA *in vivo* and lead to the selected enzymes loss of function. The efficacy of gene suppression by siRNA was optimized. Worms and larvae were exposed to siRNA with different transfection reagents by soaking and subsequently, the RNA was isolated. The suppression of tested genes was determined by qPCR analysis. Despite all the efforts, our selected enzymes were not possible to silence, therefore another method for functional testing has to be employed.

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USING MACHINE LEARNING IN EVALUATION OF *IN VITRO* VIABILITY TESTS IN *HAEMONCHUS CONTORTUS*

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Haemonchus contortus is a gastrointestinal parasite of sheep with great ability to develop resistance to anthelmintic drugs. With the worsening situation, there is a need for new therapies, testing of drug sensitivity as well as addressing the increases in nematode resistance. At present, there are several viability tests, each having some limitations. Egg hatch test (EHT) and larval development test (LDT) are commonly used *in vitro* methods which are relatively simple, nevertheless, they require labor-intensive counting of eggs or larvae at different stages under a microscope. In our study, we applied machine learning in the evaluation part of EHT and LDT, which was the object detection and classification from microscopic images. We adopted Mask Region-based Convolutional Neural Network (Mask R-CNN)[1] in Python 3, Keras, and TensorFlow model as it is the state-of-the-art approach for object recognition tasks. We leveraged a pre-existing model and re-trained it using our dataset. By generating bounding boxes and segmentation for each instance of an object, the model recognizes eggs from first-stage larvae and/or third-stage larvae. Using the Mask R-CNN methodology, we were able to automate the process to a high degree of accuracy.

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SERTRALINE AS A NEW POTENTIAL ANTHELMINTICS AGAINST *HAEMONCHUS CONTORTUS?*

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Haemonchus contortus is a parasitic nematode of small ruminants which cause problem to many farmers around the world. Grazing management, biological control of pastures, nutritional suplementation, vaccination and selective breading play important role in prevention of this disease. However, chemoterapy still represents the main strategy to control infections caused by *H. contortus*. Due to wide spread resistance to main clases of anthelmintics (benzimidazoles, macrocyclic lactones, amino-acetonitrile derivative) it is necessary to look for new structures. Synthesis of new chemical entities is complicated, long, and costly process. For this reason, exploiting old structures and finding new indications for already registered drugs represent a good alternative.[1] Sertraline is used in human medicine as antidepresant and belongs to the class of selective serotonin reuptake inhibitors. Sertraline showed to be effective against *Trichuris muris*, *Ancylostoma caninum* and *Schistosoma mansoni*.[2] In our project we wanted to know if sertraline is also effective against *H. contortus*. Moreover, we are interested about its biotransformation and hepatotoxicity in the host sheep.

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METABOLISM OF HELENALIN *IN VITRO* AND ITS INTERACTION WITH HUMAN CYTOCHROME P450 2A13

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Helenalin (HEL) is a sesquiterpene lactone, especially found in *Arnica montana* and *Arnica chamissonis*, that demonstrates potent anti-inflammatory activity mediated by direct alkylation of Cys38 within the DNA binding domain of RelA (p65), a key member of the nuclear factor-κB (NF-κB) family of transcription factors. Besides, HEL has been found to exert remarkable anticancer, antibacterial and antiprotozoal activity. HEL is an active component of herbal arnica preparations that have long been used to reduce muscle and joint pain, post-surgical pain as well as to treat minor sports injuries, bruises and swelling associated with trauma, contusions and sprains. Despite the frequent use of these products, neither the fate of HEL in the human organism, nor its interaction with xenobiotic-metabolizing enzymes has been studied so far. To address this issue, we have first investigated the metabolism of HEL and characterized the kinetics of metabolite formation using human and rat liver subcellular fractions and human recombinant cytochrome P450 (CYP) enzymes. UHPLC-MS/MS technique was employed for this purpose. HEL was oxidized into five metabolites by both human and rat liver microsomes, and the oxidation was generally far more efficient in rat microsomes. Moreover, several human CYP isoforms were found to be involved in the oxidation of HEL, namely CYP2A13, 2B6, 3A4, 3A5 and 3A7. In the following experiments, we have also tested the inhibitory potential of HEL towards human recombinant CYP enzymes. Out of all tested CYPs, the most effective inhibition (the lowest IC_{50} value) was observed for CYP2A13. In addition, the inhibition of CYP2A13 was NADPH- and time-dependent suggesting that HEL may act as a mechanism-based inhibitor of CYP2A13.

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UDP-GLYCOSYLTRANSFERASES AND ALBENDAZOLE METABOLISM IN THE JUVENILE STAGES OF *HAEMONCHUS CONTORTUS*

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The UDP-glycosyltransferases (UGTs), second phase biotransformation enzymes, inactivate xenobiotic substrates. Such transformation is involved in drug-resistance mechanisms in one of the most pathogenic parasites in small ruminants, *Haemonchus contortus*. In the previous study, we characterized the UGT family in *H. contortus*, including its nomenclature, phylogeny, and expression in adults – the parasitic life stage.[1] However, the drug-metabolism by UGTs in the free-living juvenile stages may as well represent an important defence system against anthelmintics. Due to the almost permanent contact with anthelmintic residues excreted from treated animals in the environment,[2] UGTs in juvenile stages may also contribute to drug-resistance development. Therefore, we decided to study the expression of UGTs and the metabolism of albendazole (ABZ) and the primary active ABZ metabolite ABZ sulfoxide (ABZSO) in the eggs, L1s, and L3s larvae of *H. contortus*. We have also compared the formation of ABZ metabolites and selected UGT transcripts between a sensitive isolate (ISE) and resistant isolates (IRE and multi-resistant WR). Furthermore, we explored the inducibility of UGTs by ABZ and ABZSO in *H. contortus* juvenile stages.[3]

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TESTING FERN EXTRACTS FRACTIONS ON ANTI-INFLAMMATORY AND ANTHELMINTIC PROPERTIES

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Ferns are used in traditional medicine in Asia and therapeutic effects of several fern extracts have been proven. However, there is still space for searching for new bioactive phytochemicals in ferns. Previous results showed significant anti-inflammatory activity of several selected fern species with higher potential in selective COX-1 inhibition comparing to moderate inhibition of COX-2 and 5-LOX activity. Based on these results we decided to make fractionation of extracts of three fern species (*Athyrium distentifolium*, *Dryopteris aemula* and *Blechnum spicant*) by using sequential liquid-liquid techniques.[1] Redissolved fractions (n-hexane, chloroform, ethylacetate, n-butanol) and residual aqueous fraction have been tested for anti-inflammatory and anthelmintic activity. *Dryopteris aemula* showed the highest potential of all fractions in selective COX-1 inhibition, its

n-hexane and chloroform fractions showed only moderate selective COX-2 and 5-LOX inhibition. All fern species and their fractionated extracts have been tested for anthelmintic activity using Egg Hatch Test[2] with Barber's pole worms (*Haemonchus contortus*). None of the fern samples reduced egg hatching in EHT compared with the control.

The study was supported from the project of Specific Academic Research (SVV 260 550).

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IN VITRO EVALUATION OF THE PHOTODYNAMIC ACTIVITY OF NOVEL BODIPY DYES

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Neoplastic diseases are nowadays one of the most common reasons of death in developed countries. Therefore, a great attention is dedicated to the development of new methods for the treatment of those diseases. One of such methods is the photodynamic therapy (PDT). This is a selective, minimally invasive method with a minimum side effects. Principle of PDT is the application of inactive drug, called photosensitizer (PS), followed by exposure to activating light with suitable wavelength in the presence of molecular oxygen. Therefore, the photodynamic therapy needs three basic components: the PS, light and oxygen.[1,2]

As it has been said, one of the most important part of PDT is the PS. The objective of this study is assessing the effectiveness of the novel PSs from the group of BODIPY dyes *in vitro*. All of the studied compounds were evaluated on malignant human cervical cell line (HeLa) and human melanoma cell line SK-MEL-28. Cytotoxicity after the exposure to activating light (phototoxicity) and intrinsic toxicity in the absence of light (dark toxicity) were determined. Further, the subcellular localization PSs after accumulation in cells was assessed using confocal microscopy and fluorescent organelle-specific probes. All of studied compounds proved to be efficient PSs (EC_{50} up to 0.14 ± 0.01 μM) with no dark toxicity up to their solubility limit.

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CAROTUXIMAB AFFECTS ENDOGLIN EXPRESSION AND ADHESION AND TRANSMIGRATION OF MONOCYTES VIA ENDOTHELIAL CELLS IN 7-KETOCHOLESTEROL INDUCED ENDOTHELIAL DYSFUNCTION

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Endoglin (Eng) is a transmembrane glycoprotein affecting TGF-β signaling pathway. In the previous study, we demonstrated that treatment of endothelial cells with 7-ketocholesterol (simulating oxidized LDL) leads to the development of endothelial dysfunction with upregulated levels of Eng, cell adhesion molecules (ICAM-1, P/E-selectin), and also increased adhesion and transmigration of monocytes via endothelial cells. Carotuximab (TRC105) is a novel monoclonal antibody that binds Eng with high affinity and block/inhibits its effects, currently studied in different clinical trials mostly focused on cancer. We hypothesized that carotuximab mediated inhibition of endoglin will affect 7-ketocholesterol induced endothelial dysfunction. Human aortic endothelial cells (HAEC), passage 5, were cultured with appropriate supplements until reaching 90% confluence. Afterwards, cells were treated with or without 300 μg mL^{-1} carotuximab for 1 hour followed by addition of 10 μg mL^{-1} 7-ketocholesterol for another 12 hours. Gene expression of Eng, cell adhesion molecules and Eng transcription factors (KLF6, NF-kB p65 and LXR) was measured by qRT-PCR. Protein levels of Eng, adhesion molecules, MMP-14, pSmads and adhesion assay were quantified by flow cytometry. Soluble endoglin levels were measured by ELISA. Transmigration assay was performed with cell culture inserts and assessed by flow cytometry. We demonstrated that carotuximab was able to prevent 7-ketocholesterol induced Eng and ICAM-1 expression, as wells as adhesion and transmigration of monocytes via endothelial cells. Therefore, we propose that carotuximab might be important in prevention of endothelial dysfunction induced by hypercholesterolemia, but to which extent must be further investigated.

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SOLUBLE ENDOGLIN AS A POTENTIAL BIOMARKER OF NASH DEVELOPMENT, PROMOTING NASH PROGRESSION IN MOUSE LIVER

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Nonalcoholic steatohepatitis (NASH) is characterized by liver steatosis with inflammation and fibrosis. Membrane (tissue) endoglin (Eng), a Tgf-β co-receptor, was shown to participate in fibrosis and inflammation. Increased levels of soluble endoglin (sEng) were found in patients with hypercholesterolemia and type 2 diabetes mellitus. Therefore, we aimed to determine hepatic Eng expression and sEng levels during NASH development, and explore whether higher levels of sEng modulate cholesterol and bile acids (BA) metabolism and affect NASH progression. Three-month-old transgenic male mice overexpressing human sEng (hsEng) and their wild type littermates were fed for six months with either FFC-diet (NASH diet) or chow diet. NASH, BA biochemistry, liver expression of Eng, sEng levels, inflammation, fibrosis markers, enzymes and transporters involved in hepatic cholesterol and BA metabolism were assessed by histology, LC-MS, qRT-PCR and Western blot. FFC-diet significantly increased mouse sEng levels and hepatic expression of Eng. High levels of hsEng resulted in an increased liver deposition of cholesterol (due to reduced conversion into BA) and increased TAG liver concentration (via reduced TAG elimination by β-oxidation combined with reduced hepatic efflux). We propose that sEng might be a biomarker of NASH development and that the presence of high levels of sEng might support NASH exacerbation by impairing the essential defensive mechanism protecting liver against excessive TAG and cholesterol accumulation, suggesting the importance of high sEng levels for transition of simple steatosis into NASH.

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STUDY OF DAUNORUBICIN METABOLITE DAUNORUBICINOL WITH REGARD TO ISOLATED NEONATAL RAT CARDIOMYOCYTES

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The anthracyclines (ANTs), such as doxorubicin and daunorubicin (DNR), are among the most potent anticancer drugs and play an undisputed key role in the treatment of many neoplastic diseases. The potential limitation in their use is risk of severe cardiotoxicity, which may occur anytime in the life of the survivors.[1] Several hypotheses have been proposed to elucidate mechanism(s) of ANTs cardiotoxicity. The oxidative stress hypothesis, impairment of calcium metabolism, the alcohol metabolite hypothesis and more recently the influence of topoisomerase IIβ.

The production of ANTs alcohol metabolites is well documented, nevertheless the available data about their cardiotoxicity are somehow contradictory. Therefore, the influence of DNR and daunorubicinol (DNR-ol) to isolated neonatal rat ventricular cardiomyocytes (NVCM) was investigated. The toxicity after 24 and 48 hours, predominantly observed for long term treatment, was less expressed than for DNR, with significant protection of dexrazoxane. To elucidate, whether the DNR-ol enters the cells, the pharmacokinetic study was performed. We observed that after 24 hours, the concentration of DNR-ol in NVCM was even higher after DNR-ol treatment, compared to treatment with DNR. After this treatment the concentrations of DNR and DNR-ol in incubation medium were comparable (48 hours). Visually, the cells treated with DNR-ol appeared less damaged than for DNR. Accordingly, so far obtained data in our time schedules, show a lower cardiotoxic potential of DNR-ol toward NVCM.

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ANTIPLATELET ACTIVITY OF 3,3-DIMETHYL-6-OXOPYRANO[3,4-*c*] PYRIDINES

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Both pyridine and pyrano derivatives have previously shown biologically relevant cardiovascular activities.[1,2] Incorporation of both scaffolds resulted in a series of 6-oxopyrano[3,4-*c*]pyridines. Using our established methodologies, their antiplatelet, anticoagulant and vasodilatory activity, as well as toxicity, were evaluated. None of the tested compounds demonstrated anticoagulant effect, but the most active compound **3a**

(3,3,8-trimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile) was a potent antiplatelet compound with IC_{50} twice as low as the clinically used acetylsalicylic acid. A series of further mechanistic tests showed that **3a** interferes with calcium signalling inside the cells. The compound is also non-toxic and in addition possesses vasodilatory activity. In conclusion, **3a** is an interesting compound with both antiplatelet and vasodilatory potential, worth of future *in vivo* testing.

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DO BISPHENOLS INFLUENCE ARTERIAL BLOOD PRESSURE?

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Bisphenol A and its structural analogues are environmental contaminants found in daily use products. These compounds can interfere with the physiological role of endocrine system leading to harmful effects on human health, with possible impact on the cardiovascular system. Recent epidemiological studies have shown that increased urinary BPA concentration in humans is associated with various types of cardiovascular diseases, including hypertension and both acute and chronic forms of coronary artery disease. Nevertheless, the outcomes from studies with bisphenols on blood pressure are ambiguous. Therefore, in this experimental study four bisphenols (A, F, S and AF) were tested *in vivo* for their potential effect on arterial blood pressure and hemodynamics after acute administration. Additionally, bisphenol AF was also tested in a chronic setting. Heart function was measured by pressure-volume catheter inserted in the left ventricle and the blood pressure by pressure transducer inserted into the *arteria iliaca sinistra*. The bisphenols were administered cumulatively as a *i.v.* bolus in the doses of 0.005, 0.05 and 2.5 mg kg^{-1}. Chronic effect was tested by daily intragastric gavage of a dose of 2.5 mg kg^{-1} for four weeks. In the acute study, bisphenols F, S and A significantly decreased blood pressure in female rat only in the dose 0.05 mg kg^{-1}. In male rat, bisphenol A was found as an active compound in the highest concentration used (2.5 mg kg^{-1}). Solely a tendency to decrease systolic and diastolic arterial blood pressure was also found in the chronic study.

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VASODILATORY EFFECTS OF BISPHENOLS IN ISOLATED RAT AORTA

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For decades, bisphenols have been used to manufacture polycarbonate plastics and epoxy resins, materials often utilized for food packaging. Humans are mainly exposed to bisphenols through the diet. The report of numerous adverse health outcomes associated with bisphenol A led to its replacement by surrogates. However, the safety of these new analogues remains unclear. The aim of this work was to study their effects on the cardiovascular system. For this purpose, a screening of the vasodilatory effects of fourteen bisphenols (A, AF, AP, C, B, E, F, Z, G, P, M, BP, S and PH) was performed using the rat thoracic aorta *ex vivo*. The most potent compounds BPAF demonstrated vasodilatory properties with EC_{50} values ~ 60 μmol L^{-1}. The mechanistic study addressing the influence of endothelial nitric oxide synthase, calcium-activated K^+ channels and muscarinic receptors, and the L-type Ca^{2+} channels was performed as well. Vasodilatory mechanism of BPAF demonstrated to be endothelium-independent, occurring *via* L-type Ca^{2+} channels on smooth muscle cells. The same channels also demonstrated to play a role in the action of bisphenols A, F and S.

These achieved results stay in contrast to some animal and population studies which reported that bisphenols are causing vasoconstriction. Hence more detailed *in vivo* experiments are needed in order to reveal the effect of bisphenols on the cardiovascular system.

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DEVELOPMENT OF A COMPLEX METHODOLOGY FOR SCREENING OF COBALT CHELATORS

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Cobalt as a structural part of the vitamin B_{12} is an essential microelement for living organisms including humans. However, cobalt poisoning, which can be caused among others by industrial exposure to cobalt metal dust or follows its release from metal hip prosthesis, is associated with pathological conditions. Cobalt intoxicated patients can

develop different manifestations including neurological impairment, hypothyroidism or cardiomyopathy. The aim of this work was to prepare a standardized and precise method for the complex screening of cobalt chelators including (1) detection of cobalt chelation efficiency, (2) an effect on cobalt based hydroxyl radical (•OH) formation and (3) an impact on healthy erythrocytes. In the first part, spectrophotometric detection using 1-nitroso-2-naphthol-3,6-disulfonic acid disodium salt as the indicator was used. This method enables cobalt detection with sufficient sensitivity around 500 nM or less in wavelengths from 470 to 540 nm at 4 tested pH conditions (4.5, 5.5, 6.8 and 7.5). The methodology was verified by four known metal chelators: 8-hydroxyquinoline (8HQ), nitroxoline, chloroxine and disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA). In the second part, the HPLC method based on our previous method for detection of Cu and Fe-triggered •OH generation was extended to cobalt. These experiments showed that nitroxoline in line with its dose dependently cobalt-chelating activity decreased cobalt-triggered •OH formation. In the third part, erythrocytes exhibited high resistance to even high concentration of cobalt. Interestingly, chloroxine is not toxic to red blood cells but it induced very pronounced red blood cell lysis in presence of cobalt ions in contrast to nitroxoline. On the other hand, Na_2EDTA demonstrated the ability to protect red blood cells from lysis unrelated to the presence of cobalt. In conclusion, a complex method for screening of cobalt chelation, which as far as we know, has not been available previously, was developed.

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SCREENING OF FLAVONOIDS AND THEIR METABOLITES FOR ANTICOAGULANT EFFECTS

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A number of flavonoids, such as genistein, daidzein, and tectorigenin, have proven *in vitro* antiplatelet activity with equal of even higher potency that the reference drug acetylsalicylic acid (ASA).[1,2] Flavonoids can also potentially have anticoagulant effects, since structure-activity relationship studies have suggested that the molecular presence of three aromatic rings in flavonoids was crucial in thrombin inhibition.[3] A molecule presenting both effects can be useful in some indications but can as well lead to potential serious adverse reactions, such as spontaneous hemorrhage or delayed wound healing. For these reasons, screening for the anticoagulant effects of new compounds is important. A total of sixty-two compounds were tested, twenty-nine of them were well-known flavonoids present in diet and thirty-three flavonoid metabolites. Whereas none of the tested compounds showed a significant change in prothrombin time, seven compounds demonstrated significant, but mild changes, on activated partial thromboplastin time at the highest con-

centration tested (100 μM). The obtained results indicate that the compounds have low clinical effect on coagulation pathway.

The study was supported from the project of Specific Academic Research (SVV 260 549).

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CLINICAL AND SOCIAL PHARMACY SECTION

SYSTEMATIC LITERATURE REVIEW OF STUDIES ON POTENTIALLY INAPPROPRIATE PRESCRIBING IN OLDER ADULTS IN CENTRAL AND EASTERN EUROPE AND THE PROGRESS ON THE HORIZON 2020 EUROAGEISM ESR7 PROJECT

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Potentially inappropriate prescribing (PIP) in older adults is a serious and prevalent problem associated with negative economic, clinical, and humanistic outcomes[1,2,3,4]. Before conducting analyses of international data collected in several European countries in ESR7 EuroAgeism H2020 project, we aimed this study to systematically review the literature on PIP in older adults residing in Central and Eastern Europe (CEE). We searched MEDLINE and Embase databases until June 2019 and reference lists of included studies and relevant reviews. We included studies that assessed the prevalence of PIP in older adults (aged 60 years and older) in all settings of care using validated tools. Two authors independently screened titles/abstracts and full-texts, extracted data, assessed risk of bias (by Joanna Briggs Institute's critical appraisal checklist for studies reporting prevalence data), and assessed certainty of evidence by GRADE approach. Large variations in outcome measurement precluded meta-analysis. Therefore, we synthesized data using descriptive statistics (median, interquartile range and range). We identified 1890 records, of which 27 studies were included. All studies were cross-sectional, but one that was uncontrolled before-after study. The PIP prevalence was: minimum 6.5%, maximum 95.8%, median 34.6%, interquartile range 25.9–63.2%, 26 studies, 1139693 participants, very low certainty of evidence. The median PIP prevalence varied across the settings of care – it was higher in studies conducted in long-term and outpatient care than in studies

conducted in acute and community care (71.1 and 53.8% vs 34.5 and 31.5%, respectively). This review confirmed that PIP is highly prevalent in the CEE region, furthermore, these results will serve as a starting point for our scientific works on analyses of PIP prevalence in several EU and non-EU countries participating in the ESR7 EuroAgeism H2020 project.

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PRESCRIBING OF HYPNOSEDATIVES IN SENIORS IN ACUTE AND AMBULATORY CARE IN THE CZECH REPUBLIC – INOMED AND EUROAGEISM H2020 PROJECT FINDINGS

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Insomnia is a frequent problem in older adults, particularly in those suffering from polymorbidity and treated by polypharmacy. The aim of our study was to describe the prevalence of insomnia and patterns of potentially inappropriate prescribing in hypnosedative drug use in acutely hospitalized and ambulatory care seniors in the Czech Republic. Evaluated data comprised comprehensive geriatric characteristics (CGC) of 438 acutely hospitalized and 563 ambulatory care geriatric patients (≥ 65 years) from regionally different study sites in the Czech Republic (Prague, Brno, Hradec Králové). All patients were prospectively assessed using the EuroAgeism H2020 study protocols. Descriptive statistics was applied in data analysis. On the whole, 34.6% (n = 151) of acutely hospitalized seniors and 30.6% (n = 172) in ambulatory care had recorded diagnosis of insomnia in personal history, respectively. The most frequently used hypnosedatives were: antipsychotics e/n (18.5% and 17.8%), Z-drugs (16.2% and 8.2%) and benzodiazepines (14.2% and 7.6%). We determined also non-geriatric dosing of Z-drugs (10.5% and 6,0%, respectively) and BZDs (0.5% and 2.7%), as well as non-geriatric lenght of drug therapy of Z-drugs (5.9%

and 2.7%, respectively) and BZDs (5.3% and 11%, respectively). Excessive indication of antipsychotics e/n and inappropriate geriatric dosing of Z-drugs and long-term use of BZDs e/n were documented in seniors in the Czech Republic.

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AGEING IN DEVELOPING COUNTRIES AND PHYSICIAN'S KNOWLEDGE OF EXPLICIT CRITERIA OF PIMs IN OLDER PATIENTS AND BARRIERS TOWARDS APPROPRIATE GERIATRIC PRESCRIBING

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Potentially Inappropriate Medication (PIM) use is associated with increased mortality and morbidity in older adults and prescribing patterns are strongly influenced by physicians' knowledge of geriatric prescribing. We aimed to assess the barriers among physicians to appropriate geriatric prescribing, their knowledge of specific explicit criteria of PIMs and the frequency of use of different information sources and guidelines. A descriptive pilot cross-sectional study was conducted among physicians (n = 256) in two tertiary care teaching hospitals. Knowledge and preferences in usage of different information sources and barriers to appropriate geriatric prescribing were assessed by validated clinical vignettes and by a piloted questionnaire. Pearson's chi-squared test, Student's t-test, Mann–Whitney's U test and logistic regression models were applied using statistical R software (version 4.0.3). Of 201 (78.5%) respondents, majority were males (63.2%), 74.1% received training in geriatric medicine and 39.8% were currently providing more than once a week care for older adults in long-term care facilities. Only 31.8% of physicians felt confident in appropriate geriatric prescribing and mean score of knowledge of PIMs by clinical vignettes was 3.5 ± 0.9. Multivariate logistic regression revealed the higher odds of good geriatric knowledge in females, age group of 30–39 years, and in physicians that received geriatric training. Positive trend of better scoring in clinical vignettes correlated with the extend of use of PIM criteria. Limited options in drug formularies (80.6%), lack of acceptable therapeutic alternatives (69.1%), potential drug-drug interactions (63.6%) and lack of time (62.1%) were the top cited barriers to appropriate prescribing in older adults. Educational interventions and interprofessional clinical pharmacy cooperation may improve appropriateness of geriatric prescribing in daily clinical practice.

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PREVALENCE OF POLYPHARMACY AND RISKS OF POTENTIALLY INAPPROPRIATE MEDICATION USE IN OLDER POPULATION IN A DEVELOPING COUNTRY: A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS

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Aim: This systematic literature review and meta-analysis aimed to investigate the prevalence of polypharmacy and PIM use and major risk factors associated with PIM prescribing in older adults in Ethiopia.

Methods: We searched PubMed/MEDLINE, Scopus, Embase, and Google Scholar databases to identify relevant studies published between January 1990 to October 2020. Observational studies reporting the prevalence and association of risk factors with polypharmacy and PIM use in older population were meta-analyzed. A multilevel meta-analysis was conducted to pool the prevalence estimates, and the risk of PIM use was reported as a relative risk (RR) with 95% confidence interval (CI).

Results: We identified by systematic literature review 404 articles. Of those, eight studies fulfilled inclusion criteria, comprising a total sample of 2608 participants. The overall prevalence of polypharmacy and PIM use pooled by meta-analysis in the Ethiopian older population was 33% and 37%, respectively. The risk factors of PIM use were analyzed in the meta-analysis (particularly polymorbidity, polypharmacy, gender, and older age), the older age of 65+ (RR:1.71, 95% CI: 1.16–2.51) was significantly associated with PIM use.

Conclusion: This first meta-analysis from a developing country revealed a high prevalence of polypharmacy and PIM use in the Ethiopian older population. There was no awareness to the risk of PIMs in patients with polypharmacy and polymorbidity, and only older age significantly predicted PIM use. Interventions ensuring rational geriatric pharmacotherapy are essential not only in developed countries, but also in developing countries in order to reduce the expected burden of PIM-related geriatric morbidity, higher costs, and mortality.

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RISK STRATIFICATION OF PATIENTS FOR POSTOPERATIVE INFECTION AFTER KNEE OR HIP ARTHROPLASTY – PRELIMINARY RESULTS

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Surgical site infection is a potential complication of all surgical procedures. There is double mortality in patients with developed infection who underwent a surgical procedure compared to non-infected patients. This study aimed to stratify patients according to the risk of postoperative infection and verify the findings in clinical practice. This prospective study has started in March 2020 in the Department of Orthopedics at University Hospital Hradec Králové. The study included patients aged ≥ 18 years who underwent hip or knee arthroplasty. Inflammatory markers, especially neutrophil-to-lymphocyte ratio (NLR), prognostic inflammatory and nutritional index (PINI) and intensive care infection score (ICIS), were analyzed one day before operation (−1D), two days after operation (2D) and in outpatient examination (OE). 42 patients (16 women and 26 men) with an average age of 64.04 ± 10.21 were included in the study. Hip arthroplasty underwent 31 (73.8%) and knee arthroplasty 11 (26.2%) patients. Cefazolin was administered in 88.1% and vancomycin in 11.9% of operations.

−1D: NLR > 4, PINI score > 21 and ICIS > 4 were identified in 5, 0, and 1 patient, respectively. 2D: NLR > 4, PINI score > 21 and ICIS > 4 were identified in 14, 24, and 4 patients, respectively. OE: NLR > 4, PINI score > 21 and ICIS > 4 were identified in no patient at all. ACS (American Society of Surgeons) risk score > 5% was identified in 9 patients. The postoperative infection was identified in 2 (4.7%) patients and postoperative anemia in 12 (28.6%) patients. The average length of hospitalization was 11 ± 6.7 days. Increased inflammatory markers could be suitable for detecting early postoperative infection. However, a more extensive set of patients is needed for further detailed statistical analysis.

The study was supported by the project of Specific Academic Research (SVV 260 551).

PREVALENCE AND RISK FACTORS OF CARDIOVASCULAR DRUG – DISEASE INTERACTIONS IN SENIORS IN ACUTE AND AMBULATORY CARE IN THE EUROAGEISM H2020 PROJECT

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Older patients are vulnerable to drug-disease interactions (DDIs) because of age-related pharmacological changes, polymorbidity, and polypharmacy. The aim of this study was to investigate the prevalence and risk factors of cardiovascular (CVS) DDIs in older adults in ambulatory and acute care facilities in the Czech Republic. The data presented are preliminary. Data of 1152 patients 65+ were collected using the EuroAgeism H2020 protocols in 4 acute care hospitals and 4 ambulances (Jun 2019 – Jan 2020). Prevalence and risk factors of CVS DDIs were identified according to STOPP/START criteria, the EU(7) PIM list and Beers 2019 criteria. The majority of participants were females (67.4%) and 38.7% were 85+ years old. Polypharmacy (5+ medicines) was identified in 85.3% patients and 90.0% suffered from polymorbidity (4+ diagnoses). Prevalence of at least 1 CVS DDIs was: by START criteria 75.4%, STOPP criteria 30.7%, EU(7) PIM criteria 29.0% and BEERS criteria 21.9%. Higher odds of being prescribed at least 1 CVS DDIs were in seniors suffering from 2+ CVS diagnoses (OR = 12.2, 95% CI 7.3–21.5, $p < 0.001$), in polypharmacy users (OR = 2.2, 95% CI 1.3–3.9, $p = 0.005$), and in 85+ years old seniors (OR = 3.1, 95% CI 1.6–6.1, $p = 0.001$). Our study documented high prevalence of CVS DDIs and risk factors in older patients in different care settings in the Czech Republic. Therefore, safer geriatric prescribing should be promoted, and regular medication check provided by clinical pharmacists.

The study was supported by the EuroAgeism European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 764632, by InoMed (Project No. CZ.02.1.01/0.0/0.0/18_069/0010046) co-funded by the European Union, by the Research programme Development and Study of Drugs (Progres Q42) and from the project of Specific Academic Research (SVV 260 551).

DRUG-RELATED HOSPITAL ADMISSIONS VIA THE EMERGENCY DEPARTMENT: A CROSS-SECTIONAL STUDY

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Drug-related hospital admissions (DRA) have attracted much research attention worldwide. The aims of this study were to determine the prevalence and preventability of DRA, to identify the implicated medications in DRA and to examine the preventability aspects of DRA. The study examined hospital admissions via the emergency department in the University Hospital Hradec Králové between August and September 2018. Planned hospitalisations and visits to the emergency room without inpatient hospitalisation were not included. The data were collected retrospectively using electronic medical records. The process of DRA identification included screening for potential adverse drug events which were the main or contributory reason for hospital admissions, causality assessment and assessment of contribution to hospital admission. DRA due to medication errors were considered preventable and DRA due to adverse drug reactions were considered non-preventable. Anatomical Therapeutic Chemical classification system was used for the classification of medications implicated in DRA. Out of 1204 hospital admissions 193 have been identified as DRA. 144 DRA were related to treatment safety while 49 DRA were related to treatment effectiveness. The prevalence of DRA was 16.0% (95% CI 14.0–18.1) and the preventability of DRA was 54.4% (95% CI 47.4–61.4). Medication classes most commonly involved in DRA related to treatment safety included antithrombotic agents, antiinflammatory and antirheumatic products, diuretics and antineoplastic agents. Diuretics, antihypertensives, antithrombotic agents and drugs used in diabetes represented the most common medication classes involved in DRA related to treatment effectiveness. Measuring the scope and nature of DRA is essential for the development of risk minimization measures. The relatively high preventability suggests there is a potential to reduce DRA in the future.

The study was supported by the Grant Agency of Charles University (Project No. 14120) and from the project of Specific Academic Research (SVV 260 551).

ADHERENCE AND BELIEFS ABOUT IMMUNOSUPPRESSANTS IN PATIENTS AFTER KIDNEY TRANSPLANTATION: RESULTS FROM UNICENTRIC FOLLOW-UP STUDY

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Strict medication adherence to immunosuppressants is essential during the whole time after kidney transplantation (KTx). Therefore, the aim of our study was to evaluate self-reported adherence and beliefs about immunosuppressants over time and links with clinical outcomes such as graft functioning and *de novo* malignancy. This observational study is a part of the multiphase project TAKTIS (developing, implementing, and testing

of an integrated care model for adults after KTx). Data were collected at the outpatient post-transplant clinic in the University Hospital Hradec Králové. Adult patients at least 4 weeks after KTx were invited for the structured interview, followed by self-administered questionnaire survey. Primary outcomes such as adherence and beliefs about imunosuppressants were measured by validated international tools. Two data collections conducted between 2016 and 2019 involved 134 patients. Non-adherence to immunosuppressants was reported by 18 (13.4%) patients at the baseline and by 15 (11.2%) at the follow-up. The perceived necessity of immunosuppressants decreased while concerns increased over time ($p < 0.001$). Baseline higher concerns were associated with the less than full score in adherence ($p = 0.0445$) or with the new onset of cancer. On the contrary, higher baseline needs corresponded with better kidney functioning. Both results remained significant after adjusting for age ($p = 0.0493$ and $p = 0.0059$, respectively). As a conclusion, self-reported non-adherence remained similar over the reporting period and beliefs about immunosuppressants corresponded with clinical outcomes related to both under- and over-immunosuppression.

The study was supported from the project of Specific Academic Research (SVV 260 551).

THEORY-DRIVEN DEVELOPMENT OF A MEDICATION ADHERENCE INTERVENTION DELIVERED BY E-HEALTH AND TRANSPLANT TEAM IN ALLOGENEIC STEM CELL TRANSPLANTATION: THE SMILE IMPLEMENTATION SCIENCE PROJECT

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Medication adherence to immunosuppressants in allogeneic stem cell transplantation (alloSCT) is essential to achieve favorable clinical outcomes.[1] Over 600 apps supporting medication adherence exist, yet they lack successful implementation likely because of lack of end-user involvement and theoretical underpinnings in their development and insufficient attention to implementation methods to support the use in real-life. Medication adherence has 3 phases: *initiation*, *implementation* and *persistence*.[2] We report the theory-driven development of a medication adherence intervention (implementation and

persistence phase) in alloSCT patients as a first step for future digitization and implementation in clinical setting within the SMILe project (*Development, implementation and testing of an integrated care model in allogeneic SteM cell transplantatIon faciLitated by eHealth*).

We applied the Behavior Change Wheel (BCW) and Capability-Opportunity-Motivation and Behavior (COM-B) model using 3 suggested stages[3] followed by one stage added by our team as preparation for digitization of the intervention: (I) Defining the problem in behavioral terms, (II) Identifying intervention options, (III) Identifying content and implementation options, (IV) SMILe Care Model Development. Scientific evidence, data from a contextual analysis and patients', caregivers' and clinical experts' inputs were compiled to work through these steps.

(I) *Correct immunosuppressant taking* and *timing* were defined as target behaviors. The focus of the intervention was determined within the COM-B dimensions Capability (*e.g. lack of routine*), Opportunity (*e.g. lack of cues*) and Motivation (*e.g. lack of problem solving*). (II) Five intervention functions were chosen, such as *education*, *training* and *enablement*. (III) Twenty-four behavior change techniques were selected, *e.g. action planning* and *problem solving*. (IV) Finally, 17 user stories were developed to guide the *SMILeApp's software development process*.

Our example on the theory-driven development of an eHealth powered intervention in alloSCT using a rigorous 3+1-stage approach based on BCW, COM-B and agile software development, can be used as methodological guidance for other eHealth intervention developers. Our approach has the potential to enhance the successful implementation and sustained used of eHealth solutions in real-life.

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ASSOCIATION OF MATERNAL ANTHROPOMETRIC PARAMETERS AND NUTRITION WITH MILK PRODUCTION DURING LACTATION

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Human breast milk is a unique source of newborn nutrition and provides a balanced profile of nutrients. Our study is focused on the evaluation of maternal anthropometric parameters, mainly fat-free mass (FFM), adipose tissue mass, body mass index (BMI), and

intake of nutritional energy and macronutrients (NEMI) with respect to breast milk production in Czech women, especially at the end of pregnancy and during the nine months after parturition. Fifty-one healthy Czech pregnant women (volunteers from prenatal courses of University Hospital Hradec Králové) were enrolled to this study. Measurements were done in the last pregnancy period (36th–38th gestational week) and in four lactation phases (3 weeks and 3, 6, 9 months postpartum). Seven-day nutritional records were assessed by the program NutriDan. The bioimpedance spectroscopic method was applied for body composition analysis. The milk sample was sucked by breast pump after 6 hours of non-breastfeeding. Data showed a positive correlation between NEMI and milk volume (MV) during the four periods postpartum. At the end of pregnancy, protein intake was positively associated with FFM ($p < 0.05$). Greater representation of maternal FFM is related to higher MV production. Women's adiposity and BMI negatively correlated with MV. In women with BMI above 28 kg m^{-2} at the end of pregnancy milk production was reduced below 1.5 mL per kg of women body weight 3 weeks after parturition.

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PERSISTENCE WITH LIPID LOWERING MEDICINES IN SLOVENIA

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Lipid lowering medications, specifically the hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins), are considered to be one the most frequently used medications in Europe as well as in the United States.[1] The insuficient medication persistence may lead to decompensated lipidemia or cardiovascular events, related healthcare costs and increased mortality in patients with hyperlipidemia.[2]

The aim of this study was to perform drug utilization analysis of statins (alone or in combinations) between 2015 and 2019 and to assess the medication persistence in a cohort of patients who iniciated the therapy in 2015 and continued until 2019. For this purpose the Slovenian health claims data on prescription medicines were applied. The database was obtained from the Health Insurance Institute of Slovenia and contained patient's and physician's demography, date of refill, number of drug packages refilled and number of defined daily doses (DDD) per package. Medicine consumption was computed as number of DDD per thousand inhabitans per day (DDD/TID). Discontinuation gap was defined as more than 135 days without medication refill. The analysis was conducted using IBM SPSS, ver. 26 and MS Excel.

The preliminary results show that the total annual number of statin prescriptions and patients increased during the study period from 796,514 to 890,940 and from 229,543 to 254,842 (cca 13% of Slovenian population), respectively. The patients during the study period comprised averagely 50.3% males and the mean age was 68.3 years. The total consumption of statins was 621.33 DDD/TID, highest consumption was registered for rosuvastatin (361.79 DDD/TID) and atorvastatin (179.83 DDD/TID). The study cohort of new patients on therapy in year 2015 contained 30,111 patients (who received 317,877 prescriptions in the period 2015–2019), 68.7% of these received at least one prescription in 2016. The discontinuation gap was captured in 38,067 patients between 2015–2019. The ongoing results are to be expected.

Since the worldwide population is expected to age and the most of the statins are available in generic versions, use of statins therapy could also simultaneously increase,[1] which would be a cornerstone for further research and focus to importance of optimal medication persistence.

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PHARMACEUTICAL CARE IN GHETTO THERESIENSTADT 1942–1945

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Today the term "pharmaceutical care" includes an individual approach to the patient, orientation to the patient's pharmacotherapy, and drug problems. However, this definition is applicable even in the specific conditions of the health care system of the ghetto Theresienstadt.[1] This work aims to identify medicaments in the ghetto health care system. The information was available from the archives of the Jewish Museum in Prague. The key documents date from 1944–1945. The information received will be analyzed from the point of view of dosage form, producer, country of origin, the active ingredient, therapeutic indications *etc*. I compare the analysis with the health and social situation in the ghetto.[2] In this way, I have completely analyzed the document Inventory of the drug warehouse in Theresienstadt and overviews of drug deliveries[3] and partly group 13 of the so-called "receipts" (Receipt No. 152–277, concerning medicines in Theresienstadt). From the first of these, I identified 332 drugs (manufactured in pharmaceutical factories), 34 pharmaceutical excipients, and 40 plant drugs. I have not identified 52 drugs yet. Of the dosage forms, there were most tablets and dragees (116 pieces), injections (89), and ointments and creams (22). The groups of analgesics (28), drugs for women's diseases (22), and chemotherapeu-

tics (16) had the largest share of therapeutic indications. Most drugs were manufactured in Germany (137), Czechoslovakia (87), and Austria (15).[4] From the second list, I have identified 504 items so far. I processed 280 drugs from this group in detail.

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SECTION OF BIOLOGICAL SCIENCES

MONOAMINE HOMEOSTASIS IN THE FETOPLACENTAL UNIT – EFFECT OF GESTATION AGE

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Monoamine neurotransmitters such as noradrenaline and dopamine have been implicated in numerous physiological processes within the central nervous system. Recently, emerging evidence suggested their involvement in placental development and functions and a crucial role in fetal development and programming. Nonetheless, a comprehensive characterization of monoamine handling in the fetoplacental unit is still lacking. Therefore in our study, we investigated the expression of key metabolic enzymes and transporters responsible for the synthesis, degradation, and transport of noradrenaline and dopamine in the placenta and fetus. The study was performed in early-to-late gestation in humans (first trimester vs. term placenta) and mid-to-late gestation in rats (placenta and fetal brain, intestine, liver, lungs, and heart). We provide the first evidence of monoamine pathways in the fetoplacental unit and show that the expression of key genes is significantly dependent on gestational age. We suggest these regulatory pathways control levels of noradrenaline and dopamine in the fetoplacental unit to ensure proper embryo and fetal development throughout pregnancy.

The study was supported by the Czech Science Foundation (Project No. 20/13017S) and from the project of Specific Academic Research (SVV 260 549).

BISPHENOLS, CYTOTOXICITY AND ACTIVITY ON ESTROGEN RECEPTOR

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Estrogen receptor (ER, NR3A1) is a nuclear receptor with high affinity for steroid hormones, particularly, estradiol (E2). The activation by E2 is responsible for signaling cascades involved in cell growth, differentiation, and even cellular death.[1] ER can also interact with exogenous substances with different chemical structures, generally known as endocrine-disrupting chemicals (EDCs). Among these compounds, bisphenols, organic xenobiotics with the structure similar to E2, are widely known EDCs.[2] These substances are abundantly used in industry, particularly in the production of polycarbonate plastics, epoxy resins, and lacquer coatings.[3] Consequently, they are widespread in the environment and can be present in sediments, surface waters, sewage, and indoor dust. If present in the human body, bisphenols can, among other effects, bind to the ER and mimic the natural hormone's effects, potentially leading to malignancies in specific tissues. In addition to bisphenol A (BPA), the most studied bisphenol, several derivates have been described.[4] In this project, we investigated *in vitro* cytotoxicity and genetic expression toxicity of 14 bisphenols in MCF-7 breast cancer cell line.

The study was suported by ERASMUS+ and from the project of Specific Academic Research (SVV 260 549).

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IMMUNOHISTOCHEMICAL ANALYSIS OF ENDOGLIN AND CELL ADHESION MOLECULES IN DIET-INDUCED NASH MODEL IN MOUSE LIVER

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Nonalcoholic steatohepatitis (NASH) is an aggressive form of nonalcoholic fatty liver disease, which is characterized by liver inflammation and fibrosis. It was shown that membrane endoglin (ENG) participates in fibrosis, and plasma concentrations of soluble endoglin were increased in patients with type II diabetes mellitus and hypercholesterolemia.[1] The purpose of this study was to analyze the expression of ENG, αSMA (alpha

smooth muscle actin as biomarker of fibrosis), and biomarkers of inflammation (ICAM-1, VCAM-1) in liver in mice. Wild type mice were divided into two groups (n = 8 in each group), control group was fed with chow diet and NASH group was fed with high fat diet (HFD) with fructose and glucose for 6 months. Expression of selected proteins was evaluated by means of immunohistochemistry (ImmPRESS™ and VECTASTAIN® ELITE® ABC kit) on formalin-fixed, paraffin-embedded liver sections. The results of this study showed that the expression of VCAM-1 and ICAM-1 was detected in liver sinusoids and it was higher in NASH group. Similarly, we showed higher expression of ENG in NASH group in comparison with control. Moreover, αSMA was found not only in blood vessels, but also in hepatic stellate cells in NASH group compared to control suggesting fibrotic process after NASH diet. In conclusion, we showed that biomarkers of inflammation and fibrosis were increased in NASH liver. Moreover, increased expression of ENG in NASH liver suggests its participation in the pathogenesis of this disease, however precise role will be evaluated in the prospective experiments.

The study was supported from the project of Specific Academic Research (SVV 260 549).

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NOVEL PHOTOSENSITIZERS IN THE FIGHT AGAINST RESISTANT MICROBIAL PATHOGENS

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Systematic abuse and overuse of antibiotics lead to antimicrobial resistance, which is currently a major problem worldwide. The possible impact of the spread of resistance is such a significant threat that the World Health Organization has set one of its priority goals to address this problem.[1] One possible solution is to look for an alternative treatment modalities. Antimicrobial photodynamic therapy using photosensitizers (PSs) may be a suitable alternative approach. PSs are non-toxic compounds that (under activation by visible light of an appropriate wavelenght) produce reactive oxygen species, which ultimately leads to non-selective destruction of the microorganism through oxidative stress.[2] Our research has focused on evaluating *in vitro* antimicrobial activity of four candidate (aza)phthalocyanine PSs. Three clinically significant microorganisms from the group of gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*), gram-negative bacteria (*Pseudomonas aeruginosa*), and yeasts (*Candida albicans*) were chosen for the study of microbicidal potential. Methodical approaches such as the recovery method using

a Bioscreen C instrument, the Alamar blue method to determine the metabolic activity of surviving cells, and the spread plate technique were employed in the study.

This study was supported from the projects of Specific Academic Research (SVV 260 549 and SVV 260 550).

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EFFICACY OF DISINFECTANTS BASED ON QUATERNARY AMMONIUM SALTS

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Quarternary ammonium salts are widely used in the field of disinfection and antisepsis. Already used substances, on this basis, include: benzoxonium chloride (Orofar), didecylmethylamonium chloride (Sanytol), carbethopendecinium bromide (Septonex) and many others.

This work presents the results of testing the effectiveness of 10 new substances based on quaternary ammonium salts prepared at the Biomedical Research Centre, University Hospital Hradec Králové. Activity and efficacy were tested on nosocomial bacterial strains, using the microdilution broth method. Thus, the minimum inhibitory concentration and the minimum bactericidal concentration were determined and compared with the standards.

The Gram-positive strains were more sensitive towards the tested substances than the Gram-negative ones. In the overall measurement, none of the substances exceeded the effectiveness of the standards. In comparison with some commercially introduced substances showed similar, some even higher efficacy.

In conclusion, we confirmed the effectiveness of newly synthesized substances, especially on Gram-positive bacteria. They could expand the range of disinfectants over time, replacing the current ones in case of resistance.

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CAN AMINO ACIDS BE CONSIDERED AS COPPER CHELATORS?

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Copper is a trace element playing an essential role in the human organism. Copper levels in the body have to be meticulously controlled because disruption of copper homeostasis can cause oxidative damage which may lead to various pathologies.[1] Chelation or reduction of copper can be potentially used as a therapeutic tool.[2] Physiologically, amino acids in peptides (*e.g.* glutathione) or proteins (*e.g.* copper transporters) are participating on regulation of copper kinetics.[3] Although the main source of protein is meat, they are also found in some plants.[4]

The aim of this study was to compare the copper chelation and reduction activity of several amino acids known to be involved in copper kinetics. L-histidine, L-methionine, L-cysteine, L-cystine, L-aspartic acid, L-glutamic acid and *N*-acetylcysteine were tested at (patho)physiologically relevant pH conditions (4.5, 5.5, 6.8 and 7.5) by two spectrophotometric methods. All compounds showed good ability to chelate cupric ions in less competitive hematoxylin assay. However, under more competitive conditions, most of the compounds lost the ability to chelate copper ions. It suggest that tested aminoacids are weak chelators. The exceptions are L-cysteine and L-histidine. Only L-cysteine and *N*-acetylcysteine were able to reduce cupric ion significantly. This study showed that all selected amino acids can interact with copper, however with different intensity.

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CIRCULATION OF ALBENDAZOLE IN THE ENVIRONMENT – EFFECT ON THE EXPRESSION OF LIVER ENZYMES IN SHEEP

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Albendazole (ABZ) is an anthelmintic drug used for treatment and control of gastrointestinal nematodes in small ruminants. After the treatment, the excrements contain residuals of the drug and its metabolites and if not handled properly they enter the environment. However, the subsequent fate of this drug in the environment is poorly monitored. Our project reveals the circulation of ABZ in the real farm conditions. The field with fodder plants was fertilized with excrements of treated sheep containing ABZ residuals. The grown plants were fed to infected sheep for two weeks. Afterwards, we have detected traces of ABZ in the plants, sheep rumen content, plasma and the excrements, proving the circulation of this environmentally persistent drug.

Furthermore, the effect of detected sub-lethal doses of ABZ and the metabolites on the parasites and the host was monitored. The RNA was isolated from liver of sheep exposed to albendazole doses present in fodder plants from the fertilized field for 14 days and reverse transcribed. Individual mRNAs encoding cytochrome P450 isoforms (CYPs) and UDP-glucuronosyltransferase were quantified by real-time polymerase chain reaction (RT-qPCR) and the data were evaluated by comparative delta-Ct method. Results showed that ABZ in very low dose significantly increased CYP1A2 mRNA and decreased CYP3A4 mRNA in sheep. In conclusion, the chronic effect of even very low doses of ABZ present in the environment may enhance its metabolism when proper dose administered, hence cause the ineffective treatment and facilitate the development of resistance in helminths.

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THE EFFECT OF PYRAZINE DERIVATIVE ON SECONDARY METABOLITES CONTENT IN PLANT CULTURE OF SILYBUM MARIANUM (L.) GAERTN *IN VITRO*

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The aim of this work was to determine the effect of an abiotic elicitor from the class of pyrazine derivates 1-benzyl-3-(pyrazin-2-yl)urea on the production of secondary metabolites (SM) in the plant culture *Silybum marianum* (L.) Gaertn.[1] Elicitation was performed on both callus and suspension cultures. The elicitor was used in three different concentrations. Particular samples were taken after 6, 24, 48, 72 and 168 hours of elicitor influence. After drying, the callus and suspension tissues were extracted with methanol and the content of the monitored secondary metabolites was determined by HPLC. It was also tested if SM are released into the growth medium. Flavonolignans silybinin A and silybinin B were not detected in any of the analyzed samples. The highest production of SM was achieved after elicitation of suspension cultures. Maximum values of the content were reached by flavonoid taxifolin. The most significant increase of the content was determined at silychristin in callus cultures. Only silydianin was released in significant amounts into

the growth media of the cultures. It was shown that the elicitor 1-benzyl-3-(pyrazin-2-yl) urea is able to increase the production of SM in both callus and suspension cultures of milk thistle in certain concentrations and duration of action has an effect on the excretion monitored metabolites into the nutrient medium.

The study was supported from the project of Specific Academic Research (SVV 260 550).

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AMARYLLIDACEAE ALKALOIDS AS MODEL STRUCTURES FOR THE DEVELOPMENT OF NEW POTENTIAL DRUGS

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The family Amaryllidaceae includes a large number of genera of flowering plants as *Hippeastrum*, *Narcissus* or *Zephyrantes*. All of them contain specific Amaryllidaceae alkaloids, which are characteristic of this family due to their chemical structures. The discovery and study of these alkaloids has attracted attention of many scientists due to the diverse biological activity of these compounds, for example cytotoxic, anticholinesterases, antibacterial, antiviral.[1] Plants of the genus *Hippeastrum* have been used in traditional medicine to treat tumors and inflammatory disorders. This use can be explained by the alkaloids, which it contains. It is mainly lycorine, haemantamine and pancristatine. These compounds have an antitumor effect. The species *Hippeastrum* cv. Ferrari is further rich in the alkaloid vittatine. In some research, simple semisynthetic derivatives of haemanthamine displayed promising inhibitory activities against cholinesterases. For this reason, vittatine was chosen as next lead-structure for preparation of semisyntetic derivatives.[2] Seven new derivatives were prepared by esterification of the alkaloid vittatine and two were created in the form of ethers. All of them were identified by NMR and HRMS analysis. Subsequently, the ability of the derivatives to inhibit human acetylcholinesterase and butyrylcholinesterase (BuChE) was studied. Most of the prepared derivatives showed good inhibitory potential against BuChE. The best of them shown activity with IC values 1.39 ± 0.08 μM.

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INDOLE ALKALOIDS FROM *VINCA MINOR* AND THEIR BIOLOGICAL ACTIVITY

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Vinca minor L. is an evergreen perennial plant belonging to the Apocynaceae family. Phytochemical screening of its biologically active constituents revealed a wide range of indole alkaloids (IAs). To date, more than 50 IAs have been reported in *V. minor*.[1] Several of these indole alkaloids, such as vincamine, are frequently used as a remedy due to their cerebrovasodilatory and neuroprotective activity. In addition, some bisindole alkaloids such as vincarubine, exhibit remarkable activity on leukemic cell lines.[1,2]

The summary ethanolic extract was prepared from dried aerial plants of *V. minor* (63 kg) and separated by commonly used chromatographic techniques (column chromatography, flash chromatography, preparative TLC). So far, four alkaloids have been obtained from two fractions. The isolated IAs were identified by comparison of obtained analytical data (MS, NMR, optical rotatory) with the literature data. The alkaloids isolated in a sufficient quantity were assayed for their biological activities connected to Alzheimer's disease (inhibition of cholinesterases, inhibition of prolyloligopeptidase, ability to cross the blood-brain barrier) and anticancer potential (cytotoxicity against panel of tumor and leukemic cell lines). Significant cytotoxic activity was demonstrated by a novel bisindole alkaloid named vincaferugine.

The study was supported from the project of Specific Academic Research (SVV 260 548).

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ISOLATION OF ALKALOIDS FROM *NARCISSUS POETICUS* var. RECURVUS AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

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Plants of the Amaryllidaceae family are known to contain a specific type of compounds, namely the Amaryllidaceae alkaloids. Among these alkaloids, the most important one is galanthamine that is approved for the pharmacological treatment of Alzheimer's disease (AD).[1] AD is the most prevalent neurodegenerative disease worldwide with complex eti-

ology and multifaceted pathophysiology. Based on the various causative factors of AD, several hypotheses, including cholinergic, amyloid, τ-protein, calcium dyshomeostasis, and isoprenoid change, have been put forward. Focused on the cholinergic hypothesis, a deficit of the neurotransmitter acetylcholine (ACh) in the cortex results in a cognitive deterioration. ACh levels can be maintained via inhibition of acetylcholinesterase.[2] Important source of Amaryllidaceae alkaloids mentioned above is the genus *Narcissus*. Extract from *Narcissus poeticus* var. recurvus was screened at the Department of Pharmaceutical Botany for the first time in 2013.[3] Subsequent phytochemical research began with 29 kg of fresh bulbs from which 42 g of alkaloid extract was obtained. About 6 g of lycorine was isolated from this extract. The rest of the extract was evaluated for its alkaloid profile by GS/MS and HPLC. This was followed by separation using flash chromatography. Finally, 18 fractions were obtained which in turn were evaluated by GS/MS and HPLC. Individual fractions are processed by preparative TLC.

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MONITORING OF ALBENDAZOLE TRANSFER FROM OVINE FAECES TO FODDER PLANTS

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Albendazole (ABZ) belongs to a benzimidazole group of anthelmintic drugs. These drugs are regularly and frequently used to limit and treat parasitic infections in animals. In this way, the consumption of these drugs increases and their negative effects on non-target organisms and the environment is extended.[1]

We know that the plants can uptake and even biotransform the ABZ in laboratory-controlled conditions.[2] The present study monitors the transfer of ABZ and its transformation products (TPs) from the faeces of treated sheep to common fodder plants *Medicago sativa* and *Trifolium pratense*. We wanted to know if there is a possibility of transferring these compounds into soil and plants in real field conditions.

Our study successfully revealed the occurrence of ABZ TPs (ABZ-SO and ABZ-SO_2) in fodder plants. Even two months after the first contact of fodder plants with faeces, ABZ-SO was still present. The highest concentration of TPs was observed in the 1st and

2nd week after the application of faeces. Then, the amount of TPs in plants decreased during the time, except in May, where a slight increase was observed, probably due to higher precipitation. The presence of the TPs in fodder plants represents not only a danger to herbivorous invertebrates, but also may play an additional role in the development of ABZ resistance in helminths.

The study was supported by the Grant Agency of Charles University (Project No. 1136120) and from the project of Specific Academic Research (SVV 260 550).

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SEMI-SYNTHETIC DERIVATIVES OF β-CARBOLINE ALKALOID HARMINE AND THEIR BIOLOGICAL ACTIVITY

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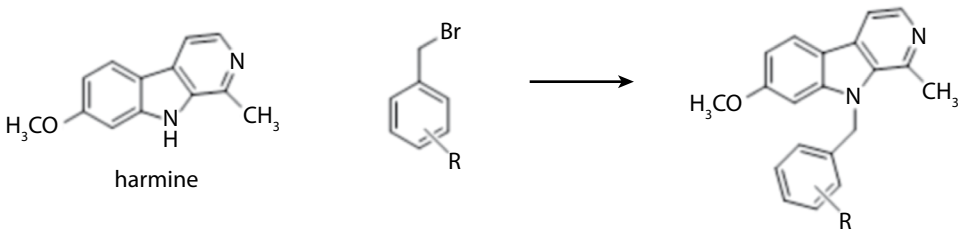
Alzheimer's disease (AD) is a serious and irreversible progressive neurodegenerative disorder, that will reach a prevalence of more than 100 million by 2050 due to the aging of population. The main pathological hallmarks of AD are intracellular neurofibrillary tangles, extracellular amyloid plaques, increased oxidative stress and cholinergic dysfunction. Cholinergic neurotransmission is terminated by acetylcholine hydrolysis regulated by two enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).[1,2]

Alkaloids play an important role in the treatment of AD. Well-known Amaryllidaceae alkaloid galanthamine is a marketed drug for AD therapy under the commercial name Reminyl©.[1] β-Carboline alkaloid harmine, isolated from *Peganum harmala* (Nitrariaceae), lacks any significant activity against human cholinesterases. However, several new derivatives of



harmine

harmine prepared by the *N*-9 derivatization showed interesting inhibition BuChE. Newly prepared compounds were identified by NMR and ESI-MS methods. The most active compounds will be studied in more detail (*e.g.* type of inhibition, docking studies, logBB *etc*.).

The study was supported from the project of Specific Academic Research (SVV 260 548).

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GILTERITINIB AS OCT1 INHIBITOR AND SUBSTRATE: POTENTIAL FOR DRUG-DRUG INTERACTIONS?

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Gilteritinib is one of the recently approved drugs which is primarily used in the treatment of relapsed/refractory acute myeloid leukemia (AML) with mutated FMS-like tyrosine kinase 3 (FLT3) receptor. In this project, gilteritinib was investigated in terms of its ability to interact with solute carriers (SLC) membrane transporters, namely with OCT1 and OCT2. These membrane proteins play a role in uptake of endogenous compounds and also drugs into the cells of main elimination organs (liver, kidney), but also to cancer cells. In particular, we wanted to examine potential interaction with daunorubicin, a drug traditionally used in AML therapy. First, we performed accumulation study and evaluated, whether gilteritinib is potential inhibitor of OCT1 studying differential uptake of daunorubicin into MDCKII-OCT1 cells based on OCT1 inhibition by gilteritinib. Secondly, the study evaluating the transfer of gilteritinib across the monolayers of MDCKII-OCT1 and control MDCKII-VK cell lines was conducted to test gilteritinib as a potential substrate of this transporter. The obtained data showed that gilteritinib has ability to inhibit the OCT1-mediated transport of daunorubicin into the MDCKII-OCT1 cells. This effect was not observed neither in control cell line, nor MDCKII-OCT2 cells. We further observed enhanced basolateral-to-apical transport of gilteritinib across monolayers of MDCKII-OCT1 cells compared to MDCKII-VK cells. This difference was abolished in the presence of OCT1 inhibitor, suggesting that gilteritinib is a substrate of OCT1. Results obtained in our study indicate that gilteritinib might be prone to OCT1-mediated pharmacokinetic drug-drug interactions. The hypothesis that combinatory treatment of AML with gilteritinib and daunorubicin could result in decreasing availability of the drugs to the leukemia cells leading to lower efficacy of the treatment should be verified.

The study was supported by the Research program Pharmacokinetic mechanisms of drug resistance in acute myeloid leukemia, their affecting and regulation (PRIMUS/20/MED/010) and from the project of Specific Academic Research (SVV 260 549).

NEW INHIBITORS OF TOPOSIOMERASE II – STUDY OF ANTIPROLIFERATIVE EFFECTS AND THEIR INFLUENCE ON ANTITUMOR ACTIVITY OF ETOPOSIDE

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Cancer is a serious societal health problem, which can affect each of us, and its incidence increases rapidly with age. Anthracycline antibiotics (ANT) are extremely effective drugs that have been used to treat a number of cancers, namely breast cancer, small cell bronchogenic cancer, recurrent ovarian cancer and many more. They have a multimodal mechanism of action, one of them is the inhibition of topoisomerase II (TOP2), an essential cellular enzyme modulating DNA topology. Currently, use of ANTs is limited due to concerns about the occurrence of cardiotoxicity. Etoposide (ETO) is an antitumor agent that acts as topoisomerase poison. ETO is often used in combination therapy with ANTs, but it is not considered cardiotoxic. Dexrazoxane (DEX) is the only approved cardioprotectant against cardiotoxicity of ANTs. However, it does not act as chelating inhibitor, as previously thought, but through inhibition of TOP2. Although DEX has been shown to be a very potent cardioprotectant, it also has its disadvantages. It has been associated with the occurrence of secondary malignancies, most often with acute myeloid leukemia, less with myelodysplastic syndrome, acute lymphoblastic leukemia or non-Hodgkin's lymfoma. Hence, it is necessary to examine other agents.[1] The aim of the study was to determine whether other inhibitors of TOP2, namely (BNS-22, XK-469, ICRF-193) can affect antiproliferative effect of ETO on the HL-60 leucemic cell line. For the tested agents, concentrations corresponding with their IC_{50} were determined and on the basis of this concentration their antiproliferative effects were evaluated both alone and in combination with ETO. For determination of cell viability, MTT aasay was used. The results seem promising and some of the agents even show synergistic effect in combination with ETO.

The study was supported by the Czech Science Foundation (Project No. 18-08169S) and by the Grant Agency of Charles University (Project No. 246219) and from the project of Specific Academic Research (SVV 260 550).

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SCREENING OF INHIBITORY ACTIVITY AGAINST CHOLINESTERASES OF VARIOUS SPECIES OF THE GENUS FICUS II

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Alkaloids are some of the most interesting substances of secondary metabolism. These compounds are synthesized in plants from amino acids or their derivatives and contain nitrogen in the structure that is incorporated into heterocycle in most cases.[1] Alkaloids exhibit a variety of biological activities as cytotoxic, anti-inflammatory, antifungal, antimalarial or anticholinesterase. The anticholinesterase effects of alkaloids include inhibition of human enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), which can be used in therapy of Alzheimer's disease (AD). AD is the most common form of dementia, manifested by impairment of cognitive and noncognitive functions that eventually lead to death.[2] The genus *Ficus* belongs to the family Moraceae, which is widespread in tropical and subtropical regions of the Western Pacific area. Plants from this genus contain large amounts of substances, including alkaloids.[3] For the phytochemical study, nine extracts from leaves and stems from 6 species of the genus *Ficus* were prepared. Dry material was ground and extracted by boiling in ethanol. Then ethanolic extracts were purified by liquid-liquid extraction (ether, ethyl acetate,chloroform). All extracts were tested for their ability to inhibit AChE and BuChE. Only extract AL-708E showed inhibition activity against human BuChE (53.29 ± 0.03% at concentration 50 μg mL^{-1}).

The study was supported by EFSA-CDN (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 548).

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SCREENING OF INHIBITORY ACTIVITY AGAINST CHOLINESTERASES OF VARIOUS SPECIES OF THE GENUS FICUS I.

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Following scientific research focuses on selected *Ficus* species and their potential usage in treatment of Alzheimer's disease (AD) thanks to alkaloids contained in them. AD is one of the most common diseases of the elderly and one of the most frequent cause of dementia. Currently, AD is incurable. The only thing that can be done is to slow down progression of the symptoms. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) plays an important role in this process.[1,2] Alkaloids are substances of secondary metabolism that are usually synthesized from amino acids or their derivatives and can be found in various plant organs. Alkaloids have a number of interesting biological effects. For example, galanthamine inhibits AChE and therefore it is used for treatment of AD.[1] Plants of genus *Ficus* belonging to Moraceae family include nearly one thousand species. These are evergreen trees that can be found mainly in tropical and subtropical areas of West Pacific. They contain alkaloids of various structures, which have antimalarial, antibacterial and anticancer activity.[3] The aim of this research was to screen several extracts as potential sources of substances for the treatment of AD. Ten extracts of 8 selected species of genus *Ficus* were subjected to a phytochemical study. Dry grinded leaves and stems were extracted by boiling in ethanol. Obtained ethanol extracts were purified by liquid-liquid extraction using ether, ethyl acetate and chloroform. All extracts were tested on the ability to inhibit AChE and BuChE. Ability to inhibit BuChE showed only the extract AL-700C ($74.82 \pm 2.78\%$ at concentration 50 $\mu g\ mL^{-1}$).

The study was supported by EFSA-CDN (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and from the project of Specific Academic Research (SVV 260 548).

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MONITORING THE SPREAD OF ALBENDAZOLE FROM THE SHEEP FAECES IN AGRICULTURAL LAND BY LC-MS

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The anthelmintic drug albendazole (ABZ) with a broad-spectrum anthelmintic effect is used for the treatment of helminthiasis caused mainly by gastrointestinal worms in veterinary and human medicine. Frequent dose, overdose or underdose make a risk of developing resistance, which can be a serious global problem in the treatment of helminthiasis. The anthelmintics can enter the environment unchanged as a parent compound or as a metabolite through the faeces of treated animals. These chemicals can be absorbed into plants, soil and groundwater and they can have a negative impact on the life and growth of smaller organisms.

This experiment aimed to monitor the distribution of albendazole and its transformation products (TPs) albendazole sulfoxide (ABZ-SO) and albendazole sulfone (ABZ-SO_2) from the faeces of treated domestic sheep in agricultural land. This was observed from different distances and depths to which the compounds could spread. In general, the concentration of the compounds in the soil was greater in the topsoil than in the bottom layer and closer to the faeces. We also observed the dependence of changes in substance concentration on rainfall (dry and rainy months). The parent drug and TPs were extracted from soil using the solid-phase dispersion extraction (QuEChERS) and identification and quantification were done by UHPLC-MS.

The study was supported by the Grant Agency of Charles University (Project No. 1136120) and from the project of Specific Academic Research (SVV 260 550).

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THE EFFECTS OF TOPOISOMERASE II BETA ON THE SENSITIVITY OF THE CANCER CELLS TO THE ANTINEOPLASTICS

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Topoisomerase II is a cellular enzyme responsible for solving topological problems of double-stranded DNA. Topoisomerase IIα and IIb isoforms are various gene products having conserved catalytic activities. The TOP IIα isoform is present in proliferating cell, while TOP IIb isoform is predominantly present in non-proliferating cells, especially neurons. Anthracycline antibiotics targeting TOP IIb are currently among the most effective anticancer drugs. The main factor limiting their clinical use is the development of side effects – especially myelotoxicity and cardiotoxicity. The mechanism of anthracycline-induced cardiotoxicity is still not fully elucidated. Nowadays, the most accepted theory is the ability of anthracyclines to produce reactive oxygen species damaging the heart muscle by oxidation. However, the mechanism is probably multifactorial. Nevertheless, the key role of inhibition of the TOP IIb enzyme, which is present in cardiomyocytes, has been increasingly discussed. The only clinically used cardioprotective is dexrazoxane. Even its mechanism of action is not fully understood. However, new studies suggest the major mechanism of action through TOP IIb located in cardiomyocytes (depletion after dexrazoxane exposure).[1] The practical aim of this project was to investigate the differences in the antiproliferative effects of daunorubicin and dexrazoxane in human cell suspension line of HL-60 tumor cells with various TOP IIb expression. Further to determine the amount of TOP IIα and TOP IIb mRNA in these cell lines by RT-qPCR and the amount of protein by immunoblotting. Primary screening of the designed primers at the TOP IIb enzyme mutation site was also performed.

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SECTION OF CHEMICAL SCIENCES

DEVELOPMENT OF SYNTHESIS OF 3-(AMINOBENZAMIDO)PYRAZINE-2-CARBOXAMIDES AS POTENTIAL INHIBITORS OF PROLYL-tRNA SYNTHETASE

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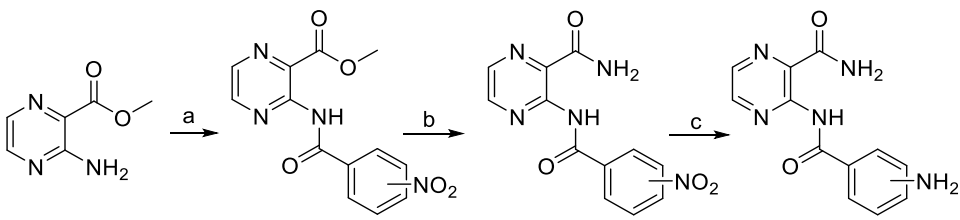
3-Benzamidopyrazine-2-carboxamides are derivates of pyrazinamide with potential inhibitory activity on prolyl-tRNA synthetase (ProRS).[1] This work is based on confirmed inhibitor of human ProRS1 and expands our previous efforts in this area.[2] The design of synthesized compounds was supported by simulations in Molecular Operating Environment (MOE, Chemical Computing Group, Canada), as a tool of Computer-Aided Drug Design (CADD). 3-Benzamidopyrazine-2-carboxamides could be used as anticancer drugs, suppressors of autoimmune responses and antifibrotic therapy.[1] Driven by structural similarity between human and mycobacterial ProRS, we are trying to develop inhibitors of ProRS as antimycobacterial compounds. The work deals with optimalization of the reaction (Scheme 1), especially with reduction of the nitro group and bad solubility.



Scheme 1. Synthesis of desired derivates

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS AND EVALUATION OF POTENTIAL ANTIMICROBIAL COMPOUNDS BASED ON MAFENIDE

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The global problem of acquired resistance of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA) and enterococci (VRE) strains and limited treatment options for infections caused by these pathogens are alarming. The development of novel compounds active against drug-resistant Gram-positive cocci and mycobacteria is challenging. Presented compounds are based on sulfa drug mafenide, which is used in the treatment of topical infections caused by Gram-positive and Gram-negative bacteria.[1]

The targeted imines were prepared from mafenide and aldehydes in one step. Most of these compounds are halogenosalicylaldehyde derivates, furthermore, derivates of 5-nitrothiophene-2-carbaldehyde and isatin were also synthesized. Thirteen compounds were prepared with a good yield (62–94%). All these compounds were tested by the broth microdilution method against Gram-positive and Gram-negative bacteria, mycobacteria, and fungi. The lowest minimum inhibitory concentration (MIC) values against bacteria, fungi as well as the highest antimycobacterial potency were found for (*E*)-4-{[(5-iodo/chloro-3-iodo-2-hydroxybenzylidene)amino]methyl}benzenesulfonamides and (*E*)-4-{[(5-nitrothiophene-2-yl)methylene)amino]methyl}benzenesulfonamide. The lowest MIC values achieved were 7.9 μmol L^{-1} for bacteria, 3.9 μmol L^{-1} for fungi and 3.9 μg mL^{-1} for mycobacteria.

In general, all the novel derivatives showed a higher *in vitro* antimicrobial effect than the original molecule of mafenide acetate.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS AND EVALUATION OF InhA INHIBITORS AS POTENTIAL ANTITUBERCULAR DRUGS

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Tuberculosis is an infectious disease that belongs to one of the top 10 causes of death worldwide. The most common cause of the disease are *Mycobacterium tuberculosis* complex strains. Antimicrobial therapy of the disease is nowadays complicated by alarming increase of strains resistant to standardly used antitubercular treatment. This is the reason of growing interest and significance in research of new potential antitubercular agents. One of the possible approaches is systematic modification of compounds with a known antimycobacterial activity, one of such compounds being triclosan. This substance acts as an uncompetitive inhibitor of enoyl-acyl-carrier protein reductase (InhA), an enzyme participating in the mycobacterial fatty acid biosynthesis pathway. It does not require activation by the mycobacterial KatG enzyme, thereby avoiding the most frequent mechanism of resistance to the frontline drug isoniazid targeting InhA too.[1] The aim of the study was to synthesise and evaluate new potential antitubercular drugs based on the structure of triclosan.

In this study we prepared eight compounds derived from triclosan, five of them were esters of carboxylic acids and three of sulfonic acids. Most of the derivatives showed a comparable activity to triclosan against *Mycobacterium tuberculosis* strain (MIC values after 14/21 days of incubation being 32/64 μmol L^{-1}, for triclosan 32/32 μmol L^{-1}), one compound showed no effect (MIC >1000 μmol L^{-1}). Comparable activity was also observed against isoniazid-resistant *Mycobacterium kansasii* strain, but not against *Mycobaterium avium* strain, where MIC values were several times higher when compared to those of triclosan.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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XANTHONE-BORONIC ACIDS: AN INSIGHT INTO THE SYNTHESIS OF BORYLATED XANTHONE DERIVATIVES

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Bortezomib is a dipeptidic derivative of boric acid, that is used in the treatment of multiple myeloma. It belongs to the proteasome inhibitors class, being one of only few in this class to be clinically used.[1] The introduction of bortezomib directed many medicinal chemists towards the boron chemistry as an innovative approach to obtain new drugs with potentially broad spectrum of activities.[1] Based on bortezomib and its interesting activity, we decided to take a deeper insight into its chemistry. The main aim of our research was to find a general procedure that could be used to obtain a library of xanthone boronic acids.[2] Such compounds were synthesized and screened for potential biological activities, such as anticancer, antimicrobial, anti-inflammatory.[3] 3,6-Dihydroxyxanthone was chosen as a precursor for borylated xanthones, mainly due to its ease of synthesis and availability. All synthesised compounds were characterised using TLC-staining methods and 1H NMR. Further details on the synthesis optimization of title compounds, mechanism of action, and structure characterisation will be discussed during the presentation.

The study was supported by the Erasmus+ and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS OF POLYFLUORINATED PSEUDOCERAMIDES AS SUBSTANCES RECOVERING PERTURBED HUMAN SKIN BARRIER

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The human skin serves as a protection against physical, chemical and microbial injury. When the skin barrier is perturbed, it can be potentially penetrated by microbes and irritants. Ceramides, as essential components of the skin lipid layer, help to maintain the barrier integrity. Skin diseases, *e.g.* atopic dermatitis and psoriasis, have been connected with decreased content of ceramides in skin. Previous findings of our group proved that a ceramide analogue with similar steric and hydrophobic parameters to skin ceramides could supplement the deficient stratum corneum ceramides by simple physico-chemical action.[1] In addition, when polyfluorination performed to known permeation enhancers, the permeation-enhancing activity was abolished.[2]

In this study, a convenient synthesis of ceramide analogues with polyfluorinated chain and potential ability to recover perturbed human skin barrier in comparison with their non-fluorinated analogues was investigated.

The study was supported by the Grant Agency of Charles University (Project No. 19-09600S) and from the project of Specific Academic Research (SVV 260 547).

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRAZIN-2-YL PHENACETAMIDE DERIVATIVES

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Tuberculosis (TB) is an infectious disease caused by *M. tuberculosis*. According to WHO report[1], it is the leading cause of death worldwide among infectious diseases. First line drugs used to treat TB are isoniazid, rifampicin, pyrazinamide, and ethambutol. This treatment is successful, yet due to poor adherence and the raising issue of antimicrobial resistance, TB is not so easy to eradicate. The series to be presented during the conference is based on pyrazine and 'retro-amides' core bound to phenacetamides bearing small substituents on the ring. Title compounds were prepared by reacting 2-aminopyrazine, 2-amino-5-chloropyrazine or 2-amino-6-chloropyrazine with different substituted phenylacetic acids or chlorides. Compounds prepared were tested for antimycobacterial activity against selected strains of mycobacteria (*M. tuberculosis* H37Ra, *M. aurum*, *M. smegmatis*), along antibacterial and antifungal activities. The minimum inhibitory concentration (MIC) was determined for all tested compounds beside isoniazid as a standard. Results and structure-activity relationships will be discussed during the presentation.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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DISCOVERY OF NEW CHEMOSENSITIZING AGENTS WITH POTENTIAL IMPLICATION IN ANTICANCER THERAPY

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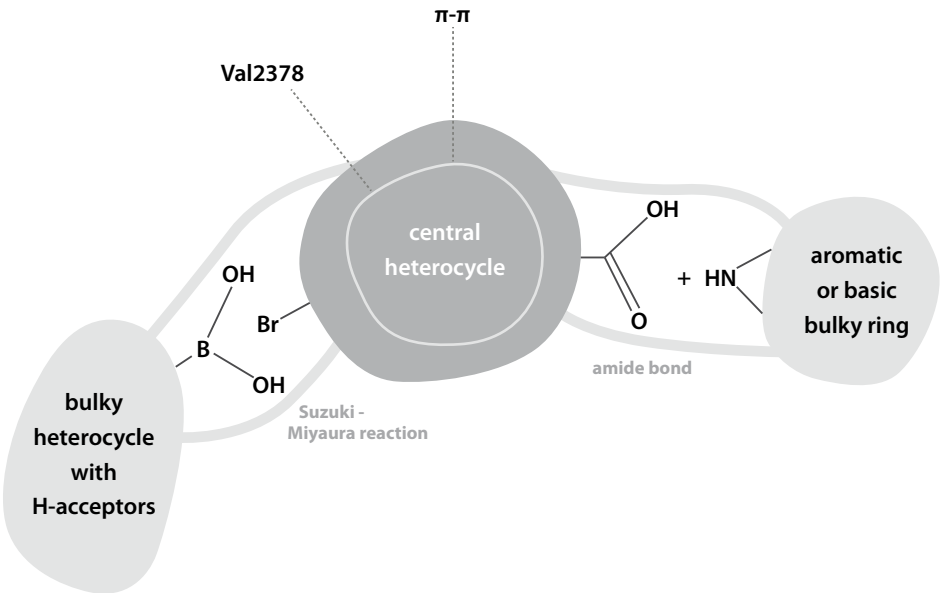
Cancer is one of the main causes of fatalities worldwide. It is attracting attention of many researchers with desire to develop strategies that selectively target one or more pathways in cancer while sparing healthy cells. As an example, the employment of one of cancer's hallmarks (impaired response to DNA damage) could serve. Probably major apical responders are three kinases belonging to the phosphatidylinositol 3-kinase-related protein kinases family: ATM (ataxia-telangiectasia mutated), ATR (ATM and Rad3-related) and DNA-PKcs (DNA-dependent protein kinase catalytic subunit). Only ATR is essential for cell viability, which emphasizes its importance as an answer for replication stress. Four ATR inhibitors have already entered clinical trials as anticancer agents – VX-970, VX-803, BAY1895344 and AZD6738. Based on their common structural features, we have designed and synthesized several new molecules that are to be tested on different cancer cell lines, both as monotherapeutics and as chemosensitizers to cisplatin.



Scheme 1. Common structural features of ATR inhibitors

The study was supported by InoMed (Project No. CZ.02.1.01/0.0/0.0/18_069/0010046) co-funded by the European Union and from the project of Specific Academic Research (SVV 260 547).

PREPARATION OF DENDRALENES SUBSTITUTED WITH ELECTRON-WITHDRAWING GROUPS

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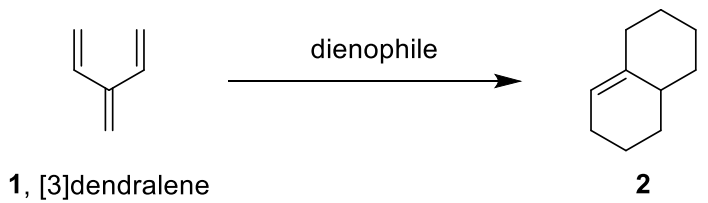
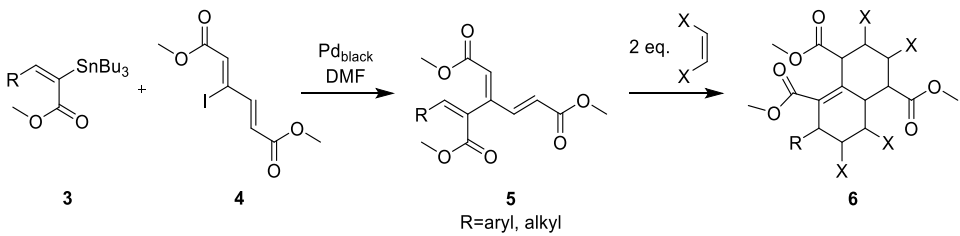
Specific type of branched, cross-conjugated polyenes is called dendralenes[1] (from Greek "Dendros" – tree). Their structure (Scheme 1, **1**) which, in theory, is not limited by the number [n] of double bonds, is ideal for cycloaddition reactions such as Diels-Alder reaction, in which complex polycyclic compounds can be obtained in single step (Scheme 1, **2**).



Scheme 1. Structure and reactivity of dendralenes

Our goal was to prepare new dendralenes (Scheme 2, **5**), substituted predominantly with electron withdrawing groups (EWG). The procedures previously developed by our research group based on Migita-Stille coupling of compounds **3** and **4**.[2] Furthermore, their ability to undergo Diels-Alder reaction was investigated (Scheme 2, **6**).



Scheme 2. Preparation and potential reactivity of EWG-[3]dendralenes

The study was supported by the Czech Science Foundation (Project No. 18-17868S) and from the project of Specific Academic Research (SVV 260 547).

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TESTING OF (−)-*N*-DODECYL-*N*-METHYLEPHEDRINIUM BROMIDE AS A CHIRAL SELECTOR IN CAPILLARY ELECTROPHORESIS ENANTIOSEPARATIONS

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Chiral ionic liquid (−)-*N*-dodecyl-*N*-methylephedrinium bromide (DMEB) was tested as a selector for chiral separations of selected drugs such as chiral quinolones, ketoprofen, and flurbiprofen by capillary electrophoresis (CE). The effect of several parameters on enantio-separation was examined: (i) type and pH of the separation buffer, (ii) type and amount of organic modifier, and (iii) the concentration of DMEB. When using this chiral selector, only the separation of ofloxacin enantiomers was observed, while the enantio-recognition of other chiral model analytes was not successful under the conditions tested.

A CE method for the assay of levofloxacin was developed to demonstrate the potential of DMEB as a chiral selector in quality control of single-isomer drugs. The best separation was achieved with 20 mmol L^{-1} tris buffer of pH 8.5 containing 100 mmol L^{-1} DMEB and 20% (v/v) of acetonitrile as the background electrolyte. The separation took place in 50 µm id fused silica capillary (80.5 cm / 72 cm) at −30 kV with UV detection at 291 nm. The resolution between the peaks of ofloxacin enantiomers was 4.22 ± 0.02 (n = 3). Linearity of the method was proved for the range 10 to 100 µg mL^{-1} of levofloxacin ($y = 0.0305x - 0.0107$, $R^2 = 0.9975$), gatifloxacin (40 µg mL^{-1}) was employed as internal standard. The method was applied to the analysis of tablets containing 500 mg of levofloxacin. The content determined was 100.1 ± 4.6% (n = 3) of the declared amount of levofloxacin. Hence, prospective applicability of DMEB as chiral selector in pharmaceutical analysis of single-isomer drugs was demonstrated.

The study was supported from the project of Specific Academic Research (SVV 260 548).

SYNTHESIS AND DYNAMICS OF DEUTERIUM-LABELED ACYLCERAMIDES

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Acylceramides are important components of skin permeability barrier where they are responsible for a formation of the long periodicity lamellar phase and corneocyte lipid envelope. These structures are indispensable for preventing water loss from human body and penetration of undesired components of the environment. Lower levels of acylceramides

usually accompany skin diseases like psoriasis and atopic dermatitis. Although acylceramides are essential for proper skin barrier function, little is known about their dynamics in skin. One of the methods allowing us to study the molecular dynamics is solid-state NMR, which requires molecules with partial or complete deuteration.

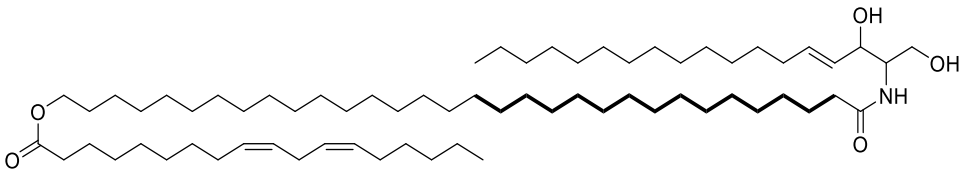

Fig. 1. Structure of acylceramides with deuteration in half of the ultralong chain

In this project, we decided to synthesize acylceramides with deuteration in their ultralong (32 carbons) chain. First, the synthesis was optimized using 1H compounds and based on this optimization, deuterated acylceramides will be prepared. The synthesis started from butyrolactone and 1,12-dibromododecane which are commercially available in their deuterated form. These starting compounds were transformed into a phosphonium salt and an aldehyde for Wittig reaction, providing a 16-carbon fragment (protected ω-hydroxylated unsaturated acid), which after a modification underwent a second Wittig reaction to create the 32-carbon long chain. This precursor was then esterified with linoleic acid and connected to a sphingoid base to form a final molecule of acylceramide. The optimized synthesis using 1H compounds was performed in 13 steps with approximately 15% overall yield, opening the possibility to synthesize acylceramides with deuteration in half of the ultralong chain (Fig. 1). In similar manner, acylceramides with deuteration in other half of the chain will be prepared.

The study was supported by the Czech Science Foundation (Project No.19-09135J) and from the project of Specific Academic Research (SVV 260 547).

SYNTHESIS AND *IN VITRO* CARDIOPROTECTIVE ACTIVITY OF DEXRAZOXANE ANALOGUES

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Anthracylines (ANTs) as daunorubicin, doxorubicin, *etc*., are used as anti-cancer drugs. ANTs have a strong anti-tumor effect. Unfortunately, a limiting factor of ANTs use in clinical practice is their serious cardiotoxicity. This side effect can lead to heart failure via irreversible damage of heart muscle cells. The only clinically approved drug used against ANTs-induced cardiotoxicity is bisdioxopiperazine derivative dexrazoxane

(DEX). Mechanism of action of DEX is intensively investigated and there are still two main theories: i) chelation of intracellular iron ions that protects hearth against oxidative damage, ii) inhibition/depletion of topoisomerase IIβ in cardiomyocytes. Synthesis and thorough evaluation of new DEX derivatives should help to clarify DEX structure–activity relationship.

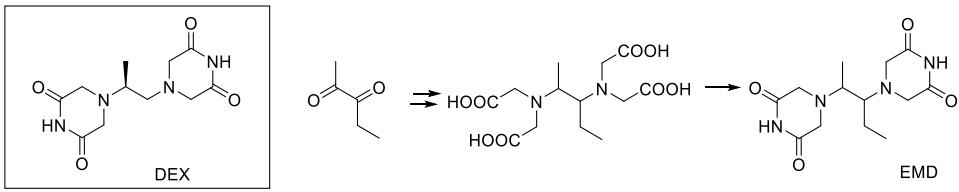

Fig. 1. Structure of DEX and synthesis of DEX analogue EMD

This study aimed at the preparation of a DEX analogue with a modified linker (EMD). The synthesis of the target compound started from pentane-2,3-dione and there were 6 more steps that lead to *erythro* and *threo* forms of 2,3-diaminopentane-*N*,*N*,*N*′,*N*′-tetraacetic acid, that were separated by column chromatography in the form of tetraesters. The final steps consisted of the cyclization of bisdioxopiperazine rings. Both final diastereomeric products (EMDa and EMDb) were studied for their ability to chelate iron ions, to inhibit TOPIIβ and to protect cardiomyocytes against ANT toxicity. The results of this work contribute to the study of DEX mechanism of action and to the discovery of new and more effective cardioprotective drugs.

The study was supported from the project of Specific Academic Research (SVV 260 547).

MONITORING OF LIBERATION TESTS FOR THE RELEASE OF CLOTRIMAZOLE FROM NANOFIBERS

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The study was focused on the analysis of release profiles of clotrimazole from different types of nanofibers using non-separation flow technique, sequential injection analysis (SIA). Finding ideal conditions for saturation of the fibers with a solution of clotrimazole was another goal. Nanofibers were produced at the Technical University of Liberec. Two types of nanofiber carriers – polymeric (polydioxanone and polycaprolactone) and inorganic (silica) – were employed. Part of the fiber of polydioxanone and polycaprolactone was saturated directly during production in different ratios of monomer and clotrimazole. The saturated fibers were then analyzed in the laboratory. The second part of polydiox-

anone and polycaprolactone together with inorganic nanofibers was produced in a pure state, *i.e.*, without the active substance. These fibers were tested in the laboratory for saturation with an ethanolic solution of clotrimazole at a certain concentration for a certain time. The saturation conditions were changed during the work to closely monitor the relation between the saturation conditions and the released concentration of clotrimazole. The measurements were performed under well-defined conditions that simulated intact healthy human skin at the temperature of 32 °C and pH of the buffer solution of 4.5. Under these conditions, nanofiber membranes with bound clotrimazole were placed in 3 Franz cells connected in parallel to a single SIA system and liberated levels of clotrimazole in the acceptor medium were monitored for 135 min. Samples were aspirated from this medium in regular 15 min intervals into a SIA system and the content of clotrimazole was determined online using UV-VIS detection. The resulting release profiles of clotrimazole from individual nanofibers were compared. The main monitored parameters were the release rate of clotrimazole into the acceptor medium, the concentration and the liberation profile of clotrimazole. More detailed results will be discussed in the presentation.

The study was supported from the project of Specific Academic Research (SVV 260 548).

SYNTHESIS OF WATER-SOLUBLE ANALOGUES OF *N*-SUBSTITUTED 1,2,4-TRIAZOLES WITH HIGH ANTIMYCOBACTERIAL ACTIVITY

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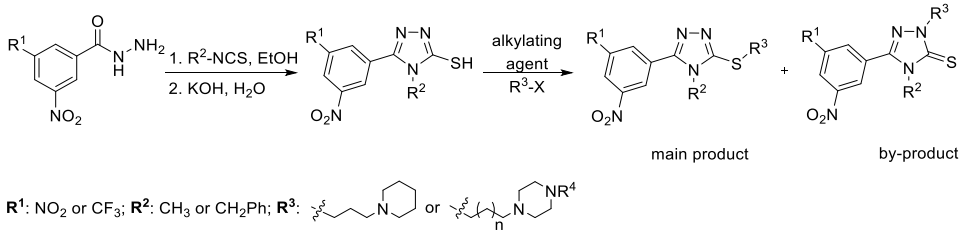
Recently, it was shown that 3,5-dinitrophenyl-1,2,4-triazoles have high activity against susceptible and drug-resistant strains of *Mycobacterium tuberculosis* with the minimum inhibitory concentration of 0.06–1 μM.[1] Thus, this work aimed at the synthesis of five water-soluble derivatives of 3,5-dinitrophenyl- and 3-nitro-5-trifluoromethylphenyl-1,2,4-triazoles. The synthesis of 4,5-substituted-1,2,4-triazole-3-thiols started from corresponding hydrazides and alkyl isothiocyanates followed by cyclization of obtained thiosemicarbazides in aqueous potassium hydroxide (Scheme 1). The alkylation of 1,2,4-triazole-3-thiols with piperidine or piperazine containing alkylation agents resulted in five final compounds in moderate yields (39–67%). Moreover, three by-products were isolated.

All prepared compounds were evaluated for their antimycobacterial activities against *M. tuberculosis* My 331/88, *M. avium* My 330/88 and *M. kansasii* My 235/80. The current work contributed to the discovery of water-soluble compounds with high efficiency that could be subjected to *in vivo* evaluation.



Scheme 1. Synthesis of target trisubstituted 1,2,4-triazoles

The study was supported from the project of Specific Academic Research (SVV 260 547).

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EVALUATION OF POSTANTIBIOTIC EFFECT AND POSTANTIBIOTIC SUB-MINIMUM INHIBITORY CONCENTRATION EFFECT OF CONVENTIONAL ANTI-INFECTIVE DRUGS ACTING AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Antimicrobial resistance is a global, multifaceted phenomenon with severe consequences, including complicated treatment, health cost, and higher mortality. Therefore, this situation needs to be properly reflected.[1] The research and discovery of new anti-infectives represent one of the options for combating this phenomenon. A wide range of parameters is determined in preclinical studies of new candidate anti-infective compounds. Among them, the postantibiotic effect (PAE) and postaantibiotic sub-minimum inhibitory concentration (PAE-SME) should be evaluated. PAE and PAE-SME are important parameters of antibiotic action, widely used as a predictor of pharmacodynamic activity.[2]

In our study, we have evaluated PAE and PAE-SME of three conventional antibiotics, namely ciprofloxacin, vancomycin and linezolid, acting against methicillin-resistant *Staphylococcus aureus*. A computerized incubator Bioscreen C was employed in the study of the two parameters mentioned above. For PAE evaluation, bacteria were exposed to three different final concentrations of drugs (5×, 10× and 20× MIC), and in the study of parameter PAE-SME, the subinhibitory concentration corresponding to 0.1×, 0.2×, 0.4×, 0.6×, and 0.8× MIC were chosen for evaluation.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y).

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CREATION OF A VIRTUAL LIBRARY OF SYNTHETIC COMPOUNDS AND ITS PRACTICAL USAGE FOR DOCKING WITH ALDOSE REDUCTASE

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Drug development is a process requiring the analysis of a large amount of data.[1] Creating a virtual database of synthesized compounds provides access to primary data concerning structure, results of biological activity studies, and molecular descriptors necessary for drug-like prediction. This work represents a continuation of the previous study.[2] Microsoft Excel was used to create the database, which includes different structural types, *e.g*. pyrazine, rhodanine, thiazolidin-2,4-dione, and 1,2,4-oxadiazole derivatives prepared in the research group Design and Development of New Antimicrobial Agents. Molecules are sorted according to structure-similarity, nevertheless, we also provide a spreadsheet containing all compounds in a line-notation ready-to-dock format. To demonstrate this database's actual usage, a molecular modelling study with enzyme aldose reductase was performed using the software Molecular Operating Environment (MOE). Aldose reductase is the first enzyme in the polyol-pathway, involved in microvascular complications of diabetes. It has become a drug target for aldose reductase inhibitors (ARI), *e.g*. epalrestat. The main goal of the project was to stress the correlation between *in vitro* and *in silico* studies. The isosteric approach was used to predict the binding mode and affinity towards the enzyme in order to test the suitability to synthesize new compounds in the upcoming research.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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MODIFICATION OF CAPILLARY WALL BY GRAPHENE FOR SEPARATION APPLICATIONS

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Capillary electrophoresis (CE) is a highly efficient separation method. Substances are separated due to different mobility in an electric field. The CE modes of operation can be modified in different ways, *e.g*. capillary electrochromatography, micellar electrokinetic chromatography.[1] On the other hand, modification of the inner wall of the capillary is believed to substantially improve both separation efficiency and selectivity. Graphene (G) is carbon with a hexagonal structure in form of two-dimensional sp^2 single-atom-thick sheets. G seems to be a suitable material for separation application due to its excellent properties such as large surface area together with affinity to carbon ring structures via π-π interactions.[2] Our work is focused on the modification of the capillary wall by graphene. The wall of the capillary was modified by the Layer-by-Layer method via layering of differently charged substances bounded by electrostatic forces.[3] Chemical coating employing covalent interactions was performed as well.[4] Different combinations of polymer (PDDA, PAH, PEI, APTES) and G were used for surface modification. Separation efficiency and selectivity of modified capillaries were studied on the model mixtures of analytes (parabens, nitrophenols, nitroanilines, purines). Obtained results were compared with commercial unmodified capillary. Improved separation together with prolonged interaction of analytes with G surface was observed.

The study was supported by the Research programme Development and Study of Drugs (Progres Q42) and from the project of Specific Academic Research (SVV 260 547).

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CHOLINESTERASES INHIBITED BY NOVICHOK AGENTS – *IN SILICO* STUDY OF REACTIVATION POSSIBILITIES

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"Novichoks" is the name of a series of nerve agents (NA) developed in the former Soviet union during the Cold War. Like other nerve agents, Novichok agents irreversibly bind acetylcholinesterase (AChE) and produce a cholinergic toxidrome, but have higher toxicity than older nerve agents.[1] Treatment of poisoning caused by nerve agents is based on oxime reactivators of AChE. Mechanism of reactivation involves nucleophilic attack (by oxime group) of phosphorus atom of inhibited AChE.[2] The aim of the work was to determine the binding energy of commercially available oxime reactivators into acetylcholinesterase inhibited by novichok, which could be used in the treatment of poisoning. Computational methods such as molecular docking and molecular dynamics were used for the study.

Structure of AChE inhibited by organophosphate (OP) were chosen from RSCB PDB (code 5HF9) and OP was replaced (using Chimera software) by 5 selected novichoks designated A230, A232, A234, A242 and A262. The protein was prepared for molecular modeling.

Four commercially available reactivators (pralidoxime, obidoxime, trimedoxime, HI-6) and one experimental reactivator (designated K203) were selected as ligands. They were prepared into docking-friendly pdbqt format (using ChemSketch, Avogadro and AutoDock Tools).

Semiflexible docking was used to determine the best ligand pose at the receptor. The most suitable pose was chosen as initial position for molecular dynamics. Then complexes protein-ligand were prepared and molecular dynamics were performed using Gromacs software. We determined the binding energy of the ligands in the protein.

The study was supported by the Faculty of Military Health Sciences and from the project of Specific Academic Research (SVV 260 547).

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SYNTHESIS OF POTENTIAL MYCOBACTERIAL INH-A AND CHOLINESTERASES INHIBITORS BASED ON TRICLOSAN

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The contribution deals with the synthesis and evaluation of new inhibitors of the mycobacterial InhA enzyme and inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). All of the prepared compounds are analogues derived from triclosan, they could be good candidates as potential drugs to use in the treatment of tuberculosis and neurodegenerative diseases, including Alzheimer's disease.[1,2]

Eight compounds were prepared in satisfactory yields. All of them were tested for antimicrobial and cholinesterase inhibitory activity. Among all the compounds that we prepared *N*-[5-chloro-2-(2,4-dichlorophenoxy)phenyl]acetamide showed the best antimicrobial effect against all three selected mycobacteria. Specifically, it had a minimum inhibitory concentration of 31.25 mg L^{-1} against *M. smegmatis*, a minimum inhibitory concentration of 15.625 mg L^{-1} against *M. aurum*, and a minimum inhibitory concentration of 7.81 mg L^{-1} against *M. tuberculosis* H37Ra. This compound also showed the lowest IC_{50} value for AChE (48.85 μmol L^{-1}) and has a better inhibitory effect against AChE than rivastigmine (56.10 μmol L^{-1}). The precursor 5-chloro-2-(2,4-dichlorophenoxy)aniline ("amino-triclosan") showed the best inhibition of BChE (IC_{50} = 11.93 μmol L^{-1}), which is a better inhibitory activity compared to rivastigmine again.

The study was supported by the Czech Science Foundation (Project No. 20-19638Y) and from the project of Specific Academic Research (SVV 260 547).

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SECTION OF PHARMACEUTICAL TECHNOLOGY

EFFECT OF COMBINATION OF MUCOADHESIVE POLYMERS ON THE BEHAVIOUR OF MATRIX TABLETS IN THE GASTRIC ENVIRONMENT

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Colon-targeted drug delivery plays a significant role in the pharmacotherapy of local diseases situated in the large intestine. In terms of sustained drug release, such formulations must be resistant to the acidic environment of the upper gastrointestinal tract. In addition, an enhanced therapeutic efficacy may be achieved by prolonging the residence time of the formulation at the absorption site by means of mucoadhesive drug delivery systems.[1] For these reasons, the presented preformulation study investigates the behaviour of matrix tablets based on mucoadhesive polymers in the gastric environment. Knowledge of the behaviour of a drug in acidic pH is important as the surroundings of the inflamed colon may be lower.

The selected polymers, guar gum (GG) and hydroxypropyl methylcellulose K15M (HPMC), were used separately or combined in ratios 85.4 : 14.6, 50 : 50 and 14.6 : 85.4. The viscosity of polymers dispersions was evaluated in biorelevant dissolution media simulating the gastric environment (FaSSGF) using a rotational rheometer. Subsequently, compacts containing model drug theophylline were prepared and evaluated for their swelling behaviour and dissolution profiles using a USP II apparatus. The obtained data shows that both polymers were able to sustain the drug release from the hydrophilic matrix tablets for up to 24 hours. The mixture 14.6 : 85.4 seems to be the most promising as it forms the strongest gel barrier during the first two hours of dissolution. On the contrary, compacts with a high amount of GG perform poorly as a pronounced burst effect may be observed due to its rapid swelling and dissolving of outer, fully hydrated layers into the gastric medium.

The study was supported by the Grant Agency of Charles University (Project No. 70119) from the project of Specific Academic Research (SVV 260 547).

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TECHNOLOGICAL SOLUTION OF FLOW BEHAVIOUR OF COMMERCIAL TABLETTING MIXTURE

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The success or failure of tablet production is particularly hinged on the flow behaviour of a tableting mixture during manufacturing process. The main aim of this experimental work was the evaluation of flow properties of the original commercial poorly flowable tableting mixture and the possible change of its composition to modified one having better flow properties. Flowability of the original fillers (lactose anhydrous, LA, and microcrystalline cellulose, MCC) and eight alternative ones, their binary mixtures and finally the newly proposed modified tableting mixture were evaluated using the standard pharmacopoeial methods such as bulk and tapped density, angle of repose, flow rate through a hopper orifice as well as by the consolidation dynamics by gravity tapping. Additionally, a powder rheometer (Freeman technology, Micromeritics Company, UK) was used to measure compressibility, cohesion, and flow stability of the samples. The result showed that the exchange of one filler, either LA or MCC, did not sufficiently improve the flow properties. Out of eight possible alternative fillers, Excipress GR 150 (lactose monohydrate) and Avicel PH 200 (microcrystalline cellulose) sufficiently solved the flowability problem. The significantly lower cohesion (72 times) and improved flow rate through the orifice with diameter of 10 mm was detected for the newly proposed modified tablet mixture comparing to the original one. As no change in the composition is possible for registration reasons, the replace of actual, poorly flowable excipients with those free flowable due to the surface modification by specific technological procedure without any change in the chemical structure is the main benefit of this work.

The study was supported from the project of Specific Academic Research (SVV 260 547).

THE STUDY OF MODEL LIPID MEMBRANES CONTAINING OMEGA-HYDROXYLATED CERAMIDES

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Acylceramides (EO-Cer) comprise a class of ceramides (Cer) with an ultralong, ω-hydroxylated acyl chain with ester-linked linoleic acid. EO-Cer are indispensable components of skin barrier, they enable formation of the long periodicity phase (LPP) and the

corneocyte lipid envelope (CLE). Disorders in EO-Cer biosynthesis are connected with many skin diseases, including some types of ichthyosis. One of the enzymes that is deficient in these ichthyoses is PNPLA1, responsible for an ω-esterification with linoleic acid, leading to insufficient EO-Cer concentrations. Instead, the precursors, *i.e.* ω-hydroxylated ceramides (O-Cer), cumulate.[1]

The aim of this project was to study the effects of O-Cer on lipid organization and barrier properties of model membranes. Membranes composed of cholesterol, fatty acids and very long Cer with physiological and non-physiological concentration of EO-Cer and O-Cer were prepared at different annealing temperatures. LPP, essential for proper barrier function, was formed in all membranes containing at least 7.5% of EO-Cer. Addition of O-Cer into membranes had mostly beneficial effect on membrane permeability. On the other hand, complete replacement of EO-Cer for their precursors (O-Cer) in membranes prepared at 90 °C led to more than three times increased permeability, which may be connected with an absence of LPP in this membrane.

The study was supported by the Czech Science Foundation (Project No. 19-09135J) and from the project of Specific Academic Research (SVV 260 547).

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STUDY OF FREE SPHINGOID BASES IN SKIN BARRIER

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Skin barrier, *stratum corneum* (SC), consists of corneocytes and intercellular matrix with three types of lipid molecules – ceramides, free fatty acids and cholesterol. Ceramides are structurally formed from the fatty acid acyl attached to sphingoid base. In minor amounts, free sphingoid bases can also be found in the skin barrier. In skin barrier disorders, there is an increase amount of free sphingoid bases.[1] Although it is assumed that presence of free sphingoid bases affects the skin barrier, it has not been elucidated why or how till today. Hence, the main goal of this work is to clarify how they influence the skin barrier. The experiment model membranes were prepared by the isolation of *ex vivo* human SC. Onto the SC, sphingosine (S), dihydrosphingosine (dS), and phytosphingosine (P) were applied. There were also used some of their mixtures in defined ratios, which mimic the condition of physiological and pathological SC. Sphingoid bases were applied as 1% suspensions in the mixture of propylene glycol and ethanol in the ratio of 7 : 3 (v/v). The experiment investigated the permeation parameters – transepidermal water loss (TEWL), electrical impedance (EI), and membrane permeability for the model permeant theo-

phylline (TH). Permeation parameters were measured for each group of samples in two phases – before and after application of suspensions or mixture of solvents on the control membranes. Based on the evaluation of results of permeation experiments, it is possible to confirm the assumption that free sphingoid bases influence all three tested parameters (increased TEWL and flux of TH, decreased EI). The individual sphingoid bases influenced the permeation parameters to a varying manner.

The study was supported by the Czech Science Foundation (Project No. 19-09135J) and from the project of Specific Academic Research (SVV 260 547).

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INFLUENCE OF MIXING CONDITIONS OF MICROCRYSTALLINE CELLULOSES WITH LUBRICANTS ON COMPRESSION PROCESS AND TABLET STRENGTH.

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This work evaluates the influence of a type of mixing device and mixing time of three types of microcrystalline cellusoses (MCC) with lubricants on the compression process and tablet strength. Microcel® MC 102, MC 200 and Prosolv® SMCC 90 are used as dry binders,[1] magnesium stearate and sodium stearyl fumarate at 1% concentration are used as lubricants. Two types of mixing devices are used to mix powders, a mixing cube and and 3D turbula mixer. The mixing time is 2 or 4 minutes. All tablets are compressed by compression force of 5 kN on the Zwick/Roell T1-FRO 50 material tester. The compressibility is evaluated by the energy profile of compression process, sensitivity to the addition of lubricants is characterized by lubricant sensitivity ratio values (LSR).[2] The total energy of compression of all MCC decreases with the addition of both types of lubricants and with increasing mixing time, more in the case of mixing in the cube. Formulations with Prosolv® SMCC 90 show the smallest decrease. Greater decrease in tablet strength is seen in all MCC at the mixing in the cube. Longer mixing times and the use of magnesium stearate also lead to greater decrease in tablet strength. Prosolv® SMCC 90 shows the lowest lubricant sensitivity, Microcel® MC 200 shows the highest.

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INFLUENCE OF MIXING CONDITIONS OF MICROCRYSTALLINE CELLULOSES WITH LUBRICANTS ON MECHANICAL PROPERTIES OF TABLETS

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The aim of the present study is to evaluate the influence of mixing conditions of three types of microcrystalline celluloses with lubricants on the mechanical properties of tablets, friability and disintegration time. The tested microcrystalline celluloses are silicified microcrystalline cellulose Prosolv® SMCC 90, microcrystalline celluloses Microcel® MC 102 and Microcel® MC 200.[1] Lubricants magnesium stearate and sodium stearyl fumarate are used at the concentration of 1%.[2] Mixtures are prepared by mixing in a mixing cube or Turbula mixer at two mixing times.[3] Tablets are prepared on Zwick/Roell Z050 material testing machine. The friability of tablets is evaluated by the pharmacopoeial method, the disintegration time by the pharmacopoeial method and the focused beam reflectance measurement method (FBRM). The highest friability values showed formulations with MCC 200, the lowest formulations with P90. The shortest disintegration times were measured for MCC 200 based on both methods, the longest for formulations with P90. The mixing cube showed a more negative effect on the mechanical properties of tablets compared to the Turbula. Magnesium stearate and mixing time of 4 minutes had more negative effect on friability and disintegration of tablets.

The study was supported from the project of Specific Academic Research (SVV 260 547).

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CORNEOCYTE LIPID ENVELOPE MODEL: EFFECTS ON THE HUMAN BARRIER SKIN LIPIDS

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Stratum corneum (SC), the uppermost layer of the skin, regulates transcutaneous water loss and protects against outer conditions and harmful substances. It consists of cornified cells – corneocytes and extracellular lipid matrix, which is responsible for barrier functions. Corneocytes are covered with covalently bound lipids creating corneocyte lipid envelope (CLE). CLE is considered to interconnect the extracellular lipids with corneocytes and to have a templating effect.[1] We aimed to create a skin lipid model simulating also the presence of CLE.

The lipidic part of the model was prepared from an equimolar mixture of isolated human skin ceramides (hCer), cholesterol and free fatty acids (FFA, either protonated or deuterated) with 5% w/w of cholesteryl sulfate. hCer were extracted from isolated human SC and purified by column chromatography. The composition of hCer was determined by high-performance thin-layer chromatography. The reverse-phase and normal phase silica gel particles served as the CLE model and negative control, respectively. X-ray diffraction revealed the periodic structure of models and showed two lamellar phases with short and long repeat distances and separated cholesterol. The long repeat distance changed in dependence on the type and amount of silica gel. The thermotropic behavior of samples with protonated or deuterated FFA was revealed by Fourier-transform infrared spectroscopy.

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STUDY OF SPHINGOSINE, DIHYDROSPHINGOSINE AND PHYTOSPHINGOSINE IN SKIN BARRIER MODELS

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The stratum corneum (SC) lipids are important for the proper function of the skin, especially ceramides (Cer), free fatty acids (FFA), and cholesterol (Chol), which are present in equimolar ratio. In the lipid matrix, the metabolic products of Cer (free sphingoid bases (FSB)) are also present, but their role in SC barrier functions is not clear. Some studies show that Cer with different sphingoid bases,[1] and increased presence of FSB, can lead to a change in the permeability of the skin barrier. This work aimed at studying the effect of permeability of sphingoid bases on the model membrane permeability. Nine types of membranes were prepared, they differed both in the presence of Cer (Cer NS vs. NdS vs. NP), but also in the presence of FSB (S vs. dS vs. P). In each membrane, there was

always a mixture of FFA (C_{16}–C_{24}), Chol, and 5% w/w proportion of cholesteryl sulfate. All S-containing membranes showed lower (compared to dS and P) permeability to water but not to ions. The hypothesis saying that "breaking" of Cer increases the permeability of model membranes was confirmed only for membranes containing S. The results of the experiment showed that for each type of Cer, there is a different permeability for water, ions, TH, and IND. The work contributed to the understanding of the importance of FSB on the permeability of model membranes, which could be useful in the study of complex models simulating a healthy/diseased skin barrier.

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STUDY OF THE CORNEOCYTE LIPID ENVELOPE

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Stratum corneum (SC) is the uppermost layer of the human epidermis. Its function is to protect the body and to prevent water loss and penetration of harmful substances or organisms from the outside environment. The structure of this layer is composed of corneocytes which are surrounded by lipid matrix. Beside the free lipids (ceramides, free fatty acids and cholesterol), in the lipid matrix are covalently bound lipids called the corneocyte lipid envelope (CLE).[1] While the role of free lipids is known, the effect of the CLE on the SC permeability is still unclear. In this work, we aimed to answer the question: "Is the CLE important in the skin permeability and does the CLE play any role as a template for the free lipid arrangement in the SC?" A partial aim of this work was to develop the valid method for the preparation of the CLE models, including delipidation and relipidation procedures. We prepared the SC models from human epidermis. First, we dermatomed the skin, then the SC was obtained by the trypsin treatment. We extracted the free lipids with organic solvents and finally we saponified the extracted SC with the alkaline methanol. The free lipids were then applied onto the extracted and saponified SC. The barrier properties of the prepared models were investigated by measuring of transepidermal water loss (TEWL), electrical impedance and the permeability of model permeant. We found out that the permeability to water of the extracted and saponified SC was very similar confirming the important role of free barrier lipids. While we observed a decrease of TEWL and flux of model permeant after the delipidation of the extracted SC, in case of saponified models with applied lipids we did not see any differences. This study confirms our hypothesis that the CLE act as a template for the orientation of the free barrier lipids and prevent permeable boundaries between the free lipids and corneocytes.

The study was supported by the Czech Science Foundation (Project No. 19-09135J) and from the project of Specific Academic Research (SVV 260 547).

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EFFECT OF COMBINATION OF MUCOADHESIVE POLYMERS ON THE BEHAVIOUR OF MATRIX TABLETS IN THE SMALL INTESTINE

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Colon-targeted drug delivery is highly desirable due to the direct treatment of local intestinal diseases, drug dose reduction, and minimization of systemic adverse effects. This effect can be achieved by several specific drug delivery systems based on different mechanisms. Generally, the most preferred are orally administered formulations due to the relatively easy manufacturing and high adherence of patients to treatment. These include matrix systems based on mucoadhesive polymers that allow both targeting to the desired site (*e.g.* colon) and controlled drug release.[1] For the above-mentioned reasons, the aim of the presented study was to evaluate the behavior of two mucoadhesive polymers, namely guar gum, hypromellose K15M and their combinations in biorelevant media simulating small intestine fluids (Fasted State Simulated Intestinal Fluid, FaSSIF). The viscosity of the polymer dispersions was measured using a rotational rheometer. Polymer formulations (compacts) containing the model drug theophylline were tested for adhesion (modified balance), swelling index (basket method) and dissolution profiles (paddle apparatus). The obtained results suggested that superior adhesion may be expected in the formulations containing a higher portion of K15M. Moreover, these compacts showed also one of the highest weight gains after 8 hours of the swelling test. Drug dissolution was characterized by controlled release from all prepared matrix tablets over a 24 hour time period. Formulations containing K15M released the drug more constantly in the initial phase in comparison to the formulation comprising guar gum.

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A STUDY OF COMPRESSIBILITY OF DIRECTLY COMPRESSIBLE TABLETING MATERIALS AND TABLETS WITH CARRAGEENAN.

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The presented communication deals with the study of compressibility of directly compressible tableting materials with ι-carrageenan.[1,2] In formulations, ι-carrageenan is mixed with chitosan, calcium alginate and hypromellose Methocel K15M in the ratios of 1 : 1, 2 : 1 and 3 : 1. The formulations with salicylic acid as a model drug at a concentration of 20% and sodium stearyl fumarate as a lubricant at a concentration of 1% are also tested. Tablets without the drug are compressed by compression forces of 3, 4 and 5 kN. Tablets with the drug are compressed by compression force of 5 kN and 8 kN. The compressibility is evaluated by energy profile of compression process, another tested parameter is tensile strength of the tablets. The total energy of compression increases with the compression force. The parameter is increased with the addition of chitosan and hypromellose in all ratios as well. With higher proportion of carrageenan in mixtures, its values decrease. The addition of chitosan, calcium alginate, and hypromellose increases values of the energy of plastic deformation and plasticity. The higher values of these parameters indicate improvement of compressibility. Only hypromellose in ratios of 1 : 1 and 2 : 1 to carrageenan increases the strength of carrageenan tablets, calcium alginate significantly reduces it. The model drug salicylic acid reduces all energy values and tensile strength of tablets.

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SECTION OF SOCIAL AND CLINICAL PHARMACY

CONSENSUS OF CZECH TERMINOLOGY IN THE FIELD OF MEDICATION ADHERENCE

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Due to the constantly changing terminology of medication adherence (MA) over the last 50 years, terms are often misused in the literature.[1] The aim was to analyze the Czech terminology of MA and to establish a consensus using a Delphi round survey. Czech literature focused on the term of MA and its synonyms was reviewed using bibliographic (PubMed, Bibliographia Medica Čechoslovaca) and full-text (Solen, ProLékaře.cz) databases. A total of 123 articles published between 1998–2020 involved terms "compliance" (69 articles, 57 definitions), "adherence" (78, 51), "persistence" (25, 23), and "concordance" (13, 11), respectively. Based on the identified literature, a list of panelists who were invited for the Delphi round survey as suggested by ABC Taxonomy[2] was created. This taxonomy determines 7 terms and their definitions in the field of MA. Out of 106 contacted panelists, 46 responded to the first round during which consensus for 2 definitions from 7 terms in the terminology of MA was established. Further rounds are an ongoing process and the rest of the terms and definitions will be determined. The disunity of using the terminology of MA and related terms showed up as a very common phenomenon of the Czech language. A consensus of Czech terminology will be established after the last round of Delphi survey.

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TRICYCLIC ANTIDEPRESSANTS CONSUMPTION IN THE CZECH REPUBLIC

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Tricyclic antidepressants are one of the oldest groups of antidepressants. Except for indication for depression, they are used in the therapy of chronic pain or enuresis nocturna in children. Nowadays, tricyclic antidepressants are drugs of the first choice only in some types of neuropathic pain, and in other indications they have been replaced by newer substances with a lower occurrence of side effects. The aim of this work is to find out how utilization of tricyclic antidepressants has developed and what share in utilization of antidepressants they represent. Data for this work were obtained from the State Institute for Drug Control of the Czech Republic, namely from distributors' report about supplies of medicinal products to pharmacies and healthcare facilities, sellers of selected medicinal products, other distributors and veterinary doctors. Utilization was evaluated for the period from 1.1.2008 to 31.12.2018 with DUR (drug utilization review). For evaluation, a defined daily dose per 1000 inhabitants per day and methods of descriptive statistics were selected.

A slight decrease of utilization of tricyclic antidepressants was observed for the watched period, as well as decrease in their share on antidepressatns utilization. The reason for the decrease could be the side effects of tricyclic antidepressants or their possible interactions with other drugs.

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RATIONALITY OF BZD USE IN PHARMACY PRACTICE IN SPAIN AND IN DIFFERENT SETTINGS OF CARE IN THE CZECH REPUBLIC: RESULTS FROM THE INOMED AND THE EUROAGEISM H2020 PROJECTS

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The aim of the study was to compare the prescription of BZDs in Spanish (SP) and Czech (CZ) sample of older adults. Data of 260 SP community-residing seniors 65+ and of 1602 CZ seniors 65+ were prospectively collected in the EuroAgeism H2020 project in 2018–2019 (by Comprehensive Geriatric Assessment) and analyzed using descriptive statistics (R-software, version 4.0.3). In SP and CZ (community pharmacy samples), there were 62.4% and 64.6% of women and the mean age of participants was 71.74 +/− 6.25 years and 76.61 +/− 7.15 years, respectively. Polypharmacy and excessive polypharmacy were documented in 24.9% and 3.8% ($p < 0.001$) (CZ) and in 41.2 % and 13.1% ($p < 0.001$) (SP) of older adults. 36.2% of seniors used at least one BZDs in SP compared to 4.9% in CZ ($p < 0.001$). Top 3 most frequently prescribed BZDs were (in SP): lorazepam (16.5%), lormetazepam (6.5%) and alprazolam (4.6%), (in CZ): alprazolam (1.1%), bromazepam (0.7%) and oxazepam (0.4%). In Spain, extensively high prevalence of BZDs in older adults (35.4%, $p < 0.001$), with expected potential negative consequences, was found in comparison with community pharmacy practice in CZ (2.4%, $p < 0.001$), acute care (18.0%, $p < 0.001$) and ambulatory care (16.7%, $p < 0.001$).

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AN ANALYSIS OF DRUG INTERACTIONS BASED ON DRUG INFORMATION CENTRE ENQUIRIES

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Drug information centre (DIC) of the Faculty of Pharmacy in Hradec Králové, Charles University and University Hospital Hradec Králové provides drug information to healthcare professionals in the form of timely and accurate answers to drug-related enquiries, including drug interactions (DI). This study aimed to analyze enquiries related to DI processed by DIC from 2015 to 2020. Data were collected from individual enquiries related to drug-drug, drug-herbal and drug-disease interactions. Various parameters assigned to each DI found were analyzed, such as clinical severity or ATC codes of interacting drugs. An analysis based on descriptive statistical methods was carried out. A total of 67 enquiries were analyzed. The three most frequent ATC codes being involved in at least one DI per enquiry were A02BC01 (omeprazole), B01AC06 (acetylsalicylic acid), and C10AA05 (atorvastatin). Warfarin, venlafaxine and omeprazole were the most frequently interacting drugs regarding the different mechanism of interactions. In contrast, citalopram, furosemide, levothyroxine and omeprazole prevailed in all interacting pairs of every interaction. The majority (62%) of DI were based on pharmacodynamics, while 31% were based on pharmacokinetics. The clinical severity of DI was graded A (Minor) in 23%, B (Moderate) in 67%, and C (Severe) in 10% of cases. The most common potential clinical outcomes of DI were increased risk of adverse effects (18%), elevated plasmatic concentration of a drug (16%), and higher risk of bleeding (13%). Additionally, a (still ongoing) qualitative analysis of five enquiries sharing a similar clinical topic was carried out. DI constitute a significant portion of enquiries processed by DIC and seem to differ in clinical severity, pharmacological mechanism and potential clinical outcomes.

The study was supported from the project of Specific Academic Research (SVV 260 551).

THE PREVALENCE OF DRUG-DRUG INTERACTIONS IN PATIENTS ADMITTED TO THE HOSPITAL VIA THE EMERGENCY DEPARTMENT

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The presence of potential drug-drug interactions (DDIs) is common in daily practice and only a small proportion of potential DDIs result in hospitalization of the patients. Nev-

ertheless, DDIs represent a significant cause of hospital admissions.[1] This cross-sectional study aims to identify DDIs in the medication history of the patients admitted to University Hospital Hradec Králové via the emergency department in August–November 2018. The objectives of this study are a) to determine the prevalence of hospital admissions with potential DDIs, b) to categorize identified potential DDIs with respect to their mechanism, severity, risk rating, level of documentation and potential outcomes and c) to determine the prevalence of hospital admissions with manifest DDIs. A sample of 375 hospital admissions has been analyzed so far. 300 hospital admissions have been screened for the presence of potential DDIs using Micromedex, Lexicomp and DrugAgency a. s. database of drug-drug interactions. 75 hospital admissions have been excluded from the screening process due to an insufficient number of medications in medication history (< 2). 2273 potential DDIs were identified in 258 hospital admissions (86% of the eligible admissions). The overall prevalence of hospital admissions with identified potential DDI in medication history was 68.8% (95% CI: 64.1–73.5). 866 different DDI pairs were involved in these potential DDIs. Manifest DDIs which contributed to hospital admission were identified in 17 (4.5%) hospital admissions. The findings will provide a deeper insight into the pharmacoepidemiologic aspects of DDIs.

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KUCHAŘOVÁ, M., BÁRTA, P., NOVÁKOVÁ, V.: Praktická cvičení z biofyziky (Practical exercises in biophysics). 4th modified ed. Prague, Karolinum Press, 2021, 52 pp. ISBN 978-80-246-4987-0.

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PATENTS

KAPKOWSKI, M., LUDYNIA, M., POLAŃSKI, J., DZIDA, M., RUDNICKA, M., BALIN, K., DOLEŽAL, M., KASTNER, P.: PL 239074 B1, 02.11.2021.

ROH, J., ŠTĚRBA, M., ŠIMŮNEK, T., ŠTĚRBOVÁ, P., KARABANOVICH, G., JIRKOVSKÁ, A., JIRKOVSKÝ, E., BAVLOVIČ PISKÁČKOVÁ, H., KUBEŠ, J., JANSOVÁ, H., KOLLÁROVÁ, P.: WO2021/144746 A1, 22.07.2021.

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DEGREES

Lectures for the Professorship Appointments, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2021

doc. RNDr. DALIBOR ŠATÍNSKÝ, Ph.D.: Associate Professor, Head of the Department of Analytical Chemistry, Faculty of Pharmacy, Hradec Králové.
Discipline: Analytical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 22. 09. 2020
Continuation: 13. 10. 2020
Title of Lecture: Moderní přístupy k úpravě vzorku v průtokových analytických metodách (Modern approaches to sample preparation in flow analytical methods), 08. 12. 2020
Appointment: 08. 05. 2021

doc. PharmDr. Mgr. DAVID VETCHÝ, Ph.D.: Associate Professor, Institute of Pharmaceutical Technology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno.
Discipline: Pharmaceutical Technology, NAU-518/2018-9
Inauguration: 11. 05. 2020
Continuation: 09. 06. 2020
Title of Lecture: Dvacet let pokroku ve farmaceutické technologii (Twenty years of progress in pharmaceutical technology), 13. 10. 2020
Appointment: 08. 05. 2021

Habilitation Theses and Lectures for Associated Professor Appointments, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2021

RNDr. JAKUB HOFMAN, Ph.D.: Senior Lecturer, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Hradec Králové.

Discipline: Human and Veterinary Pharmacology, MŠMT 24 295/2007-30/1
Inauguration: 02. 11.2020
Continuation: 08. 12. 2020
Habilitation Thesis: Farmakokinetická léková rezistence v protinádorové terapii a možnosti její modulace (Pharmacokinetic drug resistance in antitumor therapy and possibilities of its modulation), defended 16. 03. 2021
Title of Lecture: Role biotransformačních enzymů v rezistenci vůči protinádorovým léčivům (The role of biotransformation enzymes in resistance to anticancer drugs), 16. 03. 2021
Appointment: 01. 06. 2021

PharmDr. JAKUB CHLEBEK, Ph.D.: Academic Worker, Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové.
Discipline: Pharmacognosy, NAU-518/2018-9
Inauguration: 19. 02. 2021
Continuation: 16. 03. 2021
Habilitation Thesis: Isochinolinové alkaloidy jako potenciální léčiva (Isoquinoline alkaloids as potential drugs), defended 08. 06. 2021
Title of Lecture: Současný pohled na alkaloidy jako reálná a potenciální léčiva (Current view of alkaloids as real and potential drugs), 08. 06. 2021
Appointment: 01. 08. 2021

PharmDr. JAN KORÁBEČNÝ, Ph.D.: Research and Development Worker, Biomedical Research Centre, University Hospital, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 10. 09. 2020
Continuation: 13. 10. 2020
Habilitation Thesis: Multipotentní sloučeniny v terapii Alzheimerovy choroby (Multipotent compounds in Alzheimer's disease therapy), defended 08. 12. 2020
Title of Lecture: Koncepce multipotentních sloučenin v léčbě Alzheimerovy demence (The concept of multipotent compounds in the treatment of Alzheimer's dementia), 08. 12. 2020
Appointment: 01. 08. 2021

RNDr. JANA POUROVÁ, Ph.D.: Academic Worker, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Hradec Králové.
Discipline: Human and Veterinary Pharmacology, MŠMT 24 295/2007-30/1
Inauguration: 17. 02.2021
Continuation: 16. 03. 2021
Habilitation Thesis: Vliv polyfenolických látek na vaskulární systém (Effect of polyphenolic substances on the vascular system), defended 08. 06. 2021
Title of Lecture: Farmakologie vaskulárního systému (Pharmacology of the vascular system), 08. 06. 2021
Appointment: 01. 08. 2021

Doctoral Dissertation Theses to obtain the Ph.D. Degree, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2021

Mgr. BAVLOVIČ PISKÁČKOVÁ, HANA: Využití LC-MS v bioanalýze antracyklinů a potenciálních kardioprotektiv (Ultilization of LC-MS in bioanalysis of anthracyclines and potential cardioprotective compounds), 11. 05. 2021, Ph.D.
Ing. SEDLÁČEK, JAKUB: Studium distribuce substituentů v derivátech na bázi hyaluronanu (Studying the distribution of substituents in derivatives based on hyaluronane), 04. 03. 2021, Ph.D.
MSc. AL SHAMMARI, LATIFAH AJAJ: Alkaloids of genus Hippeastrum (Amaryllidaceae): isolation, identification, biological activity, 31. 05. 2021, Ph.D.

Mgr. VAGIANNIS, DIMITRIOS: Study on the role of pharmacokinetic mechanisms of drug resistance in new anticancer drugs with focus on solid tumors, 25. 06. 2021, Ph.D.

Mgr. KARAHODA, RONA: Physiological and pharmacological aspects of tryptophan and serotonin homeostasis in the fetoplacental unit, 25. 06. 2021, Ph.D.

Mgr. REIMEROVÁ, PETRA: Analýza léčiv a potenciálních léčiv v biologickém materiálu s využitím kapalinové chromatografie (Analysis of drugs and potential drugs in biological material using liquid chromatography), 19. 01. 2021, Ph.D.

Mgr. JENČO, JAROSLAV: Vývoj a optimalizácia chromatografických metód vhodných pre bioanalytickú aplikáciu (Development and optimization of chromatographic methods suitable for clinical application), 04. 03. 2021, Ph.D.

Mgr. JAROLÍMOVÁ, ŽOFIE: Studium sypného a konsolidačního chování binárních směsí Celet a plniva pro přípravu vícevrstvých tablet (Study of flow and consolidation behaviour of binar mixtures of Cellets and filler for preparation of multilayer tablets), 21. 09. 2021, Ph.D.

Mgr. NAJPAVEROVÁ, SIMONA: Zmeny nutrície, pokojového energetického výdaja a telesnej kompozície u českých žien v období gravidity a laktácie vo vzťahu k pôrodným parametrom a produkcii materského mlieka (Changes in nutrition, resting energy expenditure, and body composition of Czech women during pregnancy and lactation in relation to birth parameters and milk production), 06. 10. 2021, Ph.D.

Mgr. KUBAČKOVÁ, JANA: Preparation of pharmaceutical formulations based on polymeric and lipid carriers, 29. 06. 2021, Ph.D.

Mgr. RAABOVÁ, HEDVIKA: Nanovlákna jako moderní extrakční sorbenty pro extrakci vzorků v chromatografické analýze (Nano-fibers as modern extraction sorbents for extraction of samples in chromatographic analysis), 16. 12. 2021, Ph.D.

Mgr. ŠTEFELA, ALŽBETA: Novel bile acid derivatives as a promising therapeutic approach for liver and metabolic disorders, 03. 09. 2021, Ph.D.

Mgr. CYRUSOVÁ, TEREZA: Vliv nanočástic na metabolismus rostlin (Effect of nanoparticles on plant metabolism), 15. 02. 2021, Ph.D.

Mgr. FÁBRYOVÁ, TEREZA: Phytochemical analysis and biological activity of the alga *Haematococcus pluvialis* and *Chlorella* sp., 31. 05. 2021, Ph.D.

Mgr. KOHELOVÁ, ELIŠKA: Alkaloidy *Zephyranthes citrina* (Amaryllidaceae): izolace, strukturní identifikace, biologická aktivita (Alkaloids of *Zephyranthes citrina* (Amaryllidaceae): isolation, identification, biological activity), 15. 09. 2021, Ph.D.

Mgr. JANÁKOVÁ, BARBORA: The role of microRNA in physiology and pathology, 30. 09. 2021, Ph.D.

Mgr. NOVÁČKOVÁ, ANNA: Studium látek ovlivňujících propustnost kožní bariéry (Study of substances affecting permeability of the skin barrier), 29. 06. 2021, Ph.D.

Mgr. MAŘÍKOVÁ, JANA: NMR spektroskopie ve strukturní analýze izolovaných alkaloidů (NMR spectroscopy in structure elucidation of isolated alkaloids), 04. 05. 2021, Ph.D.

Rigorous Theses to obtain the degree PharmDr. (Doctor of Pharmacy, graduates of the study programme Pharmacy) or RNDr. (Doctor of Natural Sciences, graduates of study programme Bioanalytical Laboratory Diagnostics in Medicine), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2021

Mgr. ADAMCOVÁ, ADRIANA: Vliv glukózy na expresi endoglinu a biomarkerů endotelové dysfunkce u endotelových buněk (Effect of glucose treatment on endoglin and biomarkers of endothelial dysfunction in endothelial cells), 18. 02. 2021, PharmDr.

Mgr. ADAMIČKOVÁ, LEONA: Screening alkaloidů s cílem hledání potenciálních léčivých látek (Alkaloids screening with a focus on potential drug discovery), 28. 06. 2021, PharmDr.

Mgr. ANTALOVÁ, SIMONA: Syntéza a hodnocení potenciálních antituberkulotik na bázi inhibitorů InhA (Synthesis and evaluation of InhA inhibitors as potential antitubercular drugs), 14. 12. 2021, PharmDr.

Mgr. ASTAPENKO, MICHAELA: Syntéza a biologické hodnocení nových takrin-tryptofanových derivátů (Synthesis and biological evaluation of novel tacrine-tryptophan derivatives), 11. 11. 2021, PharmDr.

Mgr. BACSKAIOVÁ, SILVIA: Príprava biodegradabilných polymérnych nanočastíc (Biodegradable polymeric nanoparticles preparation), 22. 06. 2021, PharmDr.

Mgr. BARÁK, VLASTIMIL: Vliv formulačních faktorů na vlastnosti nanočástic s terbinafinem (Influence of formulation factors on the characteristics of terbinafine loaded nanoparticles), 14. 01. 2021, PharmDr.

Mgr. BARTOŠ, LUKÁŠ: Syntéza nitroheteroaromátů s potenciální antimykobakteriální aktivitou (Synthesis of nitroheteroaromatic compounds with potential antimycobacterial activity), 14. 12. 2021, PharmDr.

Mgr. BAVLOVIČ PISKÁČKOVÁ, HANA, Ph.D.: Využití LC-MS v bioanalýze antracyklinů a potenciálních kardioprotektiv (Utilization of LC-MS in bioanalysis of anthracyclines and potential cardioprotective compounds), 04. 06. 2021, PharmDr.

Mgr. BECA, PAVOL: Izolácia metabolitov tryptofánu z biologického materiálu (Isolation of tryptophan metabolites from biological material), 15. 01. 2021, PharmDr.

Mgr. BELCÁKOVÁ, HEDVIGA: HPMC-based liposomal mucoadhesive films with model peptide as target API, 07. 01. 2021, PharmDr.

Mgr. BENKOVÁ, MARKÉTA: Testování antimikrobiální účinnosti nově syntetizovaných látek (Antimicrobial susceptibility testing of newly synthesized compounds), 19. 02. 2021, RNDr.

Mgr. BERÁNKOVÁ, ANNA: Účinky vybraných látek *in vitro* na izolované aortě potkana (The *in vitro* effects of selected substances on isolated rat aorta), 24. 09. 2021, PharmDr.

Mgr. BINDEROVÁ, MAGDALENA: Asociace fázového úhlu s dynamometrickými a spirometrickými parametry u pacientů s CHOPN (Association of phase angle with dynamometric and spirometric parameters in patients with COPD), 01. 07. 2021, PharmDr.

Mgr. BLAHUŠOVÁ, ADRIANA: Studium sekundárních metabolitů v rostlinných explantátových kulturách I (The study of secondary metabolites in plant tissue cultures I), 17. 02. 2021, PharmDr.

Mgr. BLAŽKOVÁ, JIŘINA: Studium vlastností biodegradovatelných nanočástic na bázi polyesterů (A study of biodegradable polyesters based nanoparticles properties), 14. 01. 2021, PharmDr.

Mgr. BOBČÍKOVÁ, MARTINA: Analýza profylaktického podávání antibiotik II (Analysis of antibiotic administration in prophylaxis II), 10. 05. 2021, PharmDr.

Mgr. BOBRÍKOVÁ, MICHAELA: Vývoj a validace HPLC metody pro stanovení obsahu antokyanů v kultivarech černého rybízu (HPLC method development and validation for analysis of anthocyanins content in black currant cultivars), 05. 11. 2021, PharmDr.

Mgr. BOUČKOVÁ, KAROLÍNA: Regulace glutathionperoxidas pomocí mikroRNA (MicroRNA regulation of glutathion peroxidases), 19. 02. 2021, RNDr.

Mgr. BUBÁKOVÁ, ZUZANA: Studium vhodnosti α-bromfenyloctové kyseliny jako modelového analytu pro chirální separace s využitím kapilární elektroforézy II (Study of α-bromophenylacetic acid suitability as a model analyte for chiral separations using capillary electrophoresis as a separation technique II), 18. 06. 2021, PharmDr.

Mgr. BUJŇÁKOVÁ, TERÉZIA: Hodnotenie kvality života tehotných v Českej a Slovenskej republike pomocou špecifického dotazníka QOL-GRAV (Quality of life evaluation in pregnant women in the Czech Republic and Slovakia by means of a specific questionaire QOL-GRAV), 10. 05. 2021, PharmDr.

Mgr. BULÁKOVÁ, ANNA: Izolácia alkaloidov druhu *Geissospermum vellosii* Alemão a štúdium ich biologickej aktivity I (Isolation of alkaloids of the species *Geissospermum vellosii* Allemão and study of their biological activity I), 29. 06. 2021, PharmDr.

Mgr. BURDA, JAKUB: Využití HPLC v chirálních separacích V (The use of HPLC in the field of chiral separations V), 15. 01. 2021, PharmDr.

Mgr. BURIANOVÁ, GABRIELA: Flow-cytometrická analýza inhibičního vlivu nových cílených léčiv na aktivitu ABC lékových efluxních transportérů (Flow-cytometric analysis of inhibitory effect of novel targeted drugs on the activity of ABC drug efflux transporters), 16. 02. 2021, PharmDr.

Mgr. CIMBÁLOVÁ, EDITA: Identifikace a analýza terapie užívané těhotnými ženami III (Identification and analysis of therapy used by pregnant women III), 10. 05. 2021, PharmDr.

Mgr. COMOVÁ, KATEŘINA: *In vitro* screening nových, potenciálně antimykoticky účinných sloučenin II (*In vitro* screening of novel potentially active antimycotic compounds II), 19. 02. 2021, RNDr.

Mgr. CYMBÁL, MARTIN: Interakce alkaloidů s přechodnými kovy III (Interactions of alkaloids with transition metals III), 19. 02. 2021, RNDr.

Mgr. CYRUSOVÁ, TEREZA, Ph.D.: Vliv nanočástic na metabolismus rostlin (Effect of nanoparticles on plant metabolism), 03. 03. 2021, RNDr.

Mgr. ČECHOVÁ, LENKA: Sledování distribuce látky BZ po intramuskulárním podání (The monitoring of agent BZ after intramuscular administration), 18. 06. 2021, PharmDr.

Mgr. ČERNÝ, ONDŘEJ: Konopí pro léčebné použití v lékárně (Medical cannabis at the pharmacy), 14. 09. 2021, PharmDr.

Mgr. ČIŽMÁROVÁ, JANA: Analýza postojov seniorov na samoliečenie analgetikami so zameraním na nesteroidné antiflogistiká II (The analysis of senior's opinions on self-treatment by non-steroidal anti-inflamatory drugs II), 10. 05. 2021, PharmDr.

Mgr. DANIELISOVÁ, MONIKA: Asociace příjmu energie a parametrů energetického metabolismu těhotných a kojících žen (The association of energy intake and parameters of energy metabolism in pregnant and lactating women), 29. 09. 2021, PharmDr.

Mgr. DOLEŽÁLKOVÁ, PETRA: Vliv dlouhodobého působení solubilního endoglinu na signalizaci membránového endoglinu v myší aortě (Soluble endoglin effects on membrane endoglin signaling in mouse aorta), 18. 02. 2021, PharmDr.

Mgr. DOMECKÝ, PETR: Analýza profylaktického podávání antibiotik I (Analysis of antibiotic administration in prophylaxis I), 21. 07. 2021, PharmDr.

Mgr. DOUBEK, JIŘÍ: Kostní cementy na bázi fosforečnanu vápenatého: Syntéza, charakterizace a vlastnosti uvolňování léčivé látky (Calcium phosphate bone cements: Synthesis, characterization and drug release properties), 07. 01. 2021, PharmDr.

Mgr. DÚŽKOVÁ, MARKÉTA: Výsledky dlouhodobého sledování parazitostatu černé zvěře a jeho kontroly v oborním chovu černé zvěře (The results of long term monitoring of parazitostatus and its control in wild boar game preserve), 30. 06. 2021, PharmDr.

Mgr. FASCHINGBAUER, JAKUB: Cytotoxická a cholinesterasová inhibiční aktivita extraktů z vybraných druhů rodu Centaurea L. (Cytotoxic and cholinesterase inhibitory activity of extracts from selected species of the Centaurea L. genus), 01. 09. 2021, PharmDr.

Mgr. FEŘTOVÁ, LADA: Analýza odborných konzultací poskytovaných pacientům v lékárně I (Analysis of consultations provided to patients in a pharmacy I), 12. 03. 2021, PharmDr.

Mgr. FICAL, LUBOŠ: Vývoj UHPLC-MS/MS metody pro analýzu vybraných antivirotik v HILIC a RP módu (Development of UHPLC-MS/MS method for analysis of selected antivirotics in HILIC and RP mode), 15. 01. 2021, PharmDr.

Mgr. GALČAN, GABRIEL: Redukce nitroskupiny s využitím platinového katalyzátoru v průtokovém reaktoru (The reduction of the nitro group using a platinum catalyst in a flow reactor), 11. 11. 2021, PharmDr.

Mgr. GAVUROVÁ, LUCIE: Porovnání interakce cyanidinu a cyanidin-3-glukosidu s mědí a železem (Comparasion of interaction of cyanidin and cyanidin-3-glucoside with copper and iron), 24. 09. 2021, PharmDr.

Mgr. GRAJA, ZBYNĚK: Přírodní látky a jejich biologická aktivita VIII. Antioxidační aktivita obsahových látek nati *Rhodiola rosea* L. (Natural substances and their biological activity VIII. Antioxidant activity of substances contained in aerial parts of *Rhodiola rosea* L.), 28. 06. 2021, PharmDr.

Mgr. GRAŇÁKOVÁ, PATRÍCIA: Modulácia expresie a aktivity vybraných detoxikačných enzýmov rastlín anthelmintikami (Modulation of expression and activity of selected plant detoxifying enzymes by anthelmintics), 29. 09. 2021, PharmDr.

Mgr. GRANDE, VOJTĚCH: Vývoj nového modelu pro hodnocení kompresní fáze lisovacího procesu (Development of a new model for the evaluation of compression phase of compaction process), 09. 02. 2021, PharmDr.

Mgr. HADYSOVÁ, ZUZANA: Online extrakce na tuhé fázi pomocí sekvenční injekční analýzy (Online solid phase extraction using sequential injection analysis), 01. 02. 2021, RNDr.

Mgr. HAJŠELOVÁ, ZUZANA: Fotoprotekcia u pacientov po transplantácii obličiek (Photoprotection in patients after kidney transplantation), 10. 05. 2021, PharmDr.

Mgr. HETMAN, ANASTASIIA: Sulfates as phase II metabolites of natural phenolic compounds, 18. 02. 2021, PharmDr.

Mgr. HLUBUČKOVÁ, LUCIE: Analýza proteinové nálože extracelulárních vesiklů izolovaných z kvasinky *Candida albicans* (Analysis of protein cargo of extracellualr vesicles isolated from the yeast *Candida albicans*), 18. 02. 2021, PharmDr.

Mgr. HOLMANOVÁ, PAVLÍNA: Fotodynamická inaktivace mikroorganismů (aza)ftalocyaninovými fotosensitizéry (Photodynamic inactivation of microorganisms using (aza)phthalocyanine photosensitisers), 29. 09. 2021, RNDr.

Mgr. HOUŠKOVÁ, DENISA: Vliv generace polyamidoaminodendrimerů s ethylendiaminovým jádrem a aminoskupinami na periferii na (trans)dermální podání 5-fluorouracilu (Effect of the generation of amino-deco-

rated polyamidoamine dendrimers with ethylenediamine core at the (trans)dermal delivery of 5-fluorouracil), 15. 04. 2021, PharmDr.

Mgr. HRABINOVÁ, MARTINA, Ph.D.: Vývoj metod pro testování látek ovlivňujících CNS (Development of methods for testing drugs affecting the CNS), 16. 07. 2021, RNDr.

Mgr. HRDINOVÁ, PETRA: HPLC stanovení luteinu, vitaminu E, vitaminu E acetátu, betakarotenu v potravních doplňcích (HPLC determination of lutein, vitamin E, vitamin E acetate, beta-carotene in food supplements), 18. 06. 2021, PharmDr.

Mgr. HROMÁDKO, JIŘÍ: Vývoj UHPLC-MS/MS metody a postupu přípravy vzorků pro stanovení steroidních látek v potkaní plasmě (Development UHPLC-MS/MS method and sample preparation procedure for the determination of steroid compounds in rat plasma), 05. 11. 2021, PharmDr.

Mgr. HUDEČKOVÁ, HANA: Nanofiltrace (Nanofiltration), 26. 02. 2021, PharmDr.

Mgr. HUTLAS, ANDREJ: Growth and control of *Pseudomonas aeruginosa* in a multi-species biofilm, 18. 02. 2021, PharmDr.

Mgr. HYNKOVÁ, ANETA: Validace čištění plničky tvrdých želatinových tobolek (Cleaning validation of capsule filling machine), 09. 02. 2021, PharmDr.

Mgr. CHLADOVÁ, PAVLÍNA: Vliv koncentrace polyamidoaminodendrimerů s ethylendiaminovým jádrem a aminoskupinami na periferii na (trans)dermální podání 5-fluorouracilu (Effect of the concentration of amino-decorated polyamidoamine dendrimers with ethylenediamine core at the (trans)dermal delivery of 5-fluorouracil), 15. 04. 2021, PharmDr.

Mgr. CHŘIBEK, MATĚJ: Příprava fenylsubstituovaných benzothiazolů jako potenciálních modulátorů ABAD (Preparation of phenyl derived benzothiazoles as potential ABAD modulators), 08. 01. 2021, PharmDr.

Mgr. JANDÁČKOVÁ, ADRIANA: Stanovení tokového retenčního potenciálu silikátů pro makrogol 400 a propylenglykol (Determination of the flowable liquid retention potential of silicates for polyethylene glycol 400 and propylene glycol), 07. 01. 2021, PharmDr.

Mgr. JANDURA, DOMINIK: *In vitro* kultivace tasemnice *Hymenolepis diminuta* – 2 (*In vitro* cultivation of tapeworm *Hymenolepis diminuta* – 2), 18. 02. 2021, PharmDr.

Mgr. JANEČKOVÁ, ADRIANA: Studium vlivu množství přidaného chelatačního činidla na výsledek konjugace monoklonální protilátky (Study of the effect of the amount of chelating agent addition on the result of monoclonal antibody conjugation), 24. 09. 2021, PharmDr.

Mgr. JANOUŠKOVÁ, ADÉLA: Studium vlivu vybraných inhibitorů proteinkináz na lékovou rezistenci zprostředkovanou cytochromy P450 (Study on impact of selected protein kinase inhibitors on drug resistance mediated by cytochromes P450), 18. 02. 2021, PharmDr.

Mgr. JAROLÍMOVÁ, ŽOFIE, Ph.D.: Studium sypného a konsolidačního chování binárních směsí Celet a plniva pro přípravu vícevrstvých tablet (Study of flow and consolidation behaviour of binar mixtures of Cellets and filler for preparation of multilayer tablets), 12. 10. 2021, PharmDr.

Mgr. JELÍNKOVÁ, TEREZA: Postoje a znalosti o očkování proti HPV IV (Knowledge and attitudes to HPV immunisation IV), 10. 05. 2021, PharmDr.

Mgr. JELÍNKOVÁ, VALERIE: Hodnocení energetické bilance u spontánně dýchajících polytraumatizovaných pacientů na nutriční podpoře na JIP (The energy balance evaluation at spontaneous breathing polytrauma patients with nutritional support in ICU), 29. 09. 2021, PharmDr.

Mgr. JENČO, JAROSLAV, Ph.D.:Vývoj a optimalizácia chromatografických metód vhodných pre bioanalytickú aplikáciu (Development and optimization of chromatographic methods suitable for clinical application), 14. 04. 2021, RNDr.

Mgr. JIRKOVÁ, TEREZA: Development of dispersive liquid-liquid microextraction coupled with capillary liquid chromatography for determination of fipronil and its two metabolites, 26. 02. 2021, PharmDr.

Mgr. JOHNOVÁ, KAROLÍNA: Studium vlivu kombinace mukoadhezivních polymerů na chování matricových systémů v prostředí žaludku (Effect of combination of mucoadhesive polymers on the behaviour of matrix tablets in the gastric environment), 23. 11. 2021, PharmDr.

Mgr. KALOUSOVÁ, PAVLA: Sexual dimorphism of rat gut microbiota composition and intestinal immunity, 18. 02. 2021, PharmDr.

Mgr. KAMASOVÁ, TERÉZIA: *In vitro* effects of 3-hydroxytyrosol on renal hypoxia and inflammation, 01. 07. 2021, PharmDr.

Mgr. KÁNTOR, MICHAL: Syntéza a hodnotenie zhášačov fluorescencie zo skupiny azaftalocyaninov (Synthesis and study of azaphthalocyanine quenchers of fluorescence), 15. 04. 2021, PharmDr.

Mgr. KANTOROVÁ, TEREZA: Měď chelatující účinky flavanonů (Copper chelating properties of flavanones), 18. 02. 2021, PharmDr.

Mgr. KARAHODA, RONA, Ph.D.: Physiological and pharmacological aspects of tryptophan and serotonin homeostasis in the fetoplacental unit, 20. 12. 2021, Pharmdr.

Mgr. KARAŠČÁKOVÁ, DIANA: Biologická aktivita sekundárnych metabolitov rastlín XXX. Základný screening vybraných taxonóv na anticholinesterázovú aktivitu (Biological activity of plants secondary metabolites XXX. Basic search of selected taxons on anticholinesterase activity), 29. 06. 2021, PharmDr.

Mgr. KAREŠOVÁ, ELIŠKA: Studium přímo lisovatelných tabletovin a tablet s chitosanem (A study of directly compressible tableting materials and tablets with chitosan), 14. 01. 2021, PharmDr.

Mgr. KEPKA, ZDENĚK: Problematika stanovení neopterinu a kreatininu v moči s využitím vysokoúčinné kapalinové chromatografie se zaměřením na klinickou praxi (Difficulty of urinary neopterin and creatinine determination using high-performance liquid chromatography with focus on clinical practice), 29. 03. 2021, RNDr.

Mgr. KERNAL, JAKUB: Synthesis of isoprenoid naringenin derivatives, 08. 01. 2021, PharmDr.

Mgr. KLEČKA, MICHAL: Klidový energetický výdej v průběhu laktace (Resting energy expenditure during lacatation, 10. 05. 2021, PharmDr.

Mgr. KOBRLOVÁ, TEREZA, Ph.D.: Predikce prostupu nových léčiv přes hematoencefalickou bariéru (Prediction of the penetration of new drugs through the blood-brain barrier), 16. 07. 2021, RNDr.

Mgr. KODEDOVÁ, MARIE: Automatizace liberačních testů pro uvolňování biologicky aktivních látek z nanovláken (Automation of liberation tests for releasing biologically active substances from nanofibers), 05. 11. 2021, PharmDr.

Mgr. KOHLOVÁ, MICHAELA, Ph.D.: Development of new types of biocompatible hemodialysis membranes for separation of biomolecules, 26. 02. 2021, RNDr.

Mgr. KOPKOVÁ, NIKOLA: Meď-redukčné účinky derivátov xantén-3-ónov (Copper reducing properties of a series of xanthen-3-ones), 16. 02. 2021, PharmDr.

Mgr. KOŘÍNKOVÁ, ZUZANA: Vliv seskviterpenů na jaterní cytochromy P450 (The effect of sesquiterpenes on the hepatic cytochromes P450), 01. 07. 2021, PharmDr.

Mgr. KRASULOVÁ, KRISTÝNA, Ph.D.: Studium interakcí léčiv s enzymy metabolismu cizorodých látek (Drug interactions with xenobiotic-metabolizing enzymes), 02. 03. 2021, RNDr.

Mgr. KRTILOVÁ, KAMILA: Vliv inhibice tepotinibu, entrektinibu a sapanisertibu na aktivitu vybraných reduktas z nadrodiny AKR (Inhibitory effect of tepotinib, entrectinib, and sapanisertib on an activity of selected reductases from AKR superfamily), 01. 07. 2021, PharmDr.

Mgr. KRUPOVÁ, KRISTÍNA: Serom ako následná komplikácia po operáciách karcinómov prsníka (Seroma as a follow-up complication after breast cancer surgery), 14. 09. 2021, PharmDr.

Mgr. KUBAČKOVÁ, JANA, Ph.D.: Preparation of pharmaceutical formulations based on polymeric and lipid carriers, 05. 08. 2021, PharmDr.

Mgr. KUBÁTOVÁ, DENISA: Hodnotenie sfingozínu, dihydrosfingozínu a fytosfingozínu v modeloch kožnej bariéry (Study of sphingosine, dihydrosphingosine and phytosphingosine in skin barrier models), 23. 11. 2021, PharmDr.

Mgr. KUBÍKOVÁ, VERONIKA: Kardiovaskulární rizikové faktory a komplikace související s aterosklerózou – jejich výskyt a kontrola u seniorů v projektu EUROAGEISM H2020 (Cardiovascular risc factors and complications associated with atherosclerosis – their prevalence and control in seniors in the EUROAGEISM H2020 project), 16. 02. 2021, PharmDr.

Mgr. KUDA, LUKÁŠ: Vývoj superkritické fluidní extrakce pro izolaci biologicky aktivních látek (Development of supercritical fluid extraction for isolation of biologically active substances), 05. 11. 2021, PharmDr.

Mgr. LACUŠOVÁ, MONIKA: Anomálie viscerálnych tepien (Anomalies of visceral arteries), 14. 09. 2021, PharmDr.

Mgr. LAKOMÁ, PETRA: Vliv alisertibu a brigatinibu na aktivitu vybraných lidských karbonylredukujících enzymů (The effect of alisertib and brigatinib on the activity of selected human carbonyl reducing enzymes), 01. 07. 2021, PharmDr.

Mgr. LAMOŠOVÁ, JANA: Vplyv podmienok miesenia mikrokryštalických celulos s mazadlami na mechanické vlastnosti tabliet (Influence of mixing conditions of microcrystalline celluloses with lubricants on mechanical properties of tablets), 23. 11. 2021, PharmDr.

Mgr. LUKÁŠOVÁ, VERONIKA: Role lékových transportérů v placentárním přestupu entekaviru (Role of drug transporters in placental transfer of entecavir), 18. 02. 2021, PharmDr.

Mgr. MACKUROVÁ, MICHAELA: Měření účinnosti nových inhibitorů acetylcholinesterasy pomocí Ellmanovy metody (Evaluation of the efficacy of new acetylcholinesterase inhibitors by Ellman's method), 24. 09. 2021, PharmDr.

Mgr. MAJCHER, ADAM: Syntéza a hodnotenie ľudských 6-hydroxyceramidov (Synthesis and evaluation of human 6-hydroxyceramides), 14. 12. 2021, PharmDr.

Mgr. MARTINEC, ONDŘEJ, Ph.D.: Interakce antiretrovirálních léčiv s membránovými transportéry (Interactions of antiretroviral drugs with membrane transporters), 14. 02. 2021, PharmDr.

Mgr. MARTIŠKA, JURAJ, Ph.D.: Medikované polymerní systémy založené na větvených derivátech PLGA (Medicated polymer systems based on branched PLGA derivatives), 27. 01. 2021, PharmDr.

Mgr. MAŘÍKOVÁ, JANA, Ph.D.: NMR spektroskopie ve strukturní analýze izolovaných alkaloidů (NMR spectroscopy in structure elucidation of isolated alkaloids), 17. 06. 2021, PharmDr.

Mgr. MAZEL TONAROVÁ, MARTA: Biologicky aktivní metabolity rostlin VIII. Alkaloidy *Fumaria officinalis* a jejich biologická aktivita (Biologically active metabolites of plants VIII. Alkaloids of *Fumaria officinalis* L. and their biological activity), 28. 06. 2021, PharmDr.

Mgr. MICHÁLEK, GEORG: Analýza činnosti Lékového informačního centra IV – lékové interakce (Drug Information Centre service analysis IV – drug interactions), 14. 09. 2021, PharmDr.

Mgr. MOLNÁROVÁ, MARIE: Deriváty pyrazinu jako potenciální léčiva (Pyrazine derivatives as potential drugs), 15. 04. 2021, PharmDr.

Mgr. MORAVCOVÁ, KATARÍNA: Štúdium vplyvu povrchovo aktívnych látok na parametre polymérnych nanočastíc (Study of the surfactants effect on polymer nanoparticle parameters), 30. 09. 2021, PharmDr.

Mgr. MORAVCOVÁ, PAVLÍNA: Vývoj HPLC metody pro stanovení vybraných fenolických kyselin a flavonoidů v Tokajských vínech (Development of HPLC method for determination of selected phenolic acids and flavonoids in Tokaj wines), 01. 02. 2021, RNDr.

Mgr. MÚČKOVÁ, ĽUBICA, Ph.D.: *In vitro* charakterizácia látok modulujúcich aktivitu acetylcholinesterasy (*In vitro* characterization of acetylcholinesterase modulators), 19. 05. 2021, RNDr.

Mgr. NAJPAVEROVÁ, SIMONA, Ph.D.: Zmeny nutrície, pokojového energetického výdaja a telesnej kompozície u českých žien v období gravidity a laktácie vo vzťahu k pôrodným parametrom a produkcii materského mlieka (Changes in nutrition, resting energy expenditure, and body composition of Czech women during pregnancy and lactation in relation to birth parameters and milk production), 12. 11. 2021, PharmDr.

Mgr. NOVÁČKOVÁ, ANNA, Ph.D.: Studium látek ovlivňujících propustnost kožní bariéry (Study of substances affecting permeability of the skin barrier), 05. 08. 2021, PharmDr.

Mgr. NOVOTNÁ, JANA: Precizace predikce léčebné odpovědi protinádorové imunoterapie (Precisation prediction of therapeutic response to cancer immunotherapy), 18. 06. 2021, PharmDr.

Mgr. NOVOTNÁ, KATEŘINA: Interakce gilteritinibu s transportéry OCT1 a OCT2; vztah ke konvenční terapii akutní myeloidní leukémie (Interaction of gilteritinib with OCT1 and OCT2 transporters; relation to conventional therapy of acute myeloid leukemia), 24. 09. 2021, PharmDr.

Mgr. NOVOTNÁ, SIMONA: Pokročilé testování antibakteriální aktivity kandidátních nově syntetizovaných sloučenin (Advanced antibacterial activity testing of candidate newly synthesized compounds), 18. 02. 2021, PharmDr.

Mgr. ODVÁRKOVÁ, ANNA: Kinetic evaluation of potential inhibitors for selected cysteine proteases. 16. 02. 2021

Mgr. ONDRAŠÁKOVÁ, PETRA: Optimalizácia LbL kapsúl obsahujúcich PEI a pDNA (Optimization of PEI based LbL capsules with pDNA), 30. 06. 2021, PharmDr.

Mgr. OUZKÝ, MIROSLAV: Studium vlivu pomocných látek na disoluci léčiva z tablet (Study of excipients' influence on the drug dissolution from tablets), 14. 01. 2021, PharmDr.

Mgr. PELNÁŘOVÁ, KAROLÍNA: Hledání účinných chelátorů kobaltu – 8-hydroxychinoliny (Search of effective cobalt chelators – 8-hydroxyquinolines), 24. 09. 202, PharmDr.

Mgr. PETERKOVÁ, ALENA: Vliv inositol hexafosfátu na adhezivitu a migraci buněčných linií SW480 a SW620 (Effect of inositol hexaphosphate on adhesion and migration of cell lines SW480 and SW620), 19. 02. 2021, PharmDr.

Mgr. PCHÁLKOVÁ, TEREZA: *In vitro* screening potenciálních antimykobakteriálně účinných sloučenin na rychle rostoucích kmenech rodu *Mycobacterium* II (*In vitro* screening of potential antimycobacterial compounds against fast growing strains of *Mycobacterium* genus II), 18. 02. 2021, RNDr.

Mgr. PITROVÁ, MARKÉTA: Risks and problems associated with polypharmacy in older patients – A self-screening tool for identifying risks of pharmacotherapy by patients themselves, 10. 05. 2021, PharmDr.

Mgr. PORÁČ, JAKUB: Hodnocení vlivu inhibitorů CDK a FLT3 na aktivitu ABC efluxních transportérů *in vitro*, vztah k mnohočetné lékové rezistenci (Effect of CDK and FLT3 inhibitors on activity of ABC efflux transporters *in vitro*, relation to multidrug resistance), 16. 02. 2021, PharmDr.

Mgr. POTŮČKOVÁ, ADÉLA: Deriváty Amaryllidaceae alkaloidů a jejich biologická aktivita (Derivatives of Amaryllidaceae alkaloids and their biological activity), 17. 02. 2021, PharmDr.

Mgr. PROKOP, PAVEL: Analýza argininu a jeho methylovaných derivátů v klinických vzorcích pomocí LC-MS/MS (Analysis of arginine and its methylated derivatives in clinical samples using LC-MS/MS), 19. 02. 2021, RNDr.

Mgr. RABASOVÁ, MARKÉTA: Studium přímo lisovatelných tabletovin s kombinací chitosanu a silicifikované mikrokrystalické celulosy (A study of directly compressible tableting materials with the combination of chitosan and silicified microcrystalline cellulose), 15. 04. 2021, PharmDr.

Mgr. REIMEROVÁ, PETRA, Ph.D.: Analýza léčiv a potenciálních léčiv v biologickém materiálu s využitím kapalinové chromatografie (Analysis of drugs and potential drugs in biological material using liquid chromatography), 08. 02. 2021, PharmDr.

Mgr. RYDRYCH, MILAN: Syntéza derivátů BODIPY pro fotodynamickou terapii (Synthesis of BODIPY derivatives for photodynamic therapy), 11. 11. 2021, PharmDr.

Mgr. RZEPECKÁ, RADKA: Interakce alkaloidů s přechodnými kovy I. (Interactions of alkaloids with transition metals I.), 29. 06. 2021, PharmDr.

Mgr. SEJKOROVÁ, ILONA: Vybrané faktory komplementového systému v diagnostice intraamniálního zánětu u pacientek s předčasným porodem spojeným s předčasným odtokem plodové vody (Selected factors of the complement system in the diagnosis of intraamnial inflammation in a patient with preterm prelabor rupture of fetal membranes), 29. 09. 2021, RNDr.

Mgr. SEMRÁDOVÁ, ADÉLKA: Acidorezistentní polymerní nanočástice: příprava a hodnocení (Acidoresistant polymeric nanoparticles: preparation and assessment), 22. 06. 2021, PharmDr.

Mgr. SLOVÁKOVÁ, TEREZA: Hodnocení dusíkové bilance u ventilovaných polytraumatizovaných pacientů na JIP (The nitrogen balance evaluation at ventilated polytrauma patients in ICU), 18. 02. 2021, PharmDr.

Mgr. SOUKUPOVÁ, JANA: Adhezivní vlastnosti tenkých filmů na bázi plastifikovaných polyesterů (Adhesive properties of thin layers based on plasticized polyesters), 09. 02. 2021, PharmDr.

Mgr. STRAKOVÁ, DÁŠA: Fylogenetická diverzita kultivovatelných prokaryot z hypersalinních prostředí (Phylogenetic diversity of culturable prokaryotes from hypersaline environments), 19. 02. 2021, PharmDr.

Mgr. STUDENOVSKÁ, PETRA: Výběr a validace referenčních genů pro relativní kvantifikaci mRNA v lidských jaterních řezech (Selection and validation of reference genes for relative mRNA quantification in human liver slices), 19. 02. 2021, RNDr.

Mgr. SUCHOPÁROVÁ, LENKA: Analýza spotřeby antibiotik pro systémové podání v České republice v letech 2005–2019 (Analysis of drug utilization of antibiotics for systematic administration in the Czech Republic in 2005–2019), 10. 05. 2021, PharmDr.

Mgr. SVOBODOVÁ, LUCIE: Takrin-benzothiazolové deriváty v léčbě Alzheimerovy choroby (Tacrine-benzothiazole derivatives in Alzheimer's disease), 15. 04. 2021, PharmDr.

Mgr. ŠEBESTOVÁ, GABRIELA: Vývoj CE-C4D metody pro analýzu sacharidů v medu (Development of CE-C4D method for the analysis of saccharides in honey), 29. 03. 2021, RNDr.

Mgr. ŠEBOVÁ, DOMINIKA: Testovanie vplyvu novo nasyntetizovaných látok na viabilitu buniek *in vitro* (Testing of influence of newly synthetized compounds on viability of cells *in vitro*) 24. 09. 2021, PharmDr.

Mgr. ŠILHAVÁ, KRISTÝNA: Sledování profilu fenolických látek v různých částech jabloní pomocí HPLC (Monitoring the profile of phenolic substances in different parts of apple trees by HPLC), 08. 09. 2021, RNDr.

Mgr. ŠILHOVÁ, MARKÉTA: Interakce alkaloidů s přechodnými kovy II (Interactions of alkaloids with transition metals II), 17. 02. 2021, PharmDr.

Mgr. ŠÍPKOVÁ, PAVLA: Biologická aktivita sekundárních metabolitů rostlin X. Alkaloidy *Vinca minor* L. (Biological activity of secondary plants metabolites X. Alkaloids of *Vinca minor* L.), 17. 02. 2021, PharmDr.

Mgr. ŠIRAJOVÁ, DANIELA: Příprava polymerních fluorescenčních nanočástic (Preparation of polymeric fluorescent nanoparticles), 30. 09. 2021, PharmDr.

Mgr. ŠÍROVÁ, KAROLÍNA: Analýza nutričně významných látek v odpadních produktech ovocných stromů pomocí HPLC (Analysis of nutritionally important substances in fruit tree waste products by HPLC), 08. 09. 2021, RNDr.

Mgr. ŠLECHTA, PETR: Deriváty kombinující fragment pyrazinamidu a 4-aminosalicylové kyseliny jako antimykobakteriální sloučeniny (Derivatives combining the fragment of pyrazinamide and 4-aminosalicylic acid as antimycobacterial compounds), 08. 01. 2021, PharmDr.

Mgr. ŠOBOROVÁ, IVANA: Analýza spontánního hlášení nežádoucích účinků antiepileptik (Analysis of spontaneous adverse events reports of antiepileptic drugs), 10. 05. 2021, PharmDr.

Mgr. ŠRÁMOVÁ, ELIŠKA: Studium vlivu koncentrace cholesterolu na monovrstevné modely (Study of the effect of cholesterol concentration on monolayer models), 07. 01. 2021, PharmDr.

Mgr. ŠTEFELA, ALŽBETA, Ph.D.: Novel bile acid derivatives as a promising therapeutic approach for liver and metabolic disorders, 23. 09. 2021, PharmDr.

Mgr. ŠTĚPNIČKOVÁ, TEREZA: Formulace liposomů obsahujících imiquimod v přítomnosti dendrimerů (Formulation of imiquimod loaded liposomes in the presence of dendrimers), 23. 11. 2021,

Mgr. TRÁVNÍČEK, TOMÁŠ: Analýza terapie infekcí na dětském oddělení I (Analysis of infection therapy in paediatric department I), 10. 05. 2021, PharmDr.

Mgr. ŤUPOVÁ, LENKA, Ph.D.: Interakce vybraných antiretrovirálních léčiv a metylrtuti s membránovými transportéry placenty (Interactions of selected antiretroviral drugs and methylmercury with placental membrane transporters), 04. 01. 2021, PharmDr.

Mgr. UHROVÁ, ADÉLA: Vývoj online SPE HPLC metody pro stanovení ochratoxinu A v tokajských vínech (Development of online SPE HPLC method for determination of ochratoxin A in Tokaj wines), 01. 02. 2021, RNDr.

Mgr. URBÁNKOVÁ, TEREZA: Bioimpedanční spektroskopická analýza kompozice těla v době laktace (Bioimpedance spectroscopic body analysis during lactation), 14. 09. 2021, PharmDr.

Mgr. VAGIANNIS, DIMITRIOS, Ph.D.: Study on the role of pharmacokinetic mechanisms of drug resistance in new anticancer drugs with focus on solid tumors, 23. 09. 2021, PharmDr.

Mgr. VALENTOVÁ, IVANA: Přírodní látky a jejich biologická aktivita. Screening alkaloidních rostlin na anticholinesterasový účinek (Natural substances and their biological activity. Screening of alkaloid plants for anticholinesterase effect), 28. 06. 2021, PharmDr.

Mgr. VAŘILOVÁ, TEREZA: Vliv teploty a koncentrace roztoku na vlastnosti sprejově sušené laktosy s využitím trysky o průměru 1,4 mm (Effect of temperature and solution concentration on the properties of spray-dried lactose using the nozzle with diameter of 1.4 mm), 22. 06. 2021

Mgr. VĚŘÍŠ, ANDREA: Characterization of PLGA-based film forming systems, 15. 04. 2021, PharmDr.

Mgr. VICEN, MATEJ, Ph.D.: Membránový endoglín a jeho úloha v patogenéze endotelovej dysfunkcie v podmienkach *in vitro* (Membrane endoglin and its role in pathogenesis of endothelial dysfunction *in vitro*), 04. 01. 2021, PharmDr.

Mgr. VOLDŘICHOVÁ, LENKA: Lipidické nanočástice jako platforma pro dodání léčiv (Lipid based nanoparticles: drug delivery platform), 09. 02. 2021, PharmDr.

Mgr. VU, QUYNH ANH: Syntéza a hodnocení inhibitorů vybraných enzymů jako potenciálních léčiv (Synthesis and evaluation of selected enzyme inhibitors as potential drugs), 29. 09. 2021, RNDr.

Mgr. WINTEROVÁ, LUCIE: Homeostáza vnitrobuněčného pH v patogenních kvasinkách *Candida albicans* a *Candida glabrata* (Intracellular pH homeostasis in pathogenic yeast *Candida albicans* and *Candida glabrata*), 16. 02. 2021, PharmDr.

Mgr. ZAJÍČKOVÁ, ŠÁRKA: Extrakce amfetaminů a syntetických katinonů z mateřského mléka pomocí membránových mikroextrakčních technik (Extraction of amphetamines and synthetic cathinones from breast milk using liquid membrane microextraction techniques), 18. 06. 2021, PharmDr.

Mgr. ZELINA, DUŠAN: Deriváty Amaryllidaceae alkaloidov a ich biologická aktivita: Deriváty tazettínu II (Derivatives of Amaryllidaceae alkaloids and their biological activity: Derivatives of tazettine II), 29. 06. 2021, PharmDr.

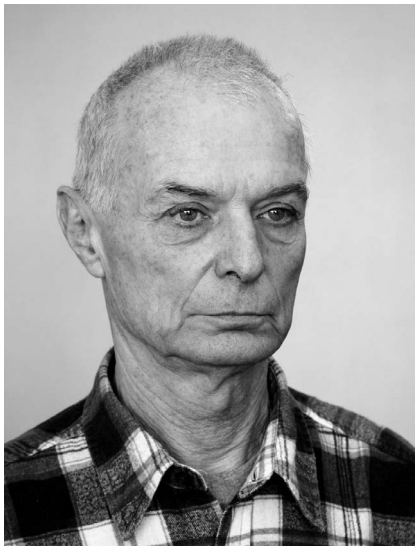
Mgr. ZENKEROVÁ, KATHARINA: Vliv evobrutinibu na rezistenci nádorových buněk k daunorubicinu způsobenou enzymy redukujícími karbonylové skupiny (Effect of evobrutinib on cancer cell resistance to daunorubicin caused by carbonyl reducing enzymes), 29. 09. 2021, PharmDr.

Mgr. ZOUHAROVÁ, MONIKA: Optimalizácia extrakcie neonikotinoidov s využitím nanovlákien v systéme sekvenčnej injekčnej analýzy (Optimization of neonicotinoids extraction using nanofibers in sequential injection analysis system), 15. 01. 2021, PharmDr.

Mgr. ŽEMLIČKOVÁ, SIMONA: Hybridní polymerní-lipidické nanočástice jako nosiče léčiv (Hybrid polymeric-lipid nanoparticles as drug carriers), 14. 01. 2021, PharmDr.

SOCIAL HAPPENINGS

IN MEMORY OF ASSOC. PROF. RNDR. PETR KLEMERA, CSC.



Petr Klemera was born on 11th August 1943 in Hradec Králové. After graduating from primary and secondary school, he studied at the Faculty of Mathematics and Physics of Charles University in Prague. Mathematics became his lifelong profession. After finishing his studies in Prague, he returned to Hradec Králové, where he worked briefly at the Faculty of Medicine of Charles University, but soon changed his workplace and from 1st September 1970 became an employee of the newly established Faculty of Pharmacy of Charles University in Hradec Králové. He was one of the first three employees of the then Department of Physical Chemistry at this faculty. He taught mathematics and mathematical statistics there. In the research field he was engaged in the development of mathematical models of biomechanical behaviour of biological systems and partly also in the use of neural networks. In 1995, he was appointed an Associate Professor of Biophysics and Biocybernetics. He remained faithful to the faculty until his retirement in 2008, but even then he liked to help the department with teaching and research.

Petr Klemera united the collective of the department, he was the one who came up with proposals for various departmental events, which he also documented photographically. He was an extremely kind person, always helpful, empathetic, very cheerful and pleasant. He was always willing to help colleagues from other departments solve mathematical and statistical problems.

His life's journey came to an end on 11th August 2021, the very day of his 78th birthday.

Honor to his memory

Monika Kuchařová

INSTRUCTIONS FOR AUTHORS
FOR FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

Manuscripts should be prepared on the A4 paper, in English, typed in Microsoft Word using Times New Roman font and spacing 1.5. It should be divided into the following sections:

Title – Times New Roman 14, left alignment (SPECTROFOTOMETRIC DETERMINATION OF ...). Write the title in lowercase letters and then format it using Font – All Caps (Písmo – Všechna velká).

Names of Authors – Times New Roman 12, center alignment (GASPARIČ, J.,[1] MILENA ČERMÁKOVÁ, M.[2]). Write the names in lowercase letters and then format them using Font – All Caps (Písmo – Všechna velká).

Names of Institutions – Times New Roman 10, center alignment ([1] Department of....., Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic).

Email address – Times New Roman 10, center alignment (e-mail: gasparic@faf.cuni.cz.)

Text – Times New Roman 12, left aligment without indents, starting a new paragraph only by Enter. Bold and Italics may be used. Manuscripts of Original Papers should be divided into sections. Headings of the sections:

ABSTRACT – 12, left alignment

KEYWORDS – maximum 5 keywords, 12, left alignment (KEYWORDS: extraction – spectrophotometry)

INTRODUCTION – 12, left alignment

EXPERIMENTAL – 12, left alignment. This part may be further subdivided, *e.g.*

Chemistry

Materials and Methods

General procedure for the preparation of the studied compounds

(E) 1-(5 tert butylpyrazin-2-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one

Bioassays

Evaluation of antimycobacterial activity

Evaluation of photosynthesis-inhibiting activity

If it is not absolutely necessary, do not use more than three levels of headlines.

Figures must be submitted in black and white and in original size (not more than 12.5 × 18 cm), separately as a supplement. Indicate the placement of the figure in the text. Captions and notes are placed below (10, left alignment)

<Koukal-Fig2.jpg>

Fig. 1. Structures of the studied compounds

Tables are placed in the text. Values in the table are written in columns without frame. Title of the table (Table 1. Antifungal activities of the studied compounds.) (10, left alignment) is above the table. Notes are below the table. The layout of the table must be submitted separately.

Chemical structures and schemes should be drawn with a suitable drawing program and inserted into the text using wrapping style (Styl obtékání) in the line with text ("V textu"). Captions and notes are placed below (10, left alignment).

RESULTS AND DISCUSSION – 12, left alignment

Acknowledgements – 12 italic, left alignment, *e.g.*
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References – 12, center alignment

References must be numbered continuously and indicated as an upper index in the text (……cancer.[1,2]). If there are more than 3 authors, use reduced format *e.g.* ŠPULÁK, M., POUROVÁ, J., VOPRŠÁLOVÁ, M. *et al.*: Eur. J. Med. Chem., 74, 2014, 65–72. Do not use automatic numbering.

Journals

1. AGRAWAL, Y. K., PATEL, D. R.: Indian J. Pharm. Sci., 47 (5), 1985, 207–209.

Books

1. NĚMCOVÁ, I., ČERMÁKOVÁ, L., GASPARIČ, J.: Spectrophotometric reactions. New York, Marcel Dekker Inc., 1996.
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Theses

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