UNIVERSITAS CAROLINA PRAGENSIS

FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

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Arranged by Assoc. Prof. RNDr. Veronika Opletalová, Ph.D.

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ABSTRACTS

9th POSTGRADUAL AND 7th POSTDOCTORAL SCIENTIFIC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ, CHARLES UNIVERSITY, HRADEC KRÁLOVÉ, 23–24 JANUARY 2019

DEDICATED TO THE 50th ANNIVERSARY OF THE FOUNDING OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

MICROBIAL RESISTANCE – ONE OF THE HOTTEST TOPICS OR THE PAST?!

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Microbial resistance is a collocation that has been frequently used all over the world. New bacterial strains that have developed resistance are noticed every year. Number of new cases is rising daily. There are rumours claiming that 10 million people will suffer and die of infectious disease caused by resistant strain in 2050. WHO has started a few programs that should lead to elimination of resistance, *e.g.* END TB or Global action plan on antimicrobial resistance.[1,2] On the other hand, resistant microbes, so called superbugs, have their own mechanisms how to survive our attempts to kill them.

One way how to be succesfull in attempts to kill these microbes is to focus on finding novel antimicrobial compounds (new mechanism of action, advantageous pharmacological properties, broader spectrum of pathogens being influenced).

Design and synthesis of these compounds is crucial part of process but it is also very important to focus on biological properties such as minimal inhibition concentration, time-kill assays or mechanism of action (MoA) determination. MoA is also very important to understand the resistance mechanisms. We are able to determine MoA of potential antibiotic agent in four biochemical pathways – inhibition of cell wall synthesis, inhibition of DNA synthesis, inhibition of RNA synthesis or inhibition of proteosynthesis. This assay is based on the incorporation of radioactively labelled compounds, which are part of studied

biochemical pathways. If detected radioactivity is lower, it means that potential antibiotic inhibits this pathway and radioactively labelled molecule cannot be incorporated into the final products. Standards used for this screening are vancomycin (inhibition of cell wall synthesis indicated by ^{3}H labelled *N*-acetylglucosamine), rifampicin (inhibition of RNA synthesis indicated by ^{3}H labelled uridine), ciprofloxacin (inhibition of DNA synthesis indicated by ^{3}H labelled thymidine), chloramphenicol (inhibition of proteosynthesis indicated by ^{3}H labelled leucine), and chlorhexidine (positive control, inhibition of all mentioned biosynthetic pathways).[3]

Currently, we are focusing on determining the possible MoA of potential antimycobacterial agents. We have revealed specific biomolecules that are connected with mycobacterial biosynthetic pathways. We have chosen ^{3}H labelled arabinose which is involved in biosynthetic pathway of mycobacterial cell wall synthesis (ethambutol is used as standard – inhibition of arabinosyl transferase), and ^{3}H labelled acetic acid, which is involved in the same pathway (isoniazid is used as standard – inhibiton of mycolic acids synthesis). We also need to optimize MoA medium for culturing mycobacteria (*Mycobacterium smegmatis*, *M. aurum* and *M. tuberculosis* H37Ra), to set MIC for standards (rifampicin, isoniazid, ciprofloxacin, streptomycin, ethambutol, gentamicin, vancomycin, chlorhexidine) and to set time-kill properties.

The study was supported by the research program Development and Study of Drugs (Progress Q42).

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NEW UNALLOYED METAL NANOCONJUGATES AS CATALYSTS IN SELECTED REDUCTION OR ACETALIZATION REACTIONS FOR GREEN CHEMISTRY

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A series of mono, bi and tri-metal nanocatalytic transition metal systems were obtained and investigated. The catalysts included combinations of metals (Re, Ru, Rh, Ir, Pd) deposited on a silica support (SiO_2) or on a metal support (Ni or Mo). These are new, never described before, catalytic nanomaterials. The studied nanocatalytic systems may be reproducibly obtained by the described synthetic methods. These heterogeneous nanocatalysts can be used in industrial processes, especially in the reaction of carbon dioxide methanation, ammonia decomposition and glycerol acetalization.

In the low-temperature ammonia decomposition reaction, a high activity was obtained by the Pd_{NPs}/Ni catalyst which can be used to generate hydrogen in fuel cells. The Ru_{NPs}/Ni catalyst has a high activity in the reaction of low-temperature methanation of carbon oxides and can be used in methane production. Obtained nanocatalysts: Re/SiO_2, ReRu/SiO_2, ReIr/SiO_2, ReRuIr/SiO_2, ReRhIr//SiO_2, ReRuRh/SiO_2, RuRhIr/SiO_2, Ru/Mo, RuRh/Mo, RuRhIr/Mo and ReRhIr/Mo show high activity in acetalization reactions. Nano-Re supported on SiO_2 is a highly active and selective catalyst for the acetalization of glycerol into five-membered cyclic acetals (*e.g.* solketal) and can be used for the processing of glycerol waste. The tested catalytic systems may have practical application and serve for the development of the described industrial chemical processes, which may bring ecological and economic benefits.

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SUPRAMOLECULAR ASSEMBLY OF TETRAPYRAZINOPORPHYRAZINES INTO J-DIMERS

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Alkylamino substituted azaphthalocyanines (AzaPcs) have interesting spectral properties – they absorb in a wide range of UV-vis spectrum from 300 to 600 nm and quench fluorescence of other compounds. In non-coordinating solvents, these AzaPcs form J-dimers (Fig. 1) due to their planar aromatic core[1] that may affect their application as quenchers in oligodeoxynucleotide probes.[2] The tendency to aggregation can be driven by peripheral substitution. The goal of this project was to study the relation between peripheral substitution and stability of J-dimers (expressed as K_D). Therefore, a series of unsymmetrical AzaPcs (Fig. 2) was synthesized. Synthetic pathway included preparation of appropriate precursors, statistical cyclotetramerization, isolation of desired ABBB congener and introduction of zinc cation to the center of metal-free AzaPc. Stability of J-dimers was investigated in toluene by titration with pyridine. Obviously, increasing bulkiness of substituent caused destabilization of J-dimers.

The study was supported by Charles University (Project No. 1168217) and from the project of Specific Academic Research (SVV 260 401).

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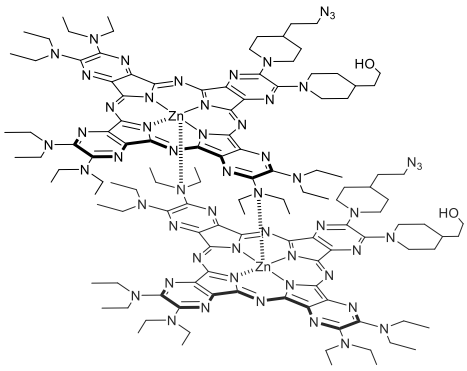

Figure 1. Example of AzaPc J-dimer

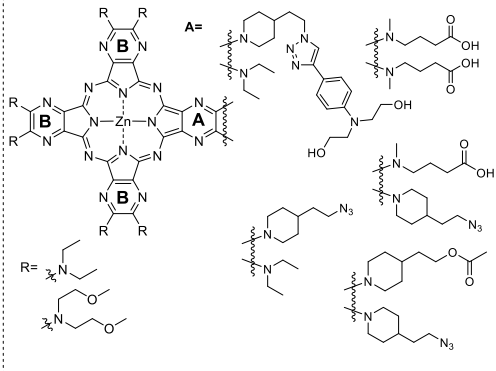

Figure 2. Structures of target unsymmetrical AzaPcs

GOLD(I)-CATALYZED SYNTHESIS OF PIPERIDINE AMINALS AND THEIR FURTHER TRANSFORMATIONS

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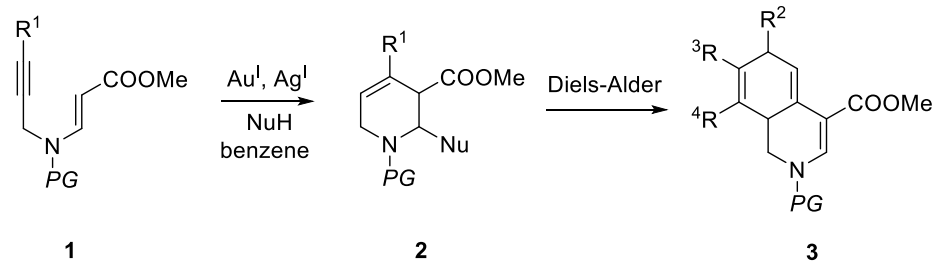
Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as catalysts. Our previous research on the cyclization of propargyl vinyl ethers to dihydropyrans[1] as well as chemoselective cyclizations of β-propargylamino acrylic esters to dihydropyridines[2] was extended to include nucleophile-assisted reactions.

The optimized synthetic protocol was applied to the preparation of a library of substituted tetrahydropyridines **2**. Their further transformations via cycloadditions gave highly substituted isoquinoline derivatives **3**. The influence of structural factors on diastereoselectivity of cyclization as well as mechanistic considerations will be discussed.



1 **2** **3**

Scheme 1. Gold(I)-Catalyzed Synthesis of Piperidine Aminals

This work was supported by the Grant Agency of Charles University (Project No. 262416), from the project of Specific Academic Research (SVV 260 401) and the Czech Science Foundation (Project No. 15-07332S).

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NARCOLEPSY AND COMPUTER-AIDED DRUG DESIGN APPROACHES

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Narcolepsy is a rare neurodegenerative disorder which is associated with a decreased capacity to regulate sleep-wake cycles, excessive daytime sleepiness, cataplexy, vivid hallucinations and paralysis. This disorder is closely dependent on balanced activity of orexinergic system in the brain, involving interactions of specialized orexin neurons and peptide neuromediators (*i.e.* orexin A and orexin B). In the past decades, it has been discovered that narcolepsy could be treated by small molecule agonists of orexin receptor 2 (OX2R), the structure of which was determined by X-ray crystallography in 2015. OX2R is a trans-membrane receptor belonging to the group of G-protein-coupled receptors. Recently, novel drug candidates could be discovered using advanced computational technology such structure-based virtual screening (SBVS). In this project, a combined SBVS of roughly 1 000 000 chemical compounds (containing for example all clinically used drugs and several thousand of bioactive substances identified in traditional Chinese medicine drugs) was performed employing a cloud platform and a peta-flops-scale supercomputer to find potential drug candidates for activation of OX2R. 11 best ligands were tested *in vitro* to evaluate their agonistic activity towards OX2R. Actually, molecular dynamic simulations of these compounds complexed with OX2R are analyzed to reveal the molecular mechanism that determine the agonistic or antagonistic effect of a ligand. The simulations involve phospholipidic membrane (containing 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, DOPC), a homologically optimized OX2R, the ligands, water and ions parameterized by a force field based on Gromos54a7. These complex computational studies have led so far to discovery of an antagonist of OX2R with IC_{50} = 2.2 μM.

The study was supported by the Czech Science Foundation (Project No. 17-08596S).

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THE STORY OF TACROXIMES; NOVEL UNIQUE COMPOUNDS FOR THE RECOVERY OF ORGANOPHOSPHORUS-INHIBITED ACETYLCHOLINESTERASE

GÓRECKI, L.,[1,2] JŮNOVÁ, L.,[1,2] KUČERA, T.,[2] HEPNAROVÁ, V.,[1,2] PRCHAL, L.,[1,2] JUN, D.,[1,2] SOUKUP, O.,[1,2] KORÁBEČNÝ, J.[1,2]

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The nerve agents, the most toxic chemical warfare agents, are known for over 70 years. Their deadly effect was demonstrated several times during history. Another member of the organophosphorus compound family, organophosphorus pesticides, likewise represent serious burden for the mankind. No reliable antidote that would offer efficient medical assurance for the intoxicated patients has not been found so far. Herein, we describe two novel compounds, tacroximes, as unique merged molecules of tacrine against organophosphorous intoxication. These reactivators of acetylcholinesterase have balanced physicochemical properties, should be able to cross blood brain barrier and have slightly lower cytotoxicity. Their efficiency was proved against dichlorvos as compared with pralidoxime and obidoxime. Tacroxime represents interesting starting point to spur the development of novel, centrally active reactivators/or prophylactic agents with potential to become interesting drug candidates for *in vivo* studies.

The study was supported by University of Defence (Faculty of Military Health Sciences Longterm Development Plan and SV/FVZ201601).

SYNTHESIS OF PHTHALOCYANINE DERIVATIVES FOR SUPRAMOLECULAR INTERACTIONS WITH CUCURBITURIL

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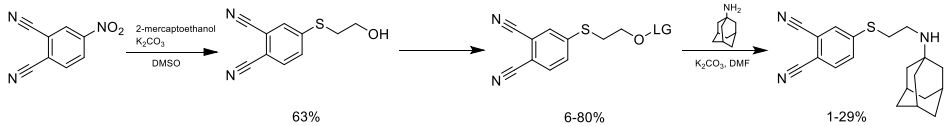
Phthalocyanines (Pc) are macrocyclic compounds structurally related to porphyrins, which are used *e.g.* as photosensitizers in photodynamic therapy in the treatment of tumor diseases. Their main disadvantage is their poor solubility in water and aggregation. Based on creating a supramolecular complex with cucurbiturils (CB) we can potentially improve those properties. CB are pumpkin-shaped macromolecules composed of methylene bridged glycoluril oligomers.[1] In this project we used one of the strongest reported supramolecular interactions between CB[7] and 1-aminoadamantane[2] as substituent on the Pc ring. We have successfully synthesized phthalocyanine precursor: adamantyl substituted phthalonitrile using reaction scheme bellow. Different synthetic approaches and optimization of this

reaction will be discussed during the presentation. Binding of this phthalonitrile to CB[7] was studied using NMR in cooperation with colleagues from Masaryk University. Crystal structure of the complex was also obtained and confirmed the predicted orientation of adamantyl moiety in cavity of CB.



The study was supported from the project of Specific Academic Research (SVV 260 401).

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SYNTHESIS OF ELECTRONICALLY TUNED [3]DENDRALENES

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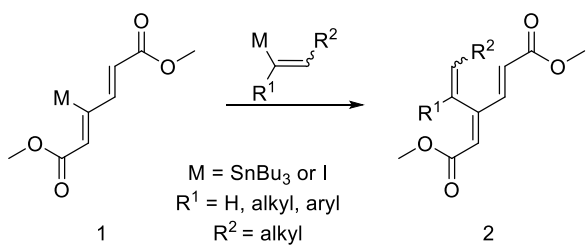
Dendralenes are acyclic cross-conjungated polyenes with an interesting, as yet unexamined reactivity and high potential for further synthesis.[1] We have focused on the synthesis of variously substituted [3]dendralenes containing electron withdrawing groups (*e.g.* carboxylic group), or a combination of electron withdrawing and donating groups. Synthesis is based on readily available *Z*-metallodienes **1**, which are subjected to Migita-Stille coupling[2] yielding the intended final products **2**. Syntheses and possible applications of new compounds in domino Diels-Alder sequences and nucleophilic additions will be discussed.



The study was supported from the project of Specific Academic Research (SVV 260 401) and by the Czech Science Foundation (Project No. 18-17868S).

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DESIGN, SYNTHESIS, AND STRUCTURE ACTIVITY RELATIONSHIPS OF HYBRID COMPOUNDS COMBINING PYRAZINAMIDE AND *p*-AMINOSALICYLIC ACID AS POTENTIAL ANTIMYCOBACTERIALS

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Tuberculosis, or as it was known in Middle Ages “the white plaque”, had killed millions of people throughout history. Later, this deadly infection was controlled with well-established drug regimen. Yet, the emergence of HIV and drug resistance have brought this old infection back to the list of the top ten causes of death worldwide.[1] Here we report the design, synthesis, and biological evaluation of a series of hybrid compounds combining pyrazinamide, which is a first line antitubercular drug, and *p*-aminosalicylic acid, which is a second line antitubercular drug, as demonstrated in the figure below. The compounds were obtained by reacting different pyrazinecarboxylic acids with *p*-aminosalicylic acid after being activated by 1,1-carbonyldiimidazole in dimethylsulfoxide as a solvent. Obtained products were *in vitro* evaluated for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv and four other non-tubercular mycobacterial strains. Furthermore, the compounds were *in vitro* screened for cytotoxicity against HepG2 liver cancer cell line. Most compounds exerted moderate to high activity against *Mycobacterium tuberculosis* H37Rv. Detailed results of biological evaluation and structure activity relationships will be discussed in the presentation.

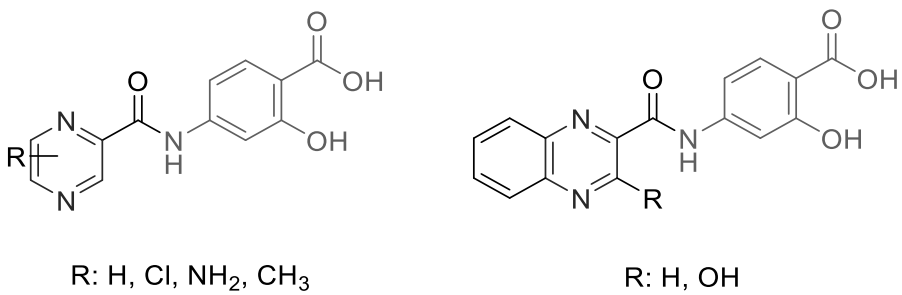

Figure 1. General structures of hybrid compounds combining pyrazinamide and *p*-aminosalicylic acid

The study was supported from the project of Specific Academic Research (SVV 260 401) and by the Grant Agency of Charles University (Project No. C-C3/1572317).

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HIGHLY SUBSTITUED PYRANONES VIA INTRAMOLECULAR TSUJI-TROST REACTION

BRŮŽA, Z.,[1] KRATOCHVÍL, J.,[1] HARVEY, J. N.,[2] RULÍŠEK, L.,[3] NOVÁKOVÁ, L.,[1] KUNEŠ, J.,[1] KOČOVSKÝ, P.,[1,3,4] POUR, M.[1]

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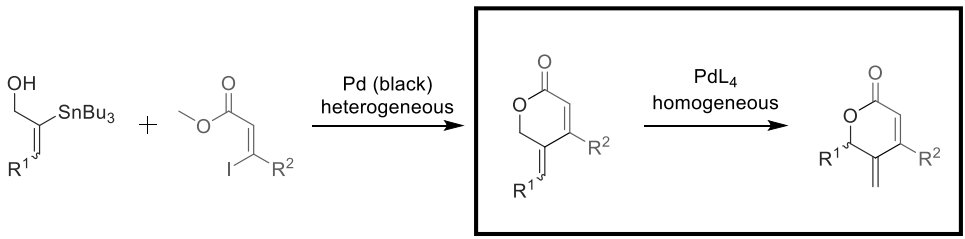
Nurturing minor reaction pathways into major transformations by implementing seemingly marginal changes of the reaction conditions may open interesting opportunities for obtaining new products from the same starting materials. To this end, we have developed an unusual isomerization of pyranones **1**[1] into 5-methylene pyranones **2** (Scheme 1).[1,2] The reaction is highly tolerant of a wide range of functional groups, and proceeds under mild conditions. Screening of chiral ligands was also performed to probe the possibility of enantiocontrol over the newly introduced chiral center. Additionally quantum chemistry calculations and kinetic simulations were performed to obtain insight into this unusual transformation.[2]



Scheme 1. General structures

The study was supported by the Grant Agency of Charles University (Project No. 1054216) and from the project of Specific Academic Research (SVV 260 401) and by the Czech Science Foundation (Project No. 18 17868S).

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SYNTHESIS OF SYMMETRICAL AND UNSYMMETRICAL ANIONIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY

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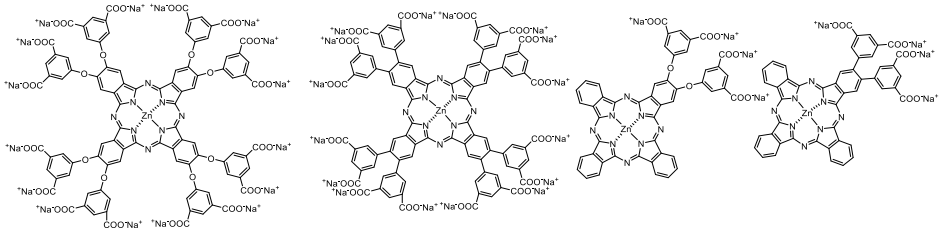
Phthalocyanines (Pcs) represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area 650–750 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. The aim of this work was to synthesize symmetrical and unsymmetrical anionic Pcs with 3,5-dicarboxylatophenyl moiety connected with Pc core directly by C-C bond or by ether bridge. Unsymmetrical compounds with amphiphilic character can be incorporated to liposomes that may protect this phthalocyanine from binding to proteins or from aggregation at low pH. Precursors for synthesis of symmetrical and unsymmetrical Pcs, 4,5-disubstituted phthalonitriles, were obtained by Suzuki coupling or nucleophilic substitution with molecules bearing 3,5-dimethyl isophthalate. Symmetrical Pcs were obtained by cyclotetramerization reaction (initiator magnesium butoxide) of one precursor while unsymmetrical Pcs were prepared by statistical condensation of phthalonitrile with 4,5-disubstituted phthalonitrile. Methylesters were completely transesterified to butyl esters during this reaction. Magnesium complexes was converted to metal-free ligands and then to zinc complexes. Basic hydrolysis of ester bonds was the last step of the synthesis. Final Pcs were tested on photodynamic activity *in vitro* on HeLa cells. Photophysical properties and binding to serum protein of Pcs were studied.



The work was supported by the Grant Agency of Charles University (Project No. 1060216) and from the project of Specific Academic Research (SVV 260 401).

SYNTHESIS AND OPTIMIZATION OF THE PREPARATION OF 32-HYDROXYDOTRIACONTANOIC ACID

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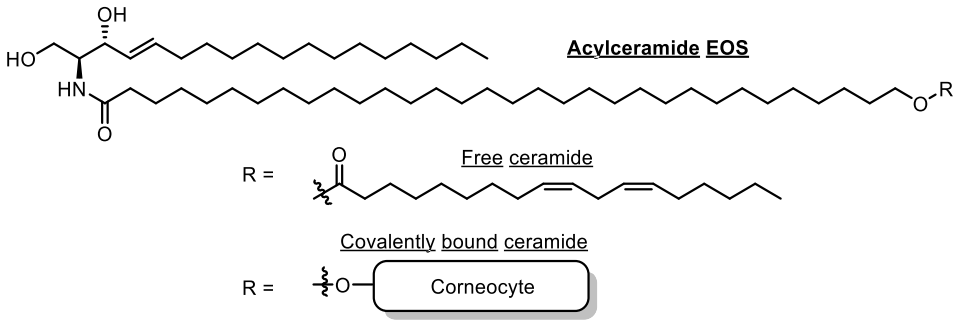
The 32-hydroxydotriacontanoic acid forms the backbone of all the acylceramides which belong among ultralong chain ceramides. They are essential components of the *stratum corneum* and play a crucial role in proper function of the skin barrier.

The carboxyl group of this acid is bound to a primary amino group of the sphingoid bases and the ω-hydroxy group is either esterified with linoleic acid to form free ceramides or it can be linked to the surface of corneocytes in the form of covalently bound ceramides.



The recent literature describes the synthesis of 32-hydroxydotriacontanoic acid with relatively small yields. The most problematic part of the synthesis is the connection of two shorter fragments leading to the ultralong chain.[1] The main aim of this project is to prepare covalently bound ceramides on solid particles and to optimize the reaction conditions. The previously used Wittig reaction was changed for other olefinations, mainly Julia and Julia-Kocienski reactions and their modifications. The highest yields were so far obtained by the modified Julia-Kocienski reaction with (1-cyclohexyl-1*H*-tetrazol-5yl)sulfonyl derivative of hexadecanoic acid as a starting material. In this case, we were able to increase the yield of this reaction even over 70%, which greatly improved the described reaction pathway.

The study was supported from the project of Specific Academic Research (SVV 260 401) and by the Czech Science Foundation (Project No. 16-25687J).

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INVESTIGATION OF THE COMPOUNDS INFLUENCING THE MELTING TEMPERATURE OF OLIGONUCLEOTIDE PROBES

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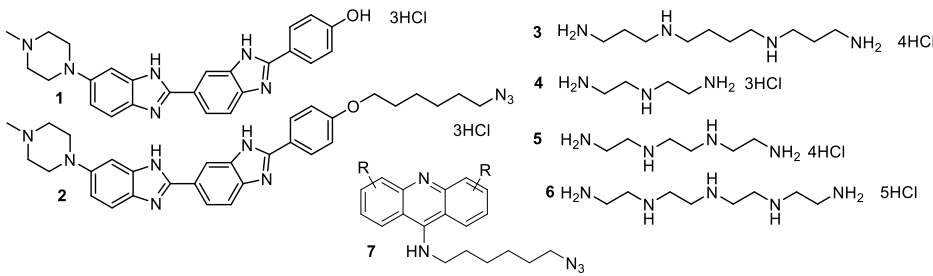
Real-time PCR is a widely used method in various research fields. The use of longer probes is often not optimal for mismatch discrimination because of low melting temperature difference between fully complementary and mismatched probes. The use of shorter oligonucleotide probes can be advantageous in this case. On the other hand, low melting temperature of these shorter probes is major complication for practical use in real-time PCR. Minor groove binders (MGB) or intercalating dyes can stabilize the duplex and increase the melting temperature, *e.g.* biogenic polyamines have strong interaction with DNA and RNA.[1] For testing the capability to increase melting temperature, several compounds were selected. Commonly known MGB Hoechst 33258 (**1**),[2] its modified derivative **2**, naturally occurring spermine **3**, three artificial polyamines (**4**–**6**) and 14 acridine derivatives (**7**) were tested for their capability to increase melting temperature of shorter probes. Modified Hoechst 33258 (**2**) and 14 acridine derivatives (**7**) were prepared in our laboratory. The tests revealed interesting increase of the melting point of the probes for several compounds.



The study was supported by the Technology Agency of the Czech Republic (TH03010251) and by the Grant Agency of Charles University (Project No. 994218).

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STABILITY EVALUATION OF MAGNESIUM COMPLEXES OF PHTHALOCYANINES AND AZAPHTHALOCYANINES UNDER ACIDIC CONDITIONS

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Phthalocyanines (Pc) and their azaanaloges (AzaPc) represent macrocyclic compounds with a large system of conjugated bonds. Due to this system, they possess interesting photophysical and photochemical properties such as strong emission of fluorescence or production of singlet oxygen, which make them widely examined as potential diagnostic and therapeutic agents.[1] Importantly, the central cation incorporated in the macrocycle changes the relaxation pathways of the excited states on the basis of heavy-atom effect.[2] Magnesium is the lightest stable central cation and for this reason its complexes are characterized by strong fluorescence; advantageously used as fluorescent probes or labels.[1] However, the instability of magnesium complexes in acidic environment (demetallation to metal-free derivatives) is the main obstacle of wider utilization of these fluorescent dyes. Once a macrocycle is demetallated, it loses its strong fluorescent properties. In this work, we decided to more closely evaluate a demetallation and other decomposition of these compounds in water solution at five different pH ranging 1–7.4. The stability was monitored by absorption spectroscopy for 24 h where characteristic splitting of the Q-band occurred after demetallation. Experiments proved that the more acidic environment, the faster process of the demetallation occurs and that Pcs are less stable than corresponding AzaPcs. We also tested possible protection provided by various delivery systems (liposomes and microemulsions). In particular liposomes showed high level protection where no changes in absorption spectra of AzaPc was detected after 24 h even at the most acidic pH 1.

The study was supported from the project of Specific Academic Research (SVV 260 401).

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PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS SECTION

AMARYLLIDACEAE ALKALOIDS FROM *NARCISSUS PSEUDONARCISSUS* cv. DUTCH MASTER AS POTENTIAL DRUGS IN 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. It is characterized by massive deposits of amyloid-β peptide, neurofibrillary tangles of the hyperphosphorylated τ-protein and inflammatory mediators leading to neuronal death. It is manifested by damage of cognitive and noncognitive functions.[1]

The genus *Narcissus* from the Amaryllidaceae family is mainly distributed in southwestern Europe and North Africa. This family contains special type of Amaryllidaceae alkaloids (AA), possessing a wide range of pharmacological properties such as antitumor, antiviral and acetylcholinesterase (AChE) inhibitory activity. AA galanthamine is used for AD therapy.[2]

Twenty-two AA of various structural types have been isolated. The bulbs were processed by extraction, followed by column and thin layer preparative chromatography and recrystallization. The chemical structures were elucidated by combination of MS, HRMS and NMR spectroscopic techniques. All isolated compounds were evaluated for their *in vitro* AChE, butyrylcholinesterase (BuChE), prolyl oligopeptidase (POP) and glycogen synthase kinase-3β (GSK-3β) inhibitory activities. The most important biological profile was demonstrated by narcimatuline ($IC_{50,\ BuChE} = 5.9 \pm 0.2$ μM, $IC_{50,\ POP} = 29.2 \pm 0.9$ μM; $IC_{50,\ GSK\text{-}3\beta} = 20.8 \pm 2.4$ μM).

This project was supported from the projects of Specific Academic Research (SVV 260 412, SVV 260 401) and by Research programme Development and Study of Drugs (Progres Q42).

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BRUNSVIGINE ISOMER AS A POTENTIAL AGENT IN THE TREATMENT OF ONCOLOGICAL DISEASES

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Plants from Amaryllidaceae family are interesting source of specific bioactive compounds –Amaryllidaceae alkaloids (AA). So far, nearly 600 AA of various structural types have been detected. Among the most important biological activities of AA belong those associated with the treatment of Alzheimer's disease (AD) and cancer. These and other diseases of affluence are becoming increasingly widespread all over the world. From this reason the development of new potential drugs is needed. Galanthamine has already been used as a reversible selective inhibitor of human erythrocytic acetylcholinesterase (HuAChE; $IC_{50\ HuAChE} = 1.5 \pm 0.2\ \mu M$)[1] in patients with AD. Many AA have been screened for their activity to inhibit the growth of different cancer cell lines and active compounds (mainly lycorine and haemanthamine) can be also used as lead structures for a preparation of their semisynthetic analogues.

From *Narcissus* cv. PROFESSOR EINSTEIN summary alkaloidal extract 25 different alkaloids have been isolated so far and they were identified by MS, HRMS and 1D- and 2D-NMR techniques and X-ray. All alkaloids were tested on their activities associated with AD (AChE, BuChE, POP, GSK-3β) and their ability to inhibit the growth of several cancer cell lines. From isolated alkaloids, the newly isolated isomer of brunsvigine gave the best results in the screening. IC_{50} determination was done ($IC_{50\ A549} = 2.29 \pm 0.43\ \mu M$, $IC_{50\ SAOS\text{-}2} = 2.20 \pm 0.25\ \mu M$) and now it is undergoing determining of the site of interference in the cell cycle.

The study was supported from the projects of Specific Academic Research (SVV 260 292 and SVV 260 412).

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PAPAVER RHOEAS: THE SOURCE OF ALKALOIDS FOR NMR ELUCIDATION

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The presented alkaloids were isolated from *Papaver rhoeas* (Papaveraceae) at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové. The Papaveraceae family is very rich in specific alkaloids derived mostly from isoquinoline.

The crude alkaloid extract displayed a promising inhibitory effect on butyrylcholinesterase. As such, it was further subjected to isolation and structural analysis of its constituents.

The isolated substances were characterized and interpreted by employing standard ^{1}H, ^{13}C, gCOSY, gHSQC, gHMBCAD and NOESY experiments on a Varian VNMR S500 spectrometer, supported by EI-MS spectra.

Each of the isolated alkaloids was later screened for biological activities on acetylcholinesterase, butyrylcholinesterase and prolyloligopeptidase. Selected compounds were also tested for cytotoxicity.

The study was supported by the Czech Science Foundation (Project No. 18-17868S) and from the project of Specific Academic Research (SVV 260 401).

ONE-STEP ISOLATION OF LUTEIN FROM GREEN MICROALGAE (*CHLORELLA VULGARIS*) BY HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY

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Lutein is a yellow carotenoid that naturally occurs in green leafy vegetables, orange-yellow fruits, and flowers. It is an antioxidant compound with eye health promoting properties and its utilization as an active ingredient in dietary supplements is currently driving the growing industrial demand for this compound. The flower petals of yellow Marigold (*Tagetes erecta* L.) represent the most important commercial source of lutein; however, their utilization is limited by seasons, climate, planting area, and the high labor costs.[1,2] Green *Chlorella vulgaris*, an eukaryotic microalga, has recently become a promising alternative feedstock for lutein. So far, the commercial lutein has mainly been obtained from Marigold flowers by solvent extraction, but this procedure has a limited specificity to the target compound. Therefore, the application of an efficient and scalable

isolation technique is pivotal for obtaining high-quality commercial lutein. In the present study, a high-performance countercurrent chromatography (HPCCC) method was developed and applied to obtain lutein from *C. vulgaris* biomass. Different two-phase solvent systems composed of *n*-heptane, ethanol and water were evaluated for their capacity to provide a proper distribution coefficient ($0.5 \leq K \leq 2.5$) of lutein and for exhibiting both an adequate density difference between the two phases (≥ 0.080 g mL^{-1}) and a short settling time (< 30 s). The two-phase solvent system composed of *n*-heptane–ethanol–water (5:4:1.5, v/v/v) was selected for the isolation of lutein. In addition, different flow rates (1–8 mL min^{-1}) and sample loadings (200–400 mg of extract) were examined to optimize the HPCCC separation conditions. Under the optimized operating conditions, the lower phase of the selected solvent system was used as the mobile phase at a flow rate of 8 mL min^{-1}, whereas the rotational speed and temperature of the separation column were 1200 rpm and 28 °C, respectively. The retention of the stationary phase at the end of the HPCCC separation was 52%. Overall, 10 mg of lutein with purity of 97% was obtained from 200 mg of *C. vulgaris* extract. The chemical identity of the target compound was confirmed by high performance liquid chromatography with UV-visible detection (HPLC-DAD) in comparison with an authentic standard. The method described in this study represents a good strategy to efficiently isolate lutein from microalgae biomass and can serve as a good reference to scale up the production of this bioactive compound at pilot and industrial size.

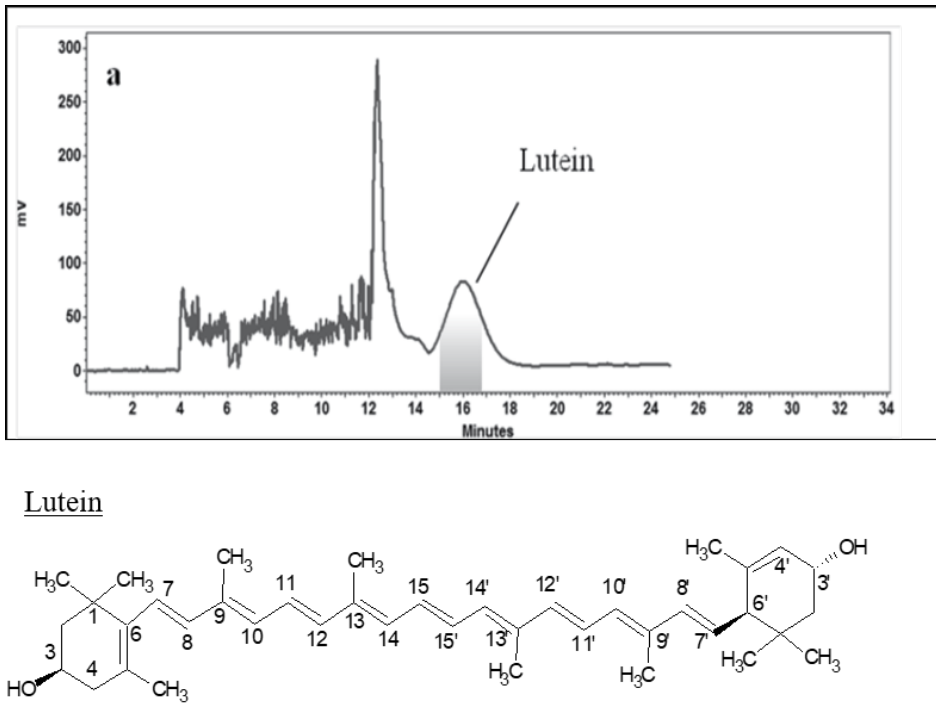

Figure 1. Chromatogram of the HPCCC isolation of lutein from microalgae *Chlorella vulgaris*.

This study was supported by the Grant Agency of Charles University (Project No. 1134217), from the project of Specific Academic Research (SVV 260 294) and by the Technological Agency of the Czech Republic (Project No. TJ01000013).

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IMMUNOMODULATORY ACTIVITY OF *SCUTELLARIA BAICALENSIS* AND *AZORELLA COMPACTA*

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Infectious diseases are an ever-present threat that can be potentially deadly. A negative role is also accounted to the gradually increasing resistance of microorganisms to antibacterial medicines. Enhancing the immunity against infectious agents is, therefore, important goal of searching for new resources of active substances. These could be found in *Scutellaria baicalensis* L. (Baical skullcap, family Lamiaceae), one of the medicinal herbs with a long history of usage in traditional Chinese medicine. Immunomodulatory activity of *S. baicalensis* has already been proven to some extent[1], but it is still unknown which kind of extract (ethanolic or aqueous) and in which concentration is more effective and which content substances have the highest share on this activity. Another possible source of immunomodulatory active substances is *Azorella compacta* Phil. (syn. *A. yareta*, Llareta, family Apiaceae), a cushion shrub grown at altitudes of the Andes in South America's puna. Experiments with aqueous extracts proved the antioxidant and immunomodulatory effect of contained polyphenols[2], but the effect of the ethanolic extract on immune cells is still unknown. In the presented study, the immunomodulatory activity of extracts from *Scutellaria baicalensis* and *Azorella compacta* was investigated through CD69 antigen activation, together with seven main flavonoids from *Scutellaria*. In addition, the content of three important flavonoids in Baical skullcap (baicalin, baicalein, wogonoside) was evaluated as well.

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ALKALOID PROFILING OF *HIPPEASTRUM* CULTIVARS BY GC-MS, ISOLATION OF AMARYLLIDACEAE ALKALOIDS, AND EVALUATION FOR THEIR CYTOTOXIC ACTIVITY

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Six alkaloidal extracts of ornamental varieties of *Hippeastrum* have been studied for their alkaloid profile by GC/MS. Twenty-one compounds with typical mass spectra of Amaryllidaceae alkaloids (AA) were detected. Nineteen of them were identified based on their mass spectra, retention times and retention indexes. Identified alkaloids belong to the crinine, haemanthamine, galanthamine, homolycorine, lycorine, montanine, and tazettine structural type of AA.

Using preparative TLC, five AA have been isolated in pure form from various *Hippeastrum* cultivars. The compounds were identified by MS, 1D and 2D NMR spectroscopic analyses and by comparison of the obtained data with the literature as montanine (**1**), vittatine (**2**), 11-hydroxyvittanine (**3**), lycorine (**4**) and hippeastrine (**5**).

Three of the isolated compounds (montanine, vittatine and hippeastrine) have been screened on a panel of human cancer cells (Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7 and SAOS-2) for their *in vitro* cytotoxic activity. In this work, montanine has been found to display strong cytotoxicity against all tested cancer cell lines, which is in accordance with previously reported results.[1] This compound has been selected for determination of IC_{50} values.

The study was supported from the project of Specific Academic Research (SVV 260 412).

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ISOLATION OF AMARYLLIDACEAE ALKALOIDS FROM *ZEPHYRANTHES CITRINA*

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Zephyranthes is a genus of bulbous perennial plants belonging to family Amaryllidaceae. The plants of this family are used by native people of different countries for treating various diseases. The genus *Zephyranthes* consists of about 90 species and only some of them have been studied for their chemical constituents. The phytochemical work on this genus revealed a diversity of compounds, especially alkaloids, having various pharmacological activities as anticancer, anticholinesterase and antiviral, antifungal and antiinflammatory. To date, ten alkaloids of various structural types have been isolated from *Zephyranthes citrina*.[1]

Summary ethanolic extract was prepared from the fresh bulbs of *Zephyranthes citrina* (30 kg) and separated by column chromatography. More than six hundred fractions were collected, and pooled together based on TLC into 21 subfractions. So far, twenty-one alkaloids have been isolated in pure form. The isolated compounds were identified by comparison of obtained analytical data (MS, NMR, optical rotatory) with the literature data. All isolated alkaloids were assayed for their biological activities connected to Alzheimer's disease (inhibition of cholinesterases, GSK-3β, ability to permeate through the blood-brain barrier), and anticancer potential (cytotoxicity against panel of cancerous and noncancerous cell lines).

The study was supported by the Grant Agency of Charles University (Project No. 178518) and from the project of Specific Academic Research (SVV 260 412).

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DERIVATIVES OF AMARYLLIDACEAE ALKALOIDS ISOLATED FROM *NARCISSUS* cv. CARLTON AND THEIR BIOLOGICAL ACTIVITY

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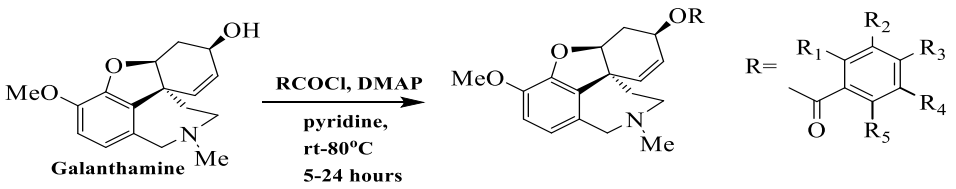
Family Amaryllidaceae comprises about 85 genera and 1100 species that are distributed widely in tropical and subtropical region of the world. *Narcissus* cv. Carlton

is an interesting species with high content of Amaryllidaceae alkaloids (AA), which belongs to this family. More than hundred alkaloids has been isolated from *Narcissus* genus. AA are classified into nine skeleton types – norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine. *Narcissus* alkaloids have remarkable therapeutic potential as antitumor, antifungal, antimalarial, antibacterial, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity.[1] Galanthamine is one of the most abundant AA and clinically used for the treatment of Alzheimer's disease as reversible inhibitor of AChE. Different derivatives of AA have been reported to display wide range of biological activities, *e.g*. anticancer, antimicrobial, antimalarial, antioxidant, AChE and BuChE inhibitory activity. Semi-synthetic derivatives of galanthamine display potential AChE inhibitory, antimicrobial and antioxidant activity. However, anticancer properties of various galanthamine derivatives have not been studied yet. The aim of this study is to prepare aromatic esters of galanthamine with substitutions in different positions on aromatic ring – methyl groups, methoxy groups, nitro groups and halogens. So far, sixteen semi-synthetic derivatives of galanthamine have been prepared and their structure has been confirmed by NMR and MS methods. All derivatives undergo different biological tests connected with Alzheimer's and oncological diseases.



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

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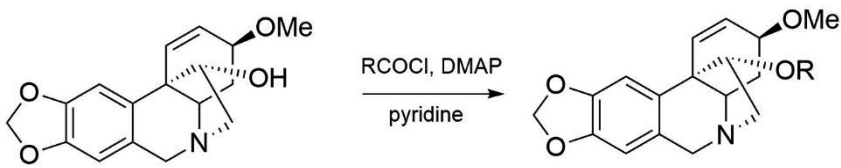
Plants of the Amaryllidaceae family contain approximately 85 genera and 1100 species and have a wide distribution through both tropical and sub-tropical regions worldwide. They have also a long and notable place in the history of traditional and Western medicine. Alzheimer's disease (AD) is the most 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 marketed drug for AD therapy under the commercial name Reminyl© (galanthamine hydrobromide). Other alkaloids like haemanthamine (HA) and lycorine have demonstrated interesting antitumor and/or apoptotic effects and other studies also pointed out various pharmacological properties of semisynthetic derivatives of some AA.

One of the most interesting AA is haemanthamine, which is widely distributed through Amaryllidaceae plants. Based on our previous results, where we reported promising anti-cholinesterase activity of pilot series of HA derivatives, we decided to continue in preparation of further semisynthetic derivatives.[1]

So far, we prepared four new aromatic esters of HA containing different substitutions on aromatic ring. New compounds were identified by 1D and 2D NMR, GC/MS and ESI-MS methods, and all substances are screened for different biological activities connected with potential treatment of AD.



This project was supported from the project of Specific Academic Research (SVV UK 260 412).

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DERIVATIVES OF AMARYLIDACEAE ALKALOIDS OF MONTANINE TYPE AND THEIR BIOLOGICAL ACTIVITIES

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Recent research on Amaryllidaceae plant family reports the isolation of more than 500 different Amaryllidaceae alkaloids (AA) with different structure types possessing wide range of biological activities. Among all biological activities they have also been investigated for their promising anti-tumor activity and have called attention for the ability to overcome apoptosis resistance. Among all AA, montanine type alkaloids were intensively studied for the cytotoxicity against different human cancer cell lines. Unfortunately montanine-type alkaloids are present in plant material only in small amount. From this reason we decided to prepare series of montanine analogues from haemanthamine, which is easily isolated from plants, via intermolecular nucleophilic attack of haemanthamine.[1]

So far, we prepared four new montanine derivatives and all compounds will be tested for their biological activities, and the most potential ones will be selected for further investigations.

The study was supported from the project of Specific Academic Research (SVV UK 260 412).

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PHARMACEUTICAL TECHNOLOGY SECTION

THE USE OF GRAVITATIONAL CONSOLIDATION FOR THE PREDICTION OF ANGLE OF INTERNAL FRICTION

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On the particle-particle basis, the angle of internal friction (AIF) commonly belongs to important material parameters characterizing behaviour of moving particles. The AIF is usually determined by measuring in a shear cell but a lot of time and a high amount of

tested material are main disadvantages.[1] Thus, the purpose of this study was to find out if the gravitational consolidation could be used for the estimation of the AIF.

Seven types of lactose powder were consolidated under a controlled number of taps. The correlation between the powder porosity and the number of taps were used for the factor porosity determination. From the arcus tangent of the slope of the linear relationship between the porosity factor and the number of taps, the AIF was estimated[2,3] and compared with that of determined by measurement using the Jenike shear cell.

A good correlation between the AIF estimated from the gravitational tapping and the AIF determined by Jenike shear cell was observed in this study. The prediction of the AIF by simple gravitational tapping would be useful as tapping belongs to standard methods of estimating tapped density and Hausner ratio used in pharmaceutical industry. However, more research in this area using different materials is necessary.

The study was supported by the Grant Agency of Charles University (Project No. 1286218/2018) and from the project of Specific Academic Research (SVV 260 401).

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BIODEGRADABLE POLYMERIC NANOPARTICLES FOR MEDICAL APPLICATIONS

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Our work is focused on polymeric nanoparticles formulation and characterization with intent of achieving prolonged response, and to reduce adverse effects of selected incorporated drugs (terbinafine hydrochloride, rifampicin, oligomer). We used originally synthesized and fully biodegradable, linear or branched polyesters based on poly(lactic-co-glycolic acid). Branched ones are synthesized with the addition of the branching unit such as poly(acrylic acid), mannitol, or erythritol derivatives. Nanoparticles were prepared using double-emulsion method, or more frequently by modified nanoprecipitation method.[1] We monitored multiple parameters (particles size, polydispersity, zeta potential) using Zetasizer Nano ZS by Malvern. The size of the nanoparticles were 100–600 nm, and could be modified by the choice of the polyester and its concentration, and mixing technique of phases. For analysis, we used HPLC, fluorometer, or spectrophotometer, according to the analyzed drug. Dissolution tests showed prolonged release of incorporated drugs, which we attributed to the gradual swelling and degradation of the polyester in an aqueous medium.[2] The release of the incorporated drug is pH dependent, and it can be accelerated by

lowering the pH value of the dissolution medium. Examined polyesters, especially the branched ones, are perspective, original, and suitable for further observation.

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THE PROPERTIES OF CHITOSAN AS A DRUG CARRIER

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Chitosan is nowadays one of the most investigated excipients used in many pharmaceutical formulations including gels, microspheres, coatings for liposomes, and tablets for its inherent properties such as biodegradability, low toxicity and good biocompatibility.[1] It is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine, prepared by hydrolysis of chitin by using several alkaline treatment.[2] The aim of the work is to investigate the properties of chitosan as a drug carrier for the further use as a hydrophilic filler to improve drug solubility in interactive mixtures or in matrix colon-targeted delivery systems. Different methods such as optical and scanning electron microscopy, laser diffraction, differential scanning calorimetry, and a shear cell measurement (Freeman powder rheometer) were used to characterize the substance. The particles were of irregular shape and rough surface (pD_F = 1.045, shape factor = 0.56), the median particle diameter x_{50} = 88.8 μm and span 1.93. Shear cell measurement showed that chitosan has poor flowability. These initial measurements were used to characterize particle properties of chitosan, however, flow and compaction properties remain to be further investigated.

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A STUDY OF THE DRUG RELEASE FROM MATRIX TABLETS WITH POLYVINYL ALCOHOL

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The aim of this study was to determine the effect of different retardant concentration on drug release from hydrophilic matrix tablets. At the same time, the influence of the type of dry binder used was investigated. Polyvinyl alcohol was used as the retarding agent at the concentrations of 30, 40 and 50%. α-Lactose monohydrate and microcrystalline cellulose in the ratio of 3:1 in the physical mixture and in MicroceLac® 100 were used as dry binders. MicroceLac® 100 is a coprocessed dry binder. Salicylic acid was used as the model less soluble drug. The tablets were prepared by direct compression method. Dissolution testing was performed using the rotating basket method. The results of the dissolution test were evaluated by nonlinear regression analysis.

Increasing additions of polyvinyl alcohol decreased drug release rate. It has been found that the type of dry binder does not affect neither the mechanism nor the release rate of salicylic acid. The dissolution behavior of tablets, which contained the physical mixture or the coprocessed dry binder and the same amount of polyvinyl alcohol was comparable.

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ORAL DELIVERY OF OLIGONUCLEOTIDES FOR LOCAL TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) includes pathological conditions characterised by inappropriate and sustained activation of the mucosal immune system of the small intestine

and/or colon.[1] Local treatment is preferred over systemic delivery often accompanied with undesired side effects. In IBD, cationic peptides are expressed in the area and phagocytic immune cells infiltrate the site of inflammation.[2] By delivery of an anti-inflammatory acting miRNA oligonucleotide, production of pro-inflammatory cytokines by these immune cells decreases, and the inflammatory process itself is suppressed. However, delivery of unstable negatively charged macromolecular oligonucleotides represents a challenge.[3] Self-nanoemulsifying drug delivery system (SNEDDS) has been utilised to deliver a hydrophobic complex of oligonucleotides orally.[4]

The complexes were prepared from a model 20-nucleotide long oligomer and a cationic lipid dimethyldioctadecylammonium bromide (DDAB) or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) with yields over 95% for molar ratio 1:60. The size of the complexes was estimated by atomic force microscopy to be 75–127 nm and 33–62 nm, for DOTAP and DDAB complex, respectively. The size of dispersed loaded SNEDDSs, ~200 nm, and negative surface charge enabling passive targeting make the formulation suitable for intended purpose.

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NOVEL FORMULATIONS FOR TOPICAL APPLICATION OF IMIQUIMOD

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Imiquimod (IMQ) is a heterocyclic imidazoquinoline used in the treatment of various viral or neoplastic skin diseases.[1] The aim of this study was to prepare suspensions, emulsions and liposomes for dermal administration of 1% IMQ and evaluate its *in vitro* permeation on human skin. IMQ is commercially available as a cream under the brand name Aldara® (5% IMQ). This formulation possesses undesired disadvantages like low skin permeation, instability and side effects on skin.[2] In order to overcome these obstacles, the formulations with lower concentration of IMQ and different permeation enhancers (L-Pro2, DDAK and oleic acid) were prepared. *In vitro* permeation experiments were carried out in Franz Diffusion Cells on human skin while Aldara® was used as a control.

After 8 and 24 hours the concentration of IMQ in each skin layer, as well as in acceptor phase (phosphate buffer, pH 7.4), was determined. In emulsions and suspensions a higher concentration of IMQ was proved in epidermis and lower concentration in the deeper compartments compared to Aldara®. The liposomal formulations did not show such high effect on delivery of IMQ to the epidermis. In the future, optimized liposomal formulations will be developed for better potential delivery of IMQ.

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LIPOSOMES FOR DERMAL DRUG DELIVERY

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Over the past years, (trans)dermal delivery of biologically active molecules has become very popular due to its advantages over other routes of administration.[1] Different methods and vehicles have been used to improve dermal delivery, of which, liposomes seem to be the most studied.[2] Recently, our research group prepared imiquimod-containing liposomal mixtures which were unable to efficiently deliver imiquimod to epidermis in higher concentrations than the commercially available cream (Aldara). Inspired by the previous results, the aim of the present study is, to prepare imiquimod loaded liposomes following an alternative approach and evaluate their ability to deliver the active substance to the skin layers.

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PHARMACEUTICAL ANALYSIS AND BIOANALYTICAL CHEMISTRY SECTION

APPLICATION OF MONOLITHIC COLUMNS IN CLINICAL PRACTICE

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Monolithic stationary phases and columns have rapidly become highly popular separation media for liquid chromatography. Today, various types of monoliths have been developed. They have different forms and are synthesized by different preparation processes. Their unique properties distinguish them from all other columns, especially the tolerance to high flow rates achievable using only moderate pressures and high speed. This characteristics achieved excellent separation and make the columns irreplaceable in certain areas.[1,2] Monolithic columns are used for example in the analysis of biologically active substances such as neopterin, kynurenine, tryptophan and creatinine.

Elevated levels of tryptophan, its metabolite kynurenine and neopterin have been observed in disease associated with activation of immune system. Determination of these substances in biological fluids, serum, urine, wound exudates or amniotic fluid, serves to determine the patient response to therapy and clinical status. Monoliths allows large separation capacity, better separation efficiency with low back pressure and analysis of more samples of biological material compared to columns with particles.[3,4] Using of monoliths will be presented in determination of different analytes (immune system activation markers) in various biological fluids (serum, amniotic fluid, wound liquid, exudates) used in many clinical studies.

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DETERMINATION OF IMPORTANT BIOMARKERS DURING TREATMENT WITH RHEOHEMAPHERESIS IN AGE-RELATED DRY FORM OF MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the most frequent cause of severe visual lost in people older than 55 years in industrial countries. The disease has two variants, dry and wet form. The most common form is the slowly progressing dry (atrophic) form. Rare (10–20%) rapidly progressing wet form with typical neovascular choroidal membrane is in 80–90% the cause of blindness. Rheohemapheresis is used in the treatment of dry form of AMD with soft drusen. It is a double plasma filtration in which specific high-molecular substances (LDL cholesterol, IgM, α2 macroglobulin, fibronectin, fibrinogen, von Willebrand factor) are removed. Reduction of levels of these substances leads to an improvement of the choroidal microcirculation and reduction of accumulation of lipoproteins which are the major component of soft drusen.[1] The potential risc of this treatment is a decrease not only in cholesterol but also in antioxidants, such as vitamin E and other important biomolecules such as vitamin A and D. For this reason, we measured levels of vitamin E (α-tocopherol), the vitamin E/cholesterol ratio in serum and lipoproteins (VLDL, LDL, HDL). Serum vitamin A (retinol) and vitamin D were also measured. These parameters were determined before and after rheohemapheresis.[2] The individual lipoprotein layers were obtained by ultracentrifugation. The vitamins were extracted from matrix using liquid-liquid extraction, protein precipitation and filtration. For determination of vitamin E and A in serum a reversed-phase high performance liquid chromatography method using monolithic column and diode-array detection was developed and validated.[3] For determination of vitamin D, ultra high performance liquid chromatography coupled with mass spectrometry detection (MS/MS) was used.[4] First results from Ministry of Health project NV17-29241A will be presented.

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UHPLC-MS/MS IN AN INVESTIGATION OF NOVEL CARDIOPROTECTIVE AGENT JAS-2

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JAS-2 [4,4′-(Butane-2,3-diyl)bis(piperazine-2,6-dione)] is a novel analogue of dexrazoxane (DEX) which is the only approved cardioprotective agent protecting myocardium against anthracycline-induced toxicity. Despite pilot studies indicate that JAS-2 is more effective in protection of neonatal rat cardiomyocytes from toxic effect of anthracyclines as compared with DEX, its use is limited by poor solubility. Therefore, a prodrug with a code name GK667 was prepared to improve the solubility of JAS-2. A modern analytical method is required to investigate stability of GK-667, its conversion to the active form – JAS-2 as well as its further metabolism. The aim of this work was UHPLC-MS/MS analysis of samples from *in vitro* experiments aimed at investigation of the prodrug activation as well as plasma from a pilot *in vivo* study. The analyses were performed using a UHPLC system (Nexera, Shimadzu,) coupled with a triple quadrupole mass spectrometer with ESI ion source (LCMS-8030, Shimadzu). The separation was achieved on Luna Omega Polar column (100 × 3.0 mm, 2.5 µm, Phenomenex) protected with a guard column. A mixture of ammonium formate and acetonitrile in a gradient mode was used as a mobile phase. Plasma samples were treated with precipitation. Cell culture medium was simply diluted with ultra-pure water. Stability study on GK-667 (100 µM, 37 °C) showed similar profile in plasma and DMEM medium. GK-667 is rapidly converted to JAS-2 which is slowly degraded to JAS-2_{met}. Stability of JAS-2 (100 µM, 37 °C) in both matrixes was compared with DEX. After a pilot *i.v.* administration of GK-667 to rabbits (5 mg/kg *i.v.*, n =2), only JAS-2 was detected ($c_{max} \approx 10$ µM). GK-667 and $JAS2_{met}$ were bellow LLOQ. The method will be further modified to enhance sensitivity, mainly for JAS-2, to be capable for full PK study in rabbits.

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DETERMINATION OF SUDAN DYES IN CHILLI PRODUCTS BY MEKC-MS/MS USING A MS FRIENDLY SURFACTANT

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Sudan dyes are phenyl-azoic derivatives widely used as synthetic organic colorants because of their colour fastness and low price. Azo dyes have been extensively used in many industrial applications including oils, solvents, plastics, *etc.* Sudan I, II, III, and IV have been employed for many years as food colorants in different products, such as chilli powder and sauces, to mimic, intensify, and prolong the appearance of natural red hues because of their intense red-orange colour. The use of these colorants can constitute a serious health risk, thus they are banned for food usage in the European Union since 2004.[1] The aim of this work is to develop a fast and sensitive method for the simultaneous determination of Sudan dyes (I, II, III, and IV) in chilli products such as powder, sauce and paste by micellar electrokinectic chromatography-mass spectrometry (MEKC-MS) employing ammonium perfluorooctanoate as volatile surfactant. MEKC separation and MS detection conditions have been optimized in order to achieve a fast, efficient, and sensitive separation of the four dyes. Target compounds were extracted from chilli samples with acetonitrile and purified using freezing-lipid filtration, achieving excellent results in terms of sample throughput. Analytical performance of the method is satisfactory, obtaining limits of quantification lower than 5 μg kg^{-1} in all cases. The precision, expressed as relative standard deviation (%, RSD) was below 15.7%. The extraction efficiency for fortified samples ranged from 86.5 to 99.8%, with RSDs lower than 10.3%. Matrix effect was evaluated for all samples studied, being lower than 17% in all cases. Its applicability has been successfully tested in 20 chilli products. The concentration was calculated from the corresponding matrix-matched calibration curve, detecting Sudan I and IV in several samples at 50 and 450 μg kg^{-1} respectively.

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PROTEOMIC ANALYSIS OF ACETYLOME

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In terms of numbers, acetylation of lysine ε-amino group (AcK) represents the second most important protein posttranslational modification involved in cell signaling next to phosphorylation.[1] Unfortunately, functional role of the majority of acetylated sites (*i.e.* acetylome) is unknown. The position of protein acetylation remains enigmatic even for immune cells whose signaling cascades must be tightly regulated to prevent immunopathology. In particular, there are no rigorous data regarding acetylome of dendritic cells (DCs) – the most effective antigen-presenting cells responsible for priming of adaptive immunity. Therefore, we decided to analyze acetylation events in primary murine bone marrow-derived DCs (BMDCs) using proteomics. First, we compared AcK sites from BMDCs to those obtained from murine liver tissue. While we were able to identify >3,500 AcK sites in liver, only ~1,900 were found in BMDCs. This was probably an issue of sensitivity as AcK sites from some BMDC compartments (*e.g.* mitochondria) were underrepresented, suggesting low AcK stoichiometry. To further improve the depth of BMDC acetylome, we performed HPLC pre-fractionation of BMDC peptides prior immunoprecipitation step, which helped to increase the number of identified BMDC AcK sites to ~3,000. As expected from transcriptionally active immune cells, about 3% of detected acetylated BMDC proteins were transcription factors and >10% of all AcK sites were reported substrates of nuclear CBP/p300 acetyltransferase.

The study was supported by Ministry of Health of the Czech Republic – conceptual development of research organization (UHHK, 00179906).

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CHAOTROPIC AGENTS EFFECTING SEPARATION OF CHIRAL DRUG CANDIDATE K1277

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Chirality of drugs is one of major concerns since the half of the last century, when the toxicity of one enantiomer of thalidomide caused deformations in millions of new-born

children in many developed countries. As a model drug for enantiomeric separation we used derivate of tacrine conjugated with tryptophan labelled K1277, which was synthetized by our colleagues as a potential anti-Alzheimer drug. The chromatographic separation was performed with Dionex 3000RS UHPLC system with UV detection (254 nm) in reverse phase isocratic mode with chiral Lux Cellulose1 column as a stationary phase and a mobile phase consisting of acetonitrile and water in 45:55 or 40:60 ratio with an addition of various inorganic chaotropic agents in different concentrations. All used agents were sodium salts to minimize interactions of cationic part to the separation. The results we obtained were partly in conclusion with Hofmeister chaotropic series as reviewed by Phechkrajang[1] and used by Kazakevich *et al.*[2] and Pan *et al.*[3] in their research of chaotropic behaviour. However with more potent agents the separation was still inconclusive even in the low concentrations. The research was also focused on pH addition to the effects especially in the potent agents as for example BF_4^- anion, which seemed interesting. In the future we would like to analyse behaviour of K1277 with different conditions influencing enantiomeric separation as well as using computer prediction for optimal conditions and potentially experiment with common chiral drugs.

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ANALYSIS OF CANNABINOIDS IN DIETARY SUPPLEMENTS AND COSMETIC PRODUCTS USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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We describe development of an ultra-high-performance supercritical fluid chromatography (UHPSFC) method with PDA and MS detection for analysis of cannabinoids in dietary supplements and cosmetics products. Considering a high number of cosmetics and dietary products available on the market and process of cannabinoid legalization for medical use importance of methods suitable for quality control is growing. The optimization of UHPSFC method was carried out with mixture of six most abundant cannabinoids as reference standards including cannabidiol, Δ-9-tetrahydrocannabinol, cannabigerol, cannabidiolic acid, Δ-9-tetrahydrocannabinolic acid and cannabigerolic acid. Firstly, diol, 2-picolylamine, diethylamine, 1-aminoanthracene, BEH, BEH 2-ethylpyridine, CSH pentafluorophenyl and HSS C18 SB stationary phases were tested. Secondly,

mobile phase modifiers and additives, including methanol, ethanol, mixture of acetonitrile and methanol, 0.1% ammonium hydroxide and 10 mM ammonium formate were employed. Their effects on peak shape, peak resolution, selectivity, retention time and analysis time were evaluated. Other optimized parameters involved gradient elution, column temperature and pressure of back-pressure regulator. Finally, Viridis BEH 2-EP column was selected as optimal stationary phase. In order to separate the mixture of six compounds a gradient elution from 2 to 30% of solvent B was used. Mobile phase consisted of CO_2 as solvent A and methanol with 0.1% ammonium hydroxide and 2% water as solvent B. To show the applicability of the proposed method, the determination of the six cannabinoids in various cosmetics products and dietary supplements based on cannabis sativa were carried out.

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ULTRA-HIGH PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY IN PHARMACEUTICAL QUALITY CONTROL: TACKLING THE METHOD VALIDATION

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First part of the study was focused on developing general achiral screening approach for ultra-high performance supercritical fluid chromatography (UHPSFC) methods. For that, 10 pharmaceutical quality control (QC) mixtures containing active pharmaceutical ingredients (API) and their particular impurities were used. In the end, not only that the screening approach was suggested based on obtained results but also UHPSFC method for each QC mixture was obtained. Even though most of these methods seemed to be sufficient, several of them had to be further optimized to ensure their successful validation and application on API and tablet samples. Several challenges occurred during the optimizations. The necessity of the resolution of API and following impurity equal at least to 3 was the most common one which was especially difficult to solve in mixtures with structurally close compounds. However, also unrepeatable elution of compound eluting close to the dead volume or in the end of gradient set-up and/or shifts of retention times due to column aging were detected. The most frequent optimization adjustments involved changes in gradient program. Moreover, the substitution of Viridis HSS C18 SB for slightly different Acquity UPLC HSS C18 SB and/or addition of acetonitrile into the modifier solution also led to significant changes in selectivity. In case of β-estradiol mixture, the coupling of columns was necessary to ensure sufficient resolution of structurally close compounds. Finally, validation of optimized methods was carried out. Parameters recommended by ICH guidelines Q2 and Q3 including specificity, linearity, range, lower and upper limit of quantification,

limit of detection, accuracy, and precision were examined. Intermediate precision and the accuracy profile evaluation were also determined for several methods. In the end, all ten UHPSFC methods were successfully validated according ICH guidelines. Overall, from 55 analytes, only two impurities did not meet the validation criteria due to low sensitivity and low resolution, respectively.

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TARGETED AND NONTARGETED ANALYSIS OF HUMAN URINE FOR METABOLOMIC STUDY

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Swedish Cardio Pulmonary Bioimage Study (SCAPIS) is a project following 30 000 human subjects in the age of 50–64 years. The project was designed to improve risk prediction of cardiopulmonary disease. The Swedish SciLifeLab SCAPIS Wellness Profiling (S3WP) program consists of a smaller cohort with 100 individuals from this large SCAPIS population. It combines the use of imaging technologies, large scale omics, and epidemiological analyses. At four opportunities over one year, plasma and urine have been sampled from each subject. Urine metabolomics using ultra high-performance liquid chromatography-ion mobility-quadrupole with time of flight detector (UHPLC-IM-QTOF) is the goal of present project. A LC-MS based metabolomics method commonly used at Swedish Metabolomic Center was applied. The separation was carried out using gradient elution of mobile phase A: 0.1% formic acid, and B: 75% acetonitrile and 25% isopropanol with 0.1% of formic acid in 11.8 minutes using Acquity HSS T3 column (2.1 × 50 mm; 1.8 µm). Firstly, a retention time mass spectra library based on the Sigma Aldrich metabolite compound library, which contains 635 metabolites, was built. The retention time, collisional cross section, and mass information database were acquired for annotation of metabolites in the metabolomics analysis of the urine samples. Secondly, the dilute and shoot approach was used for sample preparation of urine samples after the optimization of dilution ratio. Based on comparison with number of detected metabolites and matrix effects, the human urine samples were diluted with 50% methanol with internal standards in a ratio 1:10. Finally, the analysis of biological samples was carried out on a sub-set of 50 individuals with the aim to detect metabolite variation within and between subjects. By using a targeted metabolomics data processing approach, in total 48 metabolites were annotated in both ionization modes. The nontargeted analysis showed 825 features in positive mode and

724 features in negative mode. Currently, the data are evaluated in the context of the whole project.

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DEVELOPMENT OF HILIC UHPLC-UV METHOD FOR ANALYSIS OF PHENOLIC COMPOUNDS

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Hydrophilic interaction chromatography (HILIC) is suitable for separation of the small polar and ionizable compounds which are poorly retained in classical reversed phase columns. The separation mechanism is more complex than in reversed phase. It is based on combined interactions including partitioning, ion-exchange, electrostatic interactions *etc*. The advantages of HILIC involve a better mass spectrometry response due to high percentage of acetonitrile in mobile phase, lower viscosity, and orthogonal elution order in comparison with reversed phase. Quercetin and rutin are well known phenolic compound with many health benefits. They are metabolized to smaller phenolic acids and larger flavonoid structures. The separation of these analytes is challenging due to the differences in lipophilicity, acidity, and different size of molecule. The aim of this study is to find HILIC separation conditions for analysis of 5 phenolic compounds (phloroglucinol, 3,4-dihydroxyphenylacetic acid, homovanilic acid, 3-hydroxyphenylacetic acid, 3-(3-hydroxyphenyl)propionic acid, 4-methylcatechol) and 4 flavonoids (rutin, quercetin, tamarixetin, isorhamnetin) at MS compatible conditions. The separation potential of five HILIC stationary phases was tested under different mobile phase composition (80–97% of acetonitrile) and different mobile phase additives including 0.1% formic acid, 10mM ammonium acetate pH 6.0, and 10mM ammonium formate pH 3.0. The most problematic molecules in terms of HILIC seem to be flavonoid molecules, where the wide peaks were observed. Tamarixetin and isorhamnetin were not separated from each other due to similar chemical properties, and showed also coelution with other compounds. The developed method is perfectly suitable for the analysis of phenolic acids. For the flavonoid molecules other stationary phases or separation modes should be further investigated.

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DOPAMINE UNIVERSAL COATING – A NEW POTENTIAL MODIFICATION OF NANOFIBROUS SORBENTS FOR ON-LINE EXTRACTION SYSTEMS

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The dopamine universal coating showed a great potential in surface modification. 3D-structured polycaprolacton prepared via hybrid technology combining electrospinning and meltblown was used as a basic nanofibrous polymer for modification via polydopamine. Four groups of biologically active substances including the betablockers, bisphenols, phenolic acids and nonsteroidal drugs were tested as model analytes for the best description of the extraction behavior caused by polydopamine layer. Coated and uncoated nanofibers were successfully applied as sorbents for the on-line extraction liquid chromatography set-up and compared. Both materials showed good potential for the determination of bisphenols and nonsteroidal drugs in samples. Polydopamine layer significantly increased the extraction efficiency for more polar drugs. All developed on-line SPE HPLC methods were successfully applied on real samples (river water, human urine and plasma).

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APPLICATION OF NANOFIBROUS POLYMERS FOR A BIOLOGICAL SAMPLE EXTRACTION

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The sample adjustment is often discussed topic in the field of analytical separation methods. This part of analytical process is often time-consuming and laborious and it cannot be omitted. The correct sample adjustment is important step to obtain precise and accurate results. The biological samples represent specific problematics. They contain many ballast substances interfering with analytes or causing damage of instrument. In combination with low concentrations of analytes, the biological sample adjustment is necessary for sample purification and analyte preconcentration. The methods for biological sample adjustment should be fast and easy because the handling with biological fluids and tissues may be biohazardous. Therefore automatic methods are preferred.

The use of restricted access materials (RAM) meets the previous mentioned requirements. Their unique properties allow the macromolecules exclusion and analytes extraction in one step. The connection of RAM with column-switching chromatography system leads to the fully automated analytical process. First, the analytes are extracted from matrix and retained on RAM sorbent. Then the analytes of interest are eluted onto the analytical column and determined.

The original RAM are based on carbon or silica, the molecularly imprinted polymers, nanotubes or magnetic particles are used for modern types of sorbents. Despite their promising properties, the nanofibers did not be used. Though, our first study confirmed the statement the nanofibers are possible alternative to RAM. Nanofibrous polymers provided good extraction efficiency for parabens in human serum and bovine milk. Simultaneously, they were able to remove proteins from these matrices. Based on this obtained knowledge, the methods for determination of pharmaceutically significant substances in biological matrices are being developed.

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TESTING OF NANOFIBROUS POLYMERS FOR EXTRACTION ON A MAGNETIC STIRRER

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This study was focused on nanofiber polymers and their potential use for the sorption extraction on a magnetic stirrer (SBSE – Stir Bar Sorptive Extraction). Aim of the work was to test available types of nanofibers and to prove their suitability as materials for extraction on the magnetic stirrer. The presented work included the preparation of the solutions to which we placed the magnetic stirrers coated by nanofibers where the sorption/extraction was carried out with the selected substances – Sudan dyes, NSAIDs, chlorophenols, carbamate and pyrethroid insecticides, nitrophenols, parabens and bisphenols. The chromatographic separation methods were developed for each compound of drugs separately. The substances pre-concentrated on the polymeric fibers were evaluated after chromatographic analysis and separation.

After several measurements we found out that the extreme lipophilic analytes such as Sudan dyes were irreversibly adsorbed on the polymeric fibers. The polystyrene fiber (PS) was not stable in used organic solvents and therefore it had to be prepared again before every extraction. In conclusion, selected kinds of the polymeric fibers which are suitable under the conditions of extraction were defined. Insecticides have been successfully pre-concentrated on polyamide 6 (PA6) with maximum obtained enrichment factor 69.4. Bisphenols have been successfully pre-concentrated on PA6 and also for composite

polymer PS + polycaprolacton (PCL) was the highest enrichment factor 82.3. The highest theoretical enrichment factor (100) has not been achieved at any of the used nanofiber.

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DESIGN OF EXPERIMENT IN SAMPLE PREPARATION FOR VITAMIN E

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Vitamin E including α-, β-, γ-, and δ-tocopherols and tocotrienols belongs among the fat-soluble compounds with important biological activity including cardioprotective, immunomodulatory, and blood cholesterol lowering effect essential for human health. Presence of vitamin E in urine could be observed when the glomerular filtration is damaged. Due to the high lipophilicity and protein binding, liquid-liquid extraction (LLE) and protein precipitation (PP) are suitable sample preparation methods for the isolation of vitamin E from plasma and urine. The recovery of sample preparation is affected by several variables (conditions) which can be optimized using time-consuming step-by-step procedure commonly applied in many laboratories or using design of experiment (DoE). DoE involves changing all variables affecting the method recovery, identifying their interactions and relationships. It enables to determine their effect to recovery using the minimum number of experiments including different variables at different levels. The optimal conditions are achieved after the creation and evaluation of the obtained model. In this study, univariate step-by-step optimization and optimization using DoE were carried out to optimize PP and LLE. PP agent type, solvent/agent ratio, time of incubation, and time of centrifugation were tested for PP, and the type of extraction solvent, its amount, extraction time, extraction temperature, and intensity of agitation were tested for LLE to find the best conditions in the term of recovery.

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COMPARISON OF SAMPLE CLEAN-UP EFFICIENCY USING MOLECULARLY IMPRINTED POLYMER AND REVERSED PHASE SORBENT FOR ON-LINE EXTRACTION OF ZEARALENONE

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Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* fungi. Despite its non-steroidal structure, ZEA expresses estrogen-like activity and acts as endocrine disruptor in mammals. Therefore, ZEA contamination in cereal products is regulated by European legislation (EC 1126/2007).[1] Developing modern analytical methods, automation, less handling with toxic sample and significant time reducing are favorable in routine food analysis. Presented newly developed HPLC methods allowed fully automated analysis including extraction step by column switching. On-line extraction was carried out either on guard column Ascentis Express C18 or on ZEA-selective molecularly imprinted polymer (MIP). The on-line coupling was optimized in terms of solvent choice, compatibility to chromatographic separation, time programming, gradient elution, and injection volume. Following chromatographic separation was designed the same for both on-line extraction methods for their further comparison.

On-line extractions on MIP have been published scarcely due to challenging optimization and compatibility problems. On the other hand, MIP properties such as stability, robustness, and extraction capacity make it suitable for application in column-switching system. The aim of the presented study was on-line MIP SPE-HPLC method development, and its evaluation of clean-up efficiency and selectivity compared to the analogue method using conventional C18 sorbent. However, no expected significant difference in selectivity was observed. Finally, the validated methods were applied for ZEA determination in beer samples.

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STUDY OF THE BIOACCESSIBILITY OF PHTHALATES AND BISPHENOL A FROM MICROPLASTICS IN SEAWATER USING AN ON-LINE SWITCHING VALVE HPLC SYSTEM

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For the first time, an automatic flow system hyphenated to LC is presented for dynamic extraction of microplastics (polypropylene and polyvinyl chloride) with incurred phthalates and Bisphenol A (CRMs). This flow setup was able to mimic leaching of the additives from plastic debris in marine environment. The microplastic particles were packed into a metal column holder, through which surrogate seawater was pumped using flow technique. The bioaccessible analytes were preconcentrated on-line using a 10 mm long C18 monolithic column, placed onto the injection valve of an HPLC system, by this, also removing the seawater matrix. After loading the Bisphenol A and phthalates containing leachate, the HPLC valve was switched to the inject position and the analytes were eluted via an optimized acetonitrile/water gradient followed by UV detection. The entire method including dynamic flow-through extraction from microplastics, on-line preconcentration and clean-up along with the HPLC separation took 24 min. Out of the 8 phthalates in CRM, only the 4 most polar species, as well as Bisphenol A, leached significantly by the seawater. To study dynamic extraction behaviour, 40 fractions were measured for each batch of particles and the elution profiles will be shown. RSD of measurements of standards was ≤ 5% and RSD of the dynamic leaching study was ≤ 11%.

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DETECTION TECHNIQUES FOR ANALYSIS OF PHENOLIC COMPOUNDS IN APPLES

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Apples represent one of the most worldwide available natural source of phenolic compounds responsible for overall well-being associated with their antioxidant properties.

Selection of cultivars with higher content of phenolic compounds can improve their intake by consumption. Fruit matrices complexity and structural similarity of polyphenols increase demands on detection selectivity and sensitivity. This problem can be solved by combination of different detection principles. For this reason, fast screening HPLC method coupled with tandem detection DAD-CAD and DAD-FLD was developed to evaluate phenolic profiles in twenty apple cultivars.

Both methods employed a fully porous particle column Luna Omega Polar C18 with stationary phase modified for separation of polar compounds. The use of linear gradient elution allowed rapid chromatographic separation. Benefits, disadvantages, and possibilities of tandem connection HPLC-DAD-CAD and HPLC-DAD-FLD methods for determination of phenolic compounds in fruit extract were summarized. DAD at 280 nm was used for determination of gallic acid, epicatechin, phloridzin, and phloretin; at 254 nm for detection of rutin and quercetin; at 320 nm detection of chlorogenic acid. CAD was universal for all analytes and FLD was used at λ_{em} 363 nm and λ_{ex} 278 nm for detection of gallic acid and epicatechin. The LOD and LOQ values of all detectors were compared. In contrast to DAD, FLD did not met the expectations for sensitive evaluation of analytes and selectivity of CAD was low due to absence of additional spectral data.

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INCORPORATION OF NEUTROPHIL ELASTASE INHIBITOR INTO POLYSULFONE MEMBRANE – NEW APPROACHES TO IMPROVE HEMODIALYSIS PATIENTS OUTCOMES

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Chronic kidney disease patients undergoing haemodialysis (HD) suffer from chronic inflammation caused by the disease *per se* and by the long-term contact with artificial material of HD membrane. As a consequence of inflammation, the patients have elevated risk of cardiovascular diseases, and therefore markedly higher mortality rate, compared to healthy population.

One of the promising approaches to diminish inflammation is to inhibit neutrophil elastase, which is excessively released from neutrophils during the HD treatment.[1] The elastase inhibition is mediated by the direct contact with elastase inhibitor incorporated into HD membrane. For this purpose, a neutrophil elastase inhibitor with chemical formula $C_{21}H_{20}N_2O_3S$ (K_i = 0.34 nM) was newly synthetized and incorporated into flat sheet polysulfone membrane during its fabrication process. The ability of modified membrane to

diminish elastase activity was evaluated *in vitro,* using fluorometric assay. Membrane with identical composition, without inhibitor, was used as a blank sample.

The preliminary results show promising inhibition of elastase in physiological concentration of healthy population (10 μg L^{-1}). However, the concentration of incorporated inhibitor has to be further optimized in order to reach diminution of elastase in real values found in HD patients (29.6–64.8 μg L^{-1}).[2]

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COLUMN COUPLING IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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Isomers and stereoisomers are always challenging to separate in pharmaceutical research because they are compounds with similar chemical properties, necessitating a unique selectivity. Coupling columns seems to be a promising approach to solve these selectivity problems. Supercritical Fluid Chromatography (SFC) has been established as a preferred chromatographic technique for chiral separations, and an increased interest in this technique is seen for achiral analyses in recent years. Supercritical fluids possess a lower viscosity compared to liquid mobile phases, which causes a lower pressure drop across the column and easily allows the coupling of several columns in series. However, retention mechanisms in SFC are more complex than in HPLC. The aim of this project is to propose a methodology to predict the retention behaviour of analytes in a coupled column system in SFC. Atenolol, ephedrine, propranolol, mianserin, labetalol, nadolol were used as chiral model analytes, quinine, quinidine as diastereomers, and aminophenol, aminobenzoic acid, aminocresol as examples of structural isomers. A combination of a chiral and an achiral stationary phase was selected for the column coupling, using Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, Lux Amylose-2 as chiral phases and Luna NH_2, Luna Silica, Synergi Polar RP and FluoroSep-RP Phenyl as achiral phases. The mobile phase was composed of CO_2 mixed with 20% (v/v) MeOH, which contained 0.1% (v/v) trifluoroacetic acid and 0.1% (v/v) isopropylamine. Retention factors of the coupled systems were estimated using a prediction formula.[1] That takes into account retention factors from individual stationary phases and the effective length of stationary phases. The calculated values of retention factors were compared with the experimental ones. Using

the formula, we were able to predict elution order of the analytes in the coupled column system. Future work will focus on increasing the predicting precision of the formula by incorporating other factors that influence the retention behaviour of analytes.

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THE DEVELOPMENT OF A LIQUID CHROMATOGRAPHY-QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY METHOD FOR DETERMINATION OF FLAVONOIDS, ISOFLAVONOID AND THEIR METABOLITES

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Known metabolites of flavonoids and isoflavonoids can be categorized into two major groups: compounds with preserved (iso)flavonoid core and low molecular weight phenolics. The aim of our project is to detect the metabolites of flavonol rutin and isoflavonoid tectoridin in rat plasma samples. Firstly, a suitable method employing ultra-high performance liquid chromatography with quadrupole-time-of-flight mass spectrometry (Q-TOF) for the analysis of tectoridin, rutin and the row of its known metabolites: quercetin, quercetin-3-*O*-glucuronide, tamarixetin, isorhamnetin, tectorigenin, phloroglucinol, 4-methylcatechol, 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, homovanilic acid, 3-(3-hydroxyphenyl)propionic acid was developed. A very complex optimization of MS parameters had to be carried out especially due to the low sensitivity for the low molecular weight phenolics. The measurement was carried out in MS scan. The particular setting of Q-TOF analyzer was essential for sufficient sensitivity of phenolics, which was increased 10 times on standard solution. Secondly, the optimization of suitable sample preparation step will be necessary for the application on plasma samples. Based on the purpose of this method the simple and non-selective protein precipitation will be employed. In following step, the effect of specific Q-TOF analyzer setting on the signal to noise will be verified on matrix samples. The final method will be applied to real biological samples of rat plasma for the determination of these metabolites and for the identification of other, potentially unknown, metabolites.

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BENEFITS AND RISKS OF SEPARATIONS AT ELEVATED TEMPERATURE IN PROTEOMIC ANALYSES USING A CONVENTIONAL-FLOW LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY SYSTEM

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Since nanoelectrospray was introduced nanoflow chromatography hyphenated via a nanoelectrospray to a mass spectrometer has generally been viewed as a *de facto* standard platform for proteomic applications. Indeed, leveraging nanocolumns with inner diameter of 0.075 mm instead of using conventional-flow columns with inner diameter of 2.1 mm conveys a theoretical 784-fold gain in sensitivity. Nevertheless, in our recent work we demonstrated that providing the instrumentation and method are adjusted, the amount of sample for a proteomic analysis can be only roughly 5-fold greater when using conventional-flow system.[1] Column temperature is one of the parameters that contributed significantly to increasing performance of the conventional-flow system. We noticed, however, that at some point the benefit of elevated temperature to peak shape was redeemed by lower number of identified peptides. We hypothesized that an on-column peptide degradation might occur when peptides were separated at elevated temperature using acidic mobile phases. To this end, we scrutinized the effect of temperature on the stability of a model protein trapped in a reversed phase column. We confirmed that temperature as high as 45 °C in combination with 0.1% formic acid already may induce on-column peptide bonds cleavage. We subsequently carried out data-dependent LC-MS analyses of tryptic peptides at various column temperatures. We found out that besides on-column peptide bonds cleavage, peptides trapped on a stationary reversed phase may undergo various artificial chemical modifications in the presence of 0.1% formic acid in mobile phases.

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DETERMINATION OF PHLORIDZIN AND OTHER PHENOLIC COMPOUNDS IN APPLE LEAVES, BARK AND BUDS BY HPLC

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The aim of this study was to develop a new liquid chromatography method to determine the content of phenolic compounds in raw material of apple trees – leaves, bark and buds. Raw materials from different apple cultivars were extracted in methanol acidified by 0.1% formic acid in ultrasound bath. Extracted phenolic compounds – phloridzin, phloretin, chlorogenic acid, rutin and quercitrin were subsequently analyzed by high performance liquid chromatography using YMC-Triart C18 ExRS 150 × 4.6 mm × 5µm, 8 nm analytical column. The separation was performed with gradient elution at flow rate 1 ml/min. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The detection was performed by DAD at wavelengths 280 nm, 327 nm and 354 nm. The temperature of column space was 30 °C, injection volume was 1 µl. The identification of analytes was achieved by comparing their retention time and spectra with retention time and spectra of standard solutions. The method was validated before quantification of phenolic compounds in the leaves extract. The followed validation parameters in defined ranges were obtained for the tested analytes: the linearity (R^2 = 0.994–0.998), repeatability (RSD = 0.34–1.75%), recovery (86.54–123.21%) and precision (RSD = 2.07–4.56%). Phloridzin was found as a dominating phenolic compound in concentrations ranging from 42.74 to 94.96 mg/g in leaves extracts. It covered more than 90% of total phenolic content, followed by quercitrin, chlorogenic acid, phloretin, rutin. Concentration range of phloridzin in bark extract was from 48.45 to 95.88 mg/g and in buds extract from 81.39 to 212.24 mg/g. In conclusion, the present study shows that plant material from apple trees is promising source of phytochemicals with positive effect on human health and could be potentially used for the development of dietary supplements.

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CITRININ-SELECTIVE MOLECULARLY IMPRINTED POLYMER AND ITS USE FOR ON-LINE SOLID PHASE EXTRACTION COUPLED TO LIQUID CHROMATOGRAPHY

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New selective molecularly imprinted polymer has been used for extraction in on-line SPE-HPLC to achieve the selective determination of citrinin. Four different imprinted polymers varying in combinations of components prepared by bulk polymerization were evaluated in terms of binding capacity and selectivity. Imprinted polymer prepared from the mixture comprising 1-hydroxy-2-naphtoic acid as the template molecule, acrylamide as the structural monomer, ethylene dimethacrylate as the crosslinker (in a molar ratio of 1:4:16), and acetonitrile as the porogenic solvent exhibited the best properties. Selectivity of this sorbent was confirmed by comparison with the non-imprinted counterpart prepared using the same polymerization carried out in absence of the template. Imprinted polymer was packed in a 20 × 3 mm i.d. steel cartridge and coupled to the on-line SPE-HPLC system through a six-port switching valve. The method for determination of citrinin including the on-line extraction step was then developed and validated. The sample in the form of methanolic extract was loaded, cleaned, and preconcentrated in the imprinted SPE cartridge. Following separation of citrinin from residual interferences was achieved using analytical column Kinetex Biphenyl 100 × 4.6 mm i.d., 5 µm particle size, and fluorescence detection (Ex 335, Em 500 nm). The total analysis time was only 9.50 min. Fully validated method was also applied to analysis of food supplements based on red yeast rice extracts which control is implemented in European legislation. Only minor yet acceptable contamination was found in tested samples.

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COMPARISON OF LIPID EXTRACTION METHODS FROM MILK USING 2D LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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Over the past decades, lipid samples were mainly extracted using labor intensive, non-parallelizable methods such as Bligh & Dyer and Folch extraction using toxic

non-polar solvents, *e.g.* chloroform. However, these methods were developed when high-resolution accurate mass measurements instruments were not widely available. In addition, medical interest in short and medium chained triacylglycerols was disclosed recently. Comparison of these methods with more modern ones such as single extraction solvent mixture and methyl *tert*-butyl ether extraction was carried out. Major benefit of these methods compared to traditional extraction is wider coverage of extracted lipids.

Chromatographic system used was two-dimensional liquid chromatography using non-aqueous reverse phase on C18 stationary phase in the first dimension and silver-ion stationary phase in the second dimension. Mass spectra were acquired using parallel outlet to high-resolution accurate mass spectrometer with electrospray ionization and triple-quadrupole with atmospheric pressure chemical ionization in first dimension. Second dimension contained parallel outlet to the two triple-quadrupole with to atmospheric pressure photoionization and ion trap with electrospray ionization.

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PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

ANTHELMINTICS IN PLANTS

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Anthelmintics, the drugs against parasitic worms, are widely used in human and veterinary medicine, nowadays. The usefulness of anthelmintic drugs is indisputable, but at the same time they pose a risk to ecosystems. With excrements of treated animals, anthelmintics can get into the environment and there affect non-target organisms – free-living invertebrates and wild plants. In our project, we are focused on antiparasitical drugs and their uptake, biotransformation and transcriptional response in plants. The most frequently used anthelmintics (albendazole, fenbendazole, flubendazole, ivermectin, monepantel) are followed, and different plant species are tested, also the model plant *Arabidopsis thaliana* (wild type, *Brassicaceae*).

The presented work is the part of this project. The aim of the study is to get information about the effects of anthelmintics on hydroponic cultures of *Arabidopsis thaliana* presented as changes in plant transcriptome. The broad-spectrum benzimidazole anthelmintic fenbendazole and macrocyclic lactone ivermectin were used. Hydroponic cultures were stressed by 5 μM anthelmintic. The effect was studied after 24 and 72 hours of stress.

The microarray analysis was performed. For general expression at the transcription level Agilent-based microarrays were used. Quantitative analysis of whole proteomes comparing fenbendazole treated and control samples was carried out using a LC/MS Thermo Orbitrap Fusion spectrometer.

In our study the presence of fenbendazole or ivermectin and theirs metabolites in *Arabidopsis thaliana* influenced gene expression. It was more affected by both anthelmintic in rosettes than in roots. More than 4.000 proteins were identified by proteomic analysis. Most proteins were identified in leaves of plants stressed by fenbendazole.

The study was supported by the Czech Science Foundation (Project No. 18-08452S) and from the project of Specific Academic Research (SVV 260 416).

SELECTION AND VALIDATION OF REFERENCE GENES FOR mRNA AND miRNA GENE EXPRESSION STUDIES IN HUMAN LIVER SLICES USING RT-qPCR

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Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is a frequently used technique for gene expression profiling. RT-qPCR analysis depends on data normalization by endogenous reference gene, which expression is stable and independent on experimental conditions. Precision-cut liver slices (PCLS) are an interesting model due to its multicellular composition, preserved tissue architecture and intercellular communication. Its applicability to human tissues allows us to avoid interspecies differences and directly apply human tissues into multiple experimental designs. PCLS also represent a promising model for gene expression studies. However, there has been no study validating this model for selection of a suitable reference gene (or their combination). Therefore, we decided to perform a validation study, since selection of inappropriate reference gene can influence the trend and deviation of results. Three human liver samples received from surgery were used to obtain PCLS (8 mm diameter, 150 µm thickness), which were cultivated for 24 hours and samples were collected after 0, 4, 8, 12, 18 and 24 hours. As we are interested in induction studies, two cytochrome P450 (CYP) inducers β-naphthoflavone and rifampicine were used as positive controls. To verify the viability of PCLS, ATP content and lactate dehydrogenase leakage were measured. Based on literature review, six candidates (*GAPDH*, *SDHA*, *ACTB*, *B2M*, *HPRT* and *YHWAZ*) and five candidates (miR-16-5p, miR-23b-3p, miR-93-5p, miR152-3p and U6) were selected for mRNA and miRNA validation, respectively. Stability of those genes was compared using RefFinder, a free web tool that uses several other software, such as geNorm, Normfinder, BestKeeper and the comparative Ct method. Stability of

selected reference genes for mRNA was validated on expression analysis of *CYP3A4* and *CYP1A2*.

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PHENOTYPIC SCREENING OF A CHEMICALLY DIVERSE COMPOUND LIBRARY IDENTIFIED TWO COMPOUNDS WITH ANTHELMINTIC ACTIVITY AGAINST *HAEMONCHUS CONTORTUS*

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Due to the widespread development of anthelmintic resistance in *Haemonchus contortus*, there is a continuing need to discover and develop new anthelmintic drugs to ensure sustainable control of this and related, economically important and pathogenic nematodes of ruminants. With this focus in mind, we screened a compound library consisting of 236 chemicals representing diverse classes such as heterocyclic compounds (*e.g.* thiazoles, pyrroles, quinolines, pyrimidines, benzo[1,4]diazepines), hydroxamic acid-based metalloenzyme inhibitors, peptidomimetics (bis- and tris-pyrimidoneamides, alkoxyamides) and various intermediates. In the present study, we measured the inhibition of larval motility and development of exsheathed third-stage (xL3) and fourth-stage (L4) larvae of *H. contortus* using an optimized, whole-organism phenotypic screening assay. Of the 236 compounds, we identified two active compounds (called BLK127 and HBK4) that induced phenotypic changes in the worm *ex vivo*. Compound BLK127 induced an 'eviscerated' phenotype in xL3 larvae and also inhibited L4 larvae development. Compound HBK4 exerted a 'curved' phenotype in both xL3 and L4 larvae. The findings from this study provide a sound basis for future work on assessing the activity of the compounds identified here on adult stages of *H. contortus* both *ex vivo* and *in vivo* (within the host animal), and against other parasitic worms of veterinary and medical importance.

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THE ROLE OF THE UDP-GLYCOSYLTRANSFERASES IN THE METABOLISM OF ANTHELMINTICS IN *HAEMONCHUS CONTORTUS*

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Haemonchus contortus, a hematophagous gastrointestinal parasite, endangering predominantly small ruminants, such as sheep and goats, has a great ability to develop resistance to anthelmintic drugs. Therefore, it is necessary to understand the mechanism of the resistance. The effects of anthelmintics can be reduced by xenobiotic-metabolizing enzymes decreasing the concentration of the active drugs in parasite cells. UDP-glycosyltransferases (UGTs), important enzymes in the metabolism of xenobiotics and eobiotics, could protect the helminth from toxic action of anthelmintics by their metabolism to inactive glycosides. The previous study showed that albendazole, ricobendazole and flubendazole underwent glycosidations caused by UDP-glycosyltransferases. Except sex-differences in anthelmintics metabolism, more glycosylated metabolites were observed in IRE (benzimidazol resistant) strain than ISE (sensitive) strain of *H. contortus*.[1] This analysis confirmed the connection between anthelmintics resistance and their metabolism. Using quantitative PCR, the differences in the constitutive expression of UGTs between IRE and ISE strains were analyzed. Several enzymes from the UGTs superfamily, *e.g*. UGT368B2, were significantly more expressed in IRE strain than ISE strain.[2] The enhanced expression of biotransformation enzymes could lead to increased rate of anthelmintics metabolism.

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BUPARLISIB IS A NOVEL INHIBITOR OF DAUNORUBICIN REDUCTION MEDIATED BY ALDO KETO REDUCTASE 1C3.

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Buparlisib is a pan-class I phosphoinositide 3-kinase (PI3K) inhibitor and is currently under clinical evaluation for the treatment of different cancers. Because PI3K signalling is related to cell proliferation and resistance to chemotherapy, new therapeutic approaches are focused on combining PI3K inhibitors with other anti-cancer therapeutics. Carbonyl-reducing enzymes catalyse metabolic detoxification of anthracyclines and reduce their cytotoxicity. In the present work, the effects of buparlisib were tested on five human recombinant carbonyl-reducing enzymes: AKR1A1, AKR1B10, AKR1C3, and AKR7A2 from the aldo-keto reductase superfamily and CBR1 from the short-chain dehydrogenase/reductase superfamily, all of which participate in the metabolism of daunorubicin. Buparlisib exhibited the strongest inhibitory effect on recombinant AKR1C3, with a half-maximal inhibitory concentration (IC_{50}) of 9.5 mM. Its inhibition constant K_i was found to be 9.9 mM, and the inhibition data best fitted a non-competitive mode. The same extent of inhibition was observed at the cellular level in the human colorectal carcinoma HCT 116 cell line transfected with a plasmid encoding the *AKR1C3* transcript (IC_{50} = 7.9 mM). Furthermore, we performed an analysis of flexible docking between buparlisib and AKR1C3 and found that buparlisib probably occupies a part of the binding site for a cofactor, most likely via the trifluoromethyl group of buparlisib interaction with catalytic residue Tyr55. In conclusion, our results show a novel PI3K-independent effect of buparlisib that may improve therapeutic efficacy and safety of daunorubicin by preventing its metabolism by AKR1C3.

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CYTOCHROME P450 IN *HAEMONCHUS CONTORTUS*

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Resistance to anthelmintic drugs has become a major concern worldwide in animal health. The nematode *Haemonchus contortus*, one of the most economically important parasite of small ruminants, has become multi-resistant for all classes of used and known anthelmintics. One possible mechanism of resistance development is an increased expres-

sion of drug metabolizing enzymes, cytochromes P450 (CYPs). *H. contortus* genome contains around 18 CYPs homologs and some of them might to play an important role in resistance development.[1,2] The aim of this study is to specify which CYPs expression differ in drug-sensitive and drug-resistant adults of *H. contortus*. In addition, the potential inducibility of these genes by different xenobiotics exposure was tested. Three isolates of *H. contortus* adults, susceptible ISE, resistant to ivermectin (IVM) IRE and multi-resistance WR, were obtained using agar method from lambs' abomasa and sexed based on morphology. Following incubation with different xenobiotic (IVM, ABZ, MOP, PHB, BNF) the expression of CYPs was analyzed by qPCR. The constitutive expression of several CYPs differs between sex and between all three isolates. Increased expression of *cyp7*, *cyp8* was observed in WR females, while *cyp2* is overexpressed in IRE females. In males, the overexpression of *cyp2*, *cyp7*, *cyp8* is significant in both IRE and WR isolates. Several CYPs are significantly inducible with various xenobiotics especially in susceptible isolate. Our data suggest that some CYPs might be resistance related. Identification of such CYPs would lead to important biological marker in anthelmintic development.

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17β-HSD10 INHIBITORS AS A POTENTIAL THERAPY IN NEURODEGENERATIVE DISORDERS

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Mitochondria are the unique organels of many cell processes including energetic metabolism, maintaining homeostasis or regulation of cell death, and are an important player in many neurodegenerative disorders.

17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) is a mitochondrial protein playing important role in various physiological pathways, mainly in steroid metabolism, but was also found to be overexpressed in Alzheimer's disease (AD) and several types of cancer. In AD 17β-HSD10 can bind β-amyloid resulting in increased oxidative stress, cell toxicity, and neuronal impairment.[1] Modulation of this enzyme could be a novel target for neurodegenerative disorders treatment.[2]

For testing of various novel 1-(benzo[*d*]thiazol-2-yl)-3-phenylurea-based inhibitors[3–5], the recombinant enzyme was produced in *E. coli* and purified using chromatographic meth-

ods. Enzymic activity assay was performed spectrophotometrically in a microplate reader at 37 °C, using acetoacetyl-CoA as a substrate. Kinetic parameters of the enzyme were determined and over 200 inhibitors were screened for their inhibitory ability. The most inhibiting compounds were selected and their IC_{50} constants and type of inhibition were determined. These compounds are further studied using *in vitro* and *in vivo* methods with implication to neurodegenerative disorders and/or cancer.

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EVALUATION OF PROTECTIVE PROPERTIES OF DEXRAZOXANE AND OTHER CATALYTIC INHIBITORS OF TOPOISOMERASE II AGAINST ANTHRACYCLINE CARDIOTOXICITY *IN VITRO*

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Anthracyclines (ANT) are one of the most effective anticancer drugs, but their use is limited by their irreversible cardiotoxicity. ANT belong to topoisomerase II (TOPII) poisons, giving rise to DNA double strand breaks. Poisoning of the TOPIIα in cancer cells is one of the fundamental mechanisms of their antineoplastic action, but their effect on the other isoform, TOPIIβ, could play a crucial role in development of their cardiotoxic effects. However, the exact mechanism of their cardiotoxicity is still not completely understood, which hampers the rational approach to discovery of protective strategies. Dexrazoxane (DEX) is the only compound that has shown considerable cardioprotective potential against ANT cardiotoxicity in experimental studies as well as in randomized clinical trials. Latest studies suggest that the cardioprotective activity might be associated with its catalytic inhibition of TOPII. This hypothesis is supported by the results of our previous study, where not only DEX but also structurally different TOPII catalytic inhibitor merbarone showed protective potential in neonatal rat cardiomyocytes without compromising the daunorubicin (DAU) antiproliferative effect.

Therefore, we decided to study the cardioprotective potential of several other structurally different compounds, which have been described as catalytic inhibitors of TOPII (suramin, aclarubicin and gambogic acid) or as TOPII poisons (genistein and XK-469), the latter described to be specific for the beta isoform of this enzyme). In this study, using primary cul-

tures of isolated rat neonatal cardiomyocytes, we studied their own toxicity and the protective effects against cardiotoxicity caused by DAU and compared the effects of these compounds to the protective effect of DEX in our previously established model, using clinically relevant concentrations of DAU. Aclarubicin, gambogic acid and genistein did not show any cytoprotective effect against toxicity caused by DAU. On the other hand, suramin (catalytic inhibitor) and XK-469 (topoisomerase poison) both displayed significant protective potential in neonatal cardiomyocytes, although their effect was not better than efficiency of DEX.

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SYNERGISTIC EFFECT BETWEEN MIDOSTAURIN AND DAUNORUBICIN IS RELATED TO ALDO-KETO REDUCTASE 1C3 INHIBITION

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Midostaurin is a tyrosine kinase 3 (FLT3) inhibitor (TKI) acting against targets known to be expressed in hematologic disorders.[1] FDA recently approved midostaurin combined with daunorubicin chemotherapy for the treatment of acute myeloid leukemia (AML).[2] Aldo-keto reductase 1C3 is an enzyme overexpressed in a range of cancer types and leukemia cell lines[3] which reduces daunorubicin into inactive hydroxy metabolite daunorubicinol, thus conferring resistance to this chemotherapeutic.[4] Here, we report that midostaurin is a strong inhibitor of daunorubicin reduction mediated by human recombinant AKR1C3. Moreover, we performed cytotoxic studies with KG1a, a myelogenous leukemia cell line that expresses high amounts of AKR1C3. In these studies, midostaurin significantly sensitized KG1a cells to cytotoxic effect of daunorubicin. In addition, downregulation of AKR1C3 by siRNA reduced synergistic effect between midostaurin and daunorubicin in KG1a cells. In conclusion, our results demonstrate a novel effect of midostaurin that could contribute to further understanding of the effectivity of midostaurin and daunorubicin combined therapy in clinical practice.

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THE EFFECT OF SESQUITERPENES ON ENZYMES INVOLVED IN DETOXIFICATION PROCESSES IN HUMANS

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Sesquiterpenes β-caryophyllene (CAR), caryophyllene oxide (CAO), α-humulene (HUM), farnesol (FAR), *cis*-nerolidol (cNER) and *trans*-nerolidol (tNER) possess numerous biological and pharmacological activities, among which an inhibitory effect on several phase I drug-metabolizing enzymes (DME) in human and rat subcellular fractions have been observed. Since they are often present in traditional medicinal products as well as in human diet, we intended to investigate the effect of these sesquiterpenes on the mRNA and protein expression of several DME, namely cytochrome P450 (CYP) isoforms 3A4 and 2C, carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C (AKR1C), in human liver. For the investigation, freshly prepared precision-cut liver slices were cultivated in a medium supplemented with studied sesquiterpenes at 10 μM concentration for 24 h. Real-time quantitative PCR method was used to determine the mRNA levels of the studied DME. The protein levels were detected using western blot technique. So far, the results suggest that FAR and tNER possess the ability to negatively affect the levels of mRNA of all studied DME. Moreover, CAR, CAO and HUM inhibited the expression of mRNA of all DME, except for CYP2C. Their effects, however, differ among individual human liver samples presumably due to possible inter-individual variability. Such variability may also be seen when concerning the changes in protein expression. For instance, in case of FAR and tNER, both an inhibitory as well as an inductive effect on protein expression in human liver was observed. cNER seems to have no effect on the tested DME. The influence of CAR, CAO and HUM on protein expression is yet to be studied.

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INVESTIGATION OF DNA DAMAGE IN ANTHRACYCLINE-INDUCED CARDIOTOXICITY USING THE COMET ASSAY AND H2AX PHOSPHORYLATION

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Despite their long history, anthracycline antibiotics (ANT) are still used in clinics in various antineoplastic protocols. Their usage is hampered by several life-threatening side effects such as myelotoxicity and cardiotoxicity. The mechanism of cardiotoxicity remains obscure, limiting the rational discovery of protective strategies. Anthracyclines belong to topoisomerase II (TOPII) poisons giving rise to DNA double strand breaks. Poisoning the TOPIIα in cancer cells provides the basis of their antineoplastic action, but it can also play a role in cardiotoxicity. As TOPIIα expression is minimal in post-mitotic cells, TOPIIβ is the main isoform present in quiescent cells (*e.g.* cardiomyocytes) and has distinct functions from TOPIIα that involve gene expression regulation and DNA repair. The DNA damage generated in cardiomyocytes by ANTs could lead to the longterm effect on the heart. Moreover, ANTs could also cause oxidative DNA damage through redox-cycling mechanisms. To assess the possible role of DNA damage, several distinct approaches can be employed. Directly, the DNA double strand breaks (DSB) can be assayed by single-cell gel electrophoresis "the comet assay", where purified nucleoids of single cells trapped in agarose are subjected to electric field. Each DSB loosens the loops in the nucleoid forming a comet-like image. Another method involves the assessment of histone H2AX which is phosphorylated consequently to the DSB formation. These two methods were used to investigate the level of DNA damage caused by ANTs in isolated rat neonatal ventricular cardiomyocytes and HL-60 cells. Furthermore, dexrazoxane as the only approved cardioprotective agent and TOPII catalytic inhibitor was used in analysis ANT-induced DSB formation.

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PHARMACOLOGY AND TOXICOLOGY SECTION

INSIGHT INTO A REGULATORY NETWORK OF PREGNANE X RECEPTOR EXPRESSION

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The pregnane X receptor (PXR, NR1I2) is a key transcription factor involved in the regulation of both endogenous and exogenous metabolism. As a ligand-activated nuclear receptor, PXR responds to the structurally diverse collection of compounds. Following its activation, PXR triggers transcription of CYP3A4, pivotal phase I metabolic enzyme which is estimated to metabolize approximately 50% of all marketed drugs. Of particular interest is the fact that the drug-mediated activation of PXR-CYP3A4 axis may result in clinically significant pharmacokinetic interactions.

Although PXR has been studied intensively regarding its transcription function, less is known about exact mechanisms standing behind its own regulation. As previously shown, PXR expression is under the control of activated glucocorticoid receptor (GR) which directly increases a level of PXR transcript. In present follow-up study, we demonstrate that PXR mRNA is not only induced at transcriptional level but also stabilized at post-transcriptional level after activation of GR.

An evidence that GR mediates changes in miRNA expression which may subsequently lead to stabilization of PXR mRNA via its 3′-untranslated region will be provided during the lecture. The given postulate will be supported by data gained from gene reporter studies using various reporter vectors, miRNA expression profiling, and qPCR.

Overall, major conclusion drawn from our work is that GR increases expression of PXR mRNA by dual mechanisms such as transcriptional activation of PXR from its promoter and induction of post-transcriptional stabilization.

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NOVEL OBETICHOLIC ACID KETODERIVATIVES AND ISOMERS AS POTENTIAL LIGANDS OF BILE ACID RECEPTORS

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Metabolic diseases with altered cholesterol and triglyceride levels are serious healthcare problem emerging in western population, and are tightly linked to inflammation. Recently, obeticholic acid (OCA), a potent farnesoid X receptor (FXR) agonist, has been shown to be a promising treatment against inflammatory hepatic disorders. Therefore, we aimed to synthesize new derivatives and isomers of OCA (named A–I) and assess their capacity to activate nuclear receptors involved in metabolic regulation including FXR, vitamin D receptor (VDR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR).[1,2] Gene reporter assays were performed to determine their capacity to activate nuclear receptors of interest and changes on expression of target genes were analysed by real time qPCR. Furthermore, these isomers were subjected to LC/MS analysis to determine their stability and possible conversion to OCA in HepG2 cell line and primary human hepatocytes. Our results showed that all derivatives could significantly activate FXR and PXR in therapeutic doses. Compound G is an equally strong ligand of FXR as OCA itself. We have also found that compound H is an activator of VDR. None of the tested compounds was able to activate CAR. Here, we have presented novel ligands of bile acid nuclear receptors derived from OCA. Moreover, we have found a new dual FXR/VDR agonist, compound H, which may have a promising use in the therapy of inflammatory metabolic disorder including steatohepatitis or atherosclerosis.[3]

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ACTIVATION OF HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) BY BENZODIAZEPINES WITHOUT PROLIFERATIVE EFFECT AND LIVER TUMORIGENIC EFFECTS IN HUMAN CELLS

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A large number of nongenotoxic chemicals have been shown to increase the incidence of liver tumours in mice by a mode of action involving activation of the constitutive androstane receptor (CAR). Studies with the model CAR activator phenobarbital (PB) have demonstrated that events for mouse liver tumour formation include CAR activation, increased hepatocyte replicative DNA synthesis (RDS), induction of cytochrome P450 CYP2B subfamily enzymes, liver hypertrophy, increased altered hepatic foci and hepatocellular adenomas/carcinomas.[1] However, this phenomenon is not confirmed in humans. In this study, we examined drugs widely used in clinics for their interaction with human CAR. We found that some benzodiazepines significantly activate human CAR in gene reporter assay and stimulated CAR translocation *in vitro*. However, benzodiazepines did not stimulate expression of genes involved in CAR-mediated hepatocyte proliferation in HepaRG cells. Diazepam did not stimulate cell cycle or anti-apoptotic gene expression. We did not find indication about live tumor promoting effect in databases. We can conclude that some benzodiazepines are CAR activators that unlikely stimulate proliferation or liver tumor promotion in humans.

The study was supported by the Grant Agency of Charles University (Project. No. 170/50/75006) and from the project of Specific Academic Research (SVV 260 414).

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INTERPLAY OF ABCB1, ABCC1 AND OATPs TRANSPORTERS IN TRANSFER OF MARAVIROC ACROSS THE HUMAN PLACENTA

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Maraviroc was developed as an inhibitor of HIV entry co-receptor CCR5. Due to importance of CCR5 in physiological signalling processes in organism and also between tumour cells the drug is currently evaluated as a new drug in treatment of many inflammatory and cancer diseases including states that occur in pregnant women. We have recently shown that maraviroc is transported by human ABC drug efflux transporter ABCB1 that is considered as protective component of placental barrier. In this study we aimed to verify the role of this transporter in limiting maraviroc distribution across the human placenta. The method of closed-circuit dual perfusion of maraviroc across human placental cotyledon was performed showing slight decrease of maraviroc concentration in fetal compartment. Presence of ABCB1 inhibitor elacridar and ritonavir abolished this decline, confirming ABCB1-mediated efflux of maraviroc in the feto-maternal direction. However, accelerated transport of maraviroc in materno-fetal direction was also found, suggesting contribution of another transport mechanism in the opposite direction to ABCB1. Bi-directional transport study in monolayers of MDCKII-ABCC1 cells and accumulation study in A431-OATP2B1, -OATP1A2 and -OATP1B3 were performed, identifying maraviroc as substrate of human ABCC1, OATP1A2 and OATP1B3. Gene expression of ABCC1 and OATP1A2 was subsequently confirmed in all the perfused placentas as well as in isolated trophoblast and fetal endothelial cells. Our data thereby indicate interplay between ABCB1 acting in feto-maternal direction and other transporters acting in materno-fetal direction, most probably ABCC1 with possible contribution of OATP1A2. These findings may help to understand maraviroc pharmacokinetics in pregnant women and contribute to safer therapy. Moreover, they indicate that the protective role of ABCB1 in the placenta may be covered up by other transporters promoting the transfer in the opposite, materno-fetal direction.

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PLACENTAL TRANSPORT AND METABOLISM OF SEROTONIN AND TRYPTOPHAN

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Serotonin (5-HT) is an important neurotransmitter critical for fetal brain development and programming during pregnancy. To date, however, controversies remain on the source of fetal 5-HT. Until recently, it was believed that maternal 5-HT crosses the placenta easily and plays a key role in maintaining fetal brain levels. Nevertheless, recent studies demonstrate that 5-HT can also be synthesized from tryptophan (TRP) within the trophoblast. In addition, kynurenine pathway of TRP metabolism has also been identified in the placenta, giving rise to neuroprotective and neurotoxic metabolites. Furthermore, expression and activity of the rate-limiting enzymes of TRP metabolism in the placenta may be perturbed by maternal conditions such as inflammation, stress, or depression, recently associated with various disorders in fetal neurodevelopment. In our project we aim to characterize and validate several *in vitro/ in situ/ex vivo* placental models to investigate possible effects of pathologies (*e.g.* infections) and pharmacotherapy (*e.g.* antidepressants) on transport and metabolism of TRP and 5-HT in the feto-placental unit.

qRT-PCR was utilized to analyze the expression of 29 transporters/enzymes in first trimester and term human placentas, rat term placentas and placental derived cell lines. Furthermore, HPLC analytical methods have been developed for quantification of TRP and 5-HT in biological samples. Dually perfused rat term placenta was employed to investigate placental transport and catabolism of TRP and 5-HT. Finally, 5-HT uptake was quantified using *in vitro* (BeWo choriocarcinoma cell line) and *ex vivo* (fresh villous fragments isolated from human term placenta) models.

The study was supported by Research programme Development and Study of Drugs (Progres Q42).

INTERACTIONS OF ABC TRANSPORTERS AND CYTOCHROME P450 ISOFORMS WITH THE TYROSINE KINASE INHIBITOR ENSARTINIB

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Ensartinib is a promising small molecule tyrosine kinase inhibitor which is currently evaluated in phase II/III clinical trials for the treatment of solid tumors. In our research, we focused on the interactions of ensartinib with ABC drug efflux transporters and cytochrome P450 (CYP) biotransformation enzymes, important proteins that control pharmacokinetic behavior of a number of drugs and also play the role in cancer multidrug resistance (MDR). First, we assessed the inhibitory effect of ensartinib on human ABCB1, ABCG2 and ABCC1 transporters using accumulation assays in MDCKII cells overexpressing respective ABC transporters. The results of these experiments showed that ensartinib is a potent inhibitor of ABCB1 and ABCG2. Consequently, the potential of ensartinib to overcome ABC transporter-mediated cytostatic MDR was evaluated in the follow-up MTT drug combination studies. In these assays, ensartinib effectively reversed daunorubicin and mitoxantrone resistance in MDCKII cells with ABCB1 and ABCG2 overexpression, respectively. Furthermore, qRT-PCR gene expression studies were performed in A549, CaCo2, NCI-H1299 and LS174T cellular models; we recorded no significant induction of *ABCB1*, *ABCG2* or *ABCC1* genes in all the cell lines following exposure to ensartinib. Finally, using Vivid CYP450 screening kits, we demonstrated that ensartinib is a strong inhibitor of CYP3A4, CYP3A5, CYP2C9 and CYP2C19, a moderate inhibitor of CYP2C8 and CYP2D6 and does not affect the activity of CYP1A2 and CYP2B6 isoforms. In conclusion, our findings indicate that ensartinib exhibits potential to provoke drug-drug interactions and affect MDR phenotype via changes in the activity but not in gene expression of particular ABC transporters and biotransformation enzymes. Moreover, our *in vitro* combination experiments revealed possible new effective therapeutic approach targeting ABCB1 and/or ABCG2 overexpressing tumors, which could serve as a valuable foundation for future *in vivo* studies.

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INTERACTION OF PROTEIN KINASE INHIBITORS WITH ABC TRANSPORTERS IN ACUTE MYELOID LEUKEMIA, POTENTIAL ROLE IN DRUG RESISTANCE

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Acute myeloid leukemia (AML) represents a severe hematological malignity, which is, despite the overall therapeutic advances, still characterized by poor prognosis and short survival. These unfavorable characteristics are associated also with the expression of ATP-binding cassette (ABC) transporters in undifferentiated blast cells that drive disease progression and relapses. Our aim was to investigate the interactions of modern protein kinase inhibitors abemaciclib, palbociclib, ribociclib and midostaurin, the novel drugs that have recently gained a breakthrough approval in treatment of both solid tumors and leukemia, with ABC transporters. *In vitro* and *ex vivo* approaches comprising cellular models of resistant HL-60 cells and *ex vivo* peripheral blood mononuclear cells (PBMCs) isolated from *de novo* diagnosed AML patients were employed.

Accumulation studies in HL-60 cells and their ABCB1- and ABCG2-overexpressing variants confirmed that all the tested drugs show ability to inhibit efflux of daunorubicin and mitoxantrone, the anticancer drugs approved by US Food and Drug Administration for AML therapy and at the same time substrates of both, ABCB1 and ABCG2 transporters. Furthermore, the ABCG2 inhibitors effectively enhanced number of apoptotic HL-60-ABCG2 cells, when combined with mitoxantrone, as confirmed by Annexin V/propidium iodide double staining. Abemaciclib, ribociclib and midostaurin applied in human plasma-relevant concentrations further increased mitoxantrone accumulation in PBMCs of AML patients.

To conclude, our preliminary results indicate the ability of protein kinase inhibitors abemaciclib, ribociclib and midostaurine to interact with ABC transporters in AML patient-derived cells. These data will form a basis for our follow up research on AML resistance mechanisms and approaches for their possible overcoming.

The study was supported from the project of Specific Academic Research (SVV 260 414) and by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF.

DISCOVERY OF A NOVEL MOUSE CONSTITUTIVE ANDROSTANE (CAR) RECEPTOR AGONIST THAT DOES NOT POSSESS PROLIFERATIVE ACTIVITY CONNECTED WITH NON-GENOTOXIC HEPATOCARCINOGENESIS IN MICE

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Constitutive androstane receptor (CAR) is the primary regulator of drug metabolism and detoxification. CAR activation is connected with mitogenic effects leading to liver hypertrophy and tumorigenesis in rodents. Recently, stilbenoids resveratrol and *trans*-3,4,5,4′-tetramethoxystilbene (TMS) have been shown to abrogate or alleviate *N*-nitrosodiethylamine/PB-induced liver carcinogenesis or oxidative stress signaling.

Thus, we examined if TMS may be an inverse agonist of mouse Car. Unexpectedly, we have identified TMS as a novel moderate murine Car agonist in cellular reporter gene experiments, in *in silico* docking experiments as well as in induction experiments in mouse hepatocytes, in AML-12 hepatic cells, or in mice. TMS significantly up-regulates *Cyp2b10*, *Cyp2c29* and *Cyp2c59* mRNAs, but down-regulates expression of genes involved in gluconeogenesis and lipogenesis such as *Pck1*, *G6pc*, *Scd1, Acaca* and *Fasn* in similar degree as TCPOBOP. Importantly, TMS does not induce genes involved in liver proliferation or apoptosis such as *Mki67*, *Foxm1*, *Myc*, *Mcl1*, *Pcna*, *Bcl2, Bax* or *Mdm2* in C57BL/6 mice, and has no statistically significant effects on Ki67 and Pcna labeling indexes in mice, but slightly up-regulates *Gadd45β* mRNA expression.

We can thus conclude that TMS is a novel mouse Car ligand with limited effects on hepatocyte proliferation, but controlling Car-target genes involved both in xenobiotic and endobiotic metabolism.

The study was supported by the Charles University Grant Agency (Project No. 170/50/75014).

RADIOACTIVELY LABELED RAMUCIRUMAB: *IN VITRO* BINDING AND INTERNALIZATION STUDIES IN VEGFR-2 POSITIVE CELLS

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Ramucirumab (RAM) is clinically used fully humanized monoclonal antibody directed against an extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2). The VEGFR2 is a key receptor responsible for the angiogenesis and was found to be overexpressed in some types of solid tumors (*e.g.* gastric, pancreatic, breast or lung cancer). RAM binds to the receptor with much greater affinity than its natural ligand and inhibits its function. With proper radiolabeling RAM could be potentially used either as a radiodiagnostic tool for imaging of VEGFR2-positive tumors or in a targeted radiotherapy. The aim of this work was to evaluate the influence of the radiolabeling process on the RAM immunoreactivity.

Several methods of either direct or indirect (via chelating agents) radiolabeling and nuclides (^{99m}Tc, ^{131}I, and ^{67}Ga) were employed in experiments. All prepared radiopharmaceuticals were tested for radiochemical purity and stability using HPLC and ITLC methods and for *in vitro* receptor-ligand binding affinity. Two VEGFR2 expressing human cancer cell lines (PC3, SKOV3) were used in the binding study.

All employed radiolabeling methods were suitable for the RAM labeling. However, significant differences were observed in radiochemical purity, stability and also in the deterioration of the immunoreactivity of the monoclonal antibody after the radiolabeling process. The direct ^{99m}Tc-labeling provided relatively low radiochemical purity, the iodination exhibited low stability and both of these direct labeling methods negatively influenced the immunoreactivity of RAM. All the indirect radiolabeling methods preserved RAM specific binding to the VEGFR2. However, the RAM binding kinetics was slightly altered depending on the radiolabeling conditions.

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STRESS-INDUCED SENESCENCE IN THE HEART – OPTIMALIZATION OF METHODOLOGY

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Stress-induced senescence is the specific form of cellular senescence, when mitotic cells exposed to non-lethal stress exhibit senescence-associated secretome phenotype (SASP); state characterized by permanent G1 arrest, alteration of their transcriptome and secretion of specific cytokines *etc*. Recently, stress-induced senescence was described also in post-mitotic cells, but its role is unclear. In the heart, cardiac as well as non-cardiac cells could exhibit SASP in response to non-lethal dose of cardiotoxic agents and also in a disease state.

Because this phenomenon is of great importance, the main aim of this project is to set up adequate and relevant methodology applicable in our lab for studying the SASP *in vitro* (primary rat cardiomyocytes or H9c2 cardiomyoblasts) and *in vivo*. Furthermore, changes in cardiac electrical conduction have recently been described, but only on *ex vivo* models. Therefore, secondary aim of this project is to study potential cardiotoxic effects of bisphenols.

Up to date, we have performed pilot experiments with acute administration of bisphenol A in rats to test study design and bisphenol A formulation with different co-solvents. Pilot *in vitro* experiments with using H9c2 cardiomyoblasts and neonatal rat cardiomyocytes were also performed to optimize selected antibody to common cardiokines. Finally, an isolation of adult rat cardiomyocytes using various methods were performed.

The study was supported by Research programme Development and Study of Drugs (Progres Q42).

MEMBRANE AND SOLUBLE ENDOGLIN ROLE IN EARLY DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN MICE

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Membrane endoglin (ENG) expression is linked to regulation of eNOS in endothelial cells via Smad2/3 signaling resulting in proper function of endothelium. We hypothesized that hypercholesterolemia alters endoglin expression/signaling with respect to endothelial function in aorta before formation of any atherosclerotic changes in mice.

Two-month-old hypercholesterolemic ApoE/LDLR-deficient (ApoE/LDLR$^{-/-}$) female mice and wild type C57BL/6J mice were fed chow diet. Plasma samples were tested for biochemical profile and Luminex analysis. Vascular reactivity was measured by wire myograph and expressions of NO-related markers were assessed by Western blot.

ApoE/LDLR$^{-/-}$ mice demonstrated hypercholesterolemia accompanied by significantly increased levels of sEng, pro-inflammatory P-selectin and a disruption of NO metabolism. Functional data indicated that ApoE/LDLR$^{-/-}$ mice have reduced relaxation capability of smooth muscle cells and activated compensatory mechanism generating NO from eNOS-independent pathway sources. Western blot analysis showed significantly reduced expression of membrane Eng, eNOS and its active form p-eNOS as well as phosphorylated Smad2/3 in ApoE/LDLR$^{-/-}$ mice. The expression of heme oxygenase 1, an enzyme leading to higher NO release, was significantly lower in ApoE/LDLR$^{-/-}$ group. Further, the expression of phosphorylated myosin light chain, a major regulatory component in smooth muscle contraction, was significantly decreased in aorta of ApoE/LDLR$^{-/-}$ mice.

We postulate that the reduced endoglin expression is related to vascular dysfunction in aorta prior formation of atherosclerotic lesion, suggesting an important role of endoglin in cholesterol-induced endothelial/vascular dysfunction.

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ENDOGLIN IS INVOLVED IN CHOLESTEROL-INDUCED ENDOTHELIAL DYSFUNCTION AND MONOCYTE ADHESION IN HUMAN AORTIC ENDOTHELIAL CELLS

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Endoglin (CD105, TGF-βRIII receptor), acts as auxiliary partner protein in TGF-β receptor complex being essential for proper function of endothelium, but might also participate in inflammatory infiltration of leukocytes. We hypothesized that membrane endoglin participates in 7-ketocholesterol (7K) induced development of endothelial dysfunction.

HAECs were exposed to 7K (5, 10 μg/mL) for 12 hours. Gene expression (endoglin, KLF6, RELA (NF-κB p65), NR1H3 (LXR), and ICAM-1) was evaluated using qRT-PCR. Protein levels of endoglin, ICAM-1 and selectins were evaluated by immunofluorescence flow cytometry analysis and intracellular localization of RELA, eNOS, and p-eNOS was evaluated using confocal fluorescent microscopy.

Gene expression and protein levels of endoglin, eNOS, p-eNOS and cell adhesion molecules (ICAM-1, E/P-selectin) were significantly increased after 12 hours premedication with 7K compared to non-treated cells. KLF6, RELA and NR1H3 transcription genes regulating endoglin expression were increased after 12 hours premedication with 7K in dose 10 μg/mL. Inhibition of either results in inhibition of 7K induced increase of endoglin expression. 7K was able to increase adhesion and transmigration of THP-1 monocytes, through endothelial cells monolayer (HAECs). Silencing of endoglin in HAECs by siENG inhibited adhesion and transmigration of THP-1 monocytes through endothelial monolayer.

In this study, we demonstrated that 7K is able to induce inflammation and increase endoglin expression in endothelial cells via KLF6, RELA and NR1H3 transcription genes. We also demonstrated that 7K induced adhesion and transmigration of monocytes trough endothelial monolayer depends on the expression of endoglin suggesting that endoglin might play crucial role in cholesterol (oxysterol) induced endothelial dysfunction.

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THE ANTIPLATELET EFFECT OF 4-METHYLCATECHOL WAS CONFIRMED ON *IN VIVO* (*EX OVO*) HEN'S EGG TEST ON THE CHICK AREA VASCULOSA

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The flavonoid metabolites, produced by human colon bacteria, are derivatives of benzoic, propionic and acetic acids or hydroxybenzenes, *e.g.* 4-methylcatechol (4-MC), pyrogallol, phloroglucinol or resorcinol. These metabolites reach in plasma higher concentrations than parent flavonoids[1] and could be responsible for their observed positive effects.

The antiplatelet research of selected metabolites showed that all four hydroxybenzene derivatives probably have a biologically relevant effect; moreover 4-MC was even 10 times more active on arachidonic acid induced aggregation than clinically used acetylsalicylic acid (ASA). The mechanisms of action of this compound include inhibition of platelet serotonin release and partly thromboxane A_2 synthase inhibition.

To confirm this positive antiplatelet effect in a more biological system, a shell-less hen's egg test on the chick area vasculosa was used. Here ASA pretreatment decreased the lethality after induction of aggregation from 46% to 7% after the first hour and from 60% to 27% after 24 hours. 4-MC afforded even more protection; there was no mortality after the first hour and only 7% lethality after 24 hours, which corresponded with the negative control. Moreover ASA and 4-MC showed significant improvement of the size and intensity of thrombosis in comparison to negative control.

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DEVELOPMENT OF NOVEL DISINFECTANTS AGAINST PATHOGENS OCCURING IN THE HOSPITAL ENVIRONMENT

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In the project, we have prepared 64 novel compounds with a different length of alkyl chain (C8, C10, C12, C14 and C16) based on quaternary ammonium salts. The newly synthesized compounds were tested against aerobic bacteria (Gram-positive and Gram-negative), anaerobic bacterial strain *Clostridium difficile*, yeasts and filamentous fungi, Varicella zoster virus (VZV) and green algae *Pseudokirchneriella subcapitata*. The project is designed for development of various (3–6) mixtures with strong disinfecting properties and broad spectrum of efficacy by combining individual agents with more specific efficacy. The most promising agents against aerobic bacteria were 31-C16, 32-C16, 27-C14, 20-C16 and 18-C16. Against *Clostridium difficile*, the highest efficacy showed compounds 33-C12 and 32-C14. The highest efficacy against yeasts was observed for 32-C16, 18-C16, 16-C16 and 32-C14, against filamentous fungi then for 18-C16 and 16-C16. Although, the efficacy on viruses in quaternary ammonium salts is relatively rare, 32-C14 achieved $\log_{10}$ reduction factor of 5 in the virus titre after 5-minute exposure. A little less effective was 28-C8 and 33-C14. Supplemental efficacy against green algae was very high for 32-C16, 27-C14, 18-C16, 16-C16 and 27-C12. Based on *in vitro* testing, the most effective substances were selected and four mixtures have been formulated. The mixture 4 showed the results at least comparable to AJATIN.

The study was supported by the Ministry of Health of the Czech Republic (Projects NV18-09-00181 and 15-31847A) and by the project of Specific Academic Research (SV/FVZ 201 607).

EFFECT OF 4-METHYLCATECHOL ON THE RELEASE OF SEROTONIN AND THROMBOXANE IN HUMAN PLATELETS

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Platelets are crucial for blood coagulation, which preserves the integrity of the cardiovascular system. However, dysregulation of their function may lead to many diseases, in

particular stroke and acute myocardial infarction, which are the most prevalent causes of mortality in developed countries.[1] Current antiplatelet therapy has many disadvantages, including lack of efficiency, side effects, or cost.[2,3] Flavonoids, and in particular their metabolites, seem to possess antiplatelet potential. The aim of the study was to continue in the assessment of the mechanism of action of 4-methylcatechol (4-MC), a known colonic metabolite of flavonoids. Both serotonin (5-HT) and thromboxane A_2 (TXA_2) are important factors involved in formation of a blood clot. The effect of 4-MC on the release of these two agents from platelets was determined. The amount of released substances after addition of an aggregation inducer was measured by corresponding ELISA assay kits. 4-MC affected the release of 5-HT from platelets, but at micromolar concentrations had no effect on the release of TXA_2 or its concentration inside the platelets. 4-MC does not inhibit TXA_2 release from platelets, hence the major antiplatelet mechanism of action of 4-MC is currently unknown and further investigation is needed.

The study was supported by Research programme Development and Study of Drugs (Progres Q42) and from the project of Specific Academic Research (SVV 260 414).

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BIOCHANIN A AND GLYCITEIN INDUCE AN ENDOTHELIUM-INDEPENDENT DILATION OF RAT AORTA AND PORCINE CORONARY ARTERY

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Flavonoid intake seems to be inversely associated with mortality on coronary heart disease. In particular, the consumption of isoflavonoids is increasing in general population due to the use of food supplements and a variety of soy products, legumes and nuts.

In the framework of this study, fifteen isoflavonoids were screened *ex vivo* for their vasorelaxant properties in norepinephrine pre-contracted rat aorta. Among them, biochanin A and glycitein were selected for additional experiments on isolated porcine coronary arteries and mechanistic studies. Both of them exhibited an endothelium independent relaxation of the rat aortic and porcine coronary vasculature *ex vivo*. For further investigation of the possible mechanism of action, the coronary arteries were incubated in the presence of the tested isoflavonoid (concentrations ranging from 3 to 30 μM), and then cumulatively contracted by KCl, $CaCl_2$, serotonin or U46619. Vasoconstriction produced by one or more of these stimuli was inhibited (at least partially) by both tested compounds in a dose-dependent manner, indicating a direct vasorelaxant effect on the

arterial smooth muscles. Biochanin A was found to be more potent, achieving a lower EC_{50} and blocking the effect of all four constrictors.

These results suggest a positive impact of the isoflavonoids on some cardiovascular pathologies, that needs to be further confirmed by *in vivo* studies.

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COPPER CHELATORS AND THEIR ABILITY TO BIND ZINC

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Our knowledge about the role of zinc in the human organism has been constantly expanding. It is well know that zinc has many physiological roles and is essential for many enzymes and transcription factors. Its deficiency manifests by many symptoms ranging from immune dysfunction, growth retardation to skin disorders and is mostly caused by insufficient zinc intake in the diet or derangement in GIT absorption. Less is known, that zinc deficiency can follow long-life treatment by metal chelators, which are mostly non-selective.

Hence, the aim of this work was to study possible ability of clinically and experimentally used copper chelators to chelate zinc ions by using a competitive spectrophotometric method based on dithizone. The tested compounds included D-penicillamine, trientine and ammonium tetrathiomolybdate (ATTM) that have been clinically used or tested in the treatment of Wilson's disease, and experimentally tested series of four 8-quinolinoles (8-quinolinol, 5-chloro-7-iodo-8-quinolinol, 5,7-dichloro-8-quinolinol (chloroxine) and 5-nitro-8-quinolinol (nitroxoline). Various physiologically relevant pH levels ranging from 4.5 to 7.5 were simulated.

The ability to bind zinc was observed in all of the tested compounds. The most potent clinically used chelator was trientine with approximately 65% zinc binding activity in the molar ratio 1:1 at pH 7.5. However, experimentally used 8-quinolinols showed proportional or even higher binding capacity at lower pH levels. Especially nitroxoline was a very potent zinc chelator at all pH levels. Surprisingly all of the tested compounds showed higher affinity for Zn ion in comparison with ability of D-penicillamine to bind Cu ions.

In conclusion we can assume that clinically used copper-chelators as well as ATTM and 8-quinolinols are also relatively potent zinc chelators and hence their longer administration can possibly result in adverse effects associated with zinc deficiency.

The study was supported from the project of Specific Academic Research (SVV 260 414).

DEVELOPMENT AND IMPLEMENTATION OF INTERVENTIONS TO PROMOTE MEDICATION ADHERENCE IN KIDNEY TRANSPLANT PATIENTS

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Adherence to complex therapeutic regimen is essential during the whole posttransplant period. Nevertheless, it decreases over time and changes according to patient's beliefs and regular care at the clinic.[1] The aim is to introduce the design of our study developing and implementing individual multicomponent interventions for supporting medication adherence in patients after kidney transplantation and evaluating their impact on behavioral and transplant outcomes. This single-centre, prospective, interventional study will be undertaken in the University Hospital Hradec Králové in 2019–2020, where all adults on basal immunosuppression (IS) will be approached to mitigate selection bias. Patients at risk of non-adherence to IS will be identified using laboratory (medication level variability index) as well as self-report (BAASIS® questionnaire) measurement of medication adherence, combined with reports by family member or caregivers. These patients will be invited to structured interview with a pharmacist and will receive tailored set of interventions based on their individual barriers in knowledge, medication taking routine or attitudes. Interventions will be developed based on previous research results as well as the needs of the health care facility reflecting all main causes of non-adherence to IS. Study will include the baseline visit and the follow-up after 6 and 12 months. Effectiveness of interventions will be tested on both behavioral (*e.g.* adherence to IS and self-management, beliefs and knowledge about the treatment) and transplant outcomes (*e.g.* progression of graft dysfunction, rejection episodes, graft loss). If interventions proof to be effective, they can be implemented into standard posttransplant care and serve as the template for other clinics.

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NUTRITIONAL INTAKE OF ENERGY AND SUBSTRATES, THEIR CHANGES IN PREGNANT WOMEN DURING THE LAST DECADE

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Malnutrition of the maternal organism during intrauterine fetal development may result in disease in the next life of an individual. The aim of the study was to evaluate the changes in the intake of nutritional energy and substrates (PNES) in Czech pregnant women over the last 10 years and determine the accuracy of the predictive equations for PNES.[1] Thirthy-five Czech pregnant women 29 ± 2.79 years old were attended in the pilot study. PNES in individual trimesters of pregnancy was obtained in weekly nutritional records, then evaluated by the computer program NutriDan and compared with values from the predictive equations for PNES.[1] In 10 years, PNES (according to the predictive equations) was increased in protein in the 1st trimester of pregnancy by 9.58% ($p = 0.02$) and decreased in carbohydrate intakes in all pregnancy periods by 10.06% ($p = 0.04$); by 14.60%. ($p = 0.0002$); by 12.71% ($p = 0.0003$). Differences in PNES on weekdays does not change despite the expectation. The predictive equations for PNES can be used during pregnancy even after 10 years, except for protein intake in the 1st trimester and carbohydrates throughout pregnancy.

The study was supported by the Grant Agency of Charles University (Project. No. 1306218), from the project of Specific Academic Research (SVV 260 417), by Ministry of Health, Czech Republic – conceptual development of research organization (UHHK, 00179906) and by Research programme Development and Study of Drugs (Progres Q42).

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NO FAULT VACCINE COMPENSATION PROGRAM OVERDUE IN CZECH REPUBLIC – SERIOUS THREAT FOR THE CZECH MEDICAL SYSTEM IN A PROSPECT OF THE NEW CIVIL CODE

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On the first of January 2014 the New Civil Code (Law 89/2012 Coll., Civil Code) has come into the force in the Czech Republic. It has changed many aspects of everyday

life, including the problem of liability following adverse events attributed to the no-fault administration of compulsory vaccines. Until then the proprietor of health service facility was held strictly liable, for any damages that aroused from the application of vaccine even if it was caused by the nature of the applied vaccine (§421a of Law 40/1964 Coll., Civil Code). After the new legislation has come into force this is no longer true. Since 2014, the Health service provider should be held liable only, if there is his negligence proved. Thus question comes into mind – who is responsible for such adverse effect now.

The position of the Czech government is unclear but it steers towards the manufacturers' liability. We think that this is the moral hazard. In our opinion, the manufacturer should not be held liable, because he openly states in the SPC that such effects, even rare, could occur. It is the government that demands mandatory vaccination with penalization to offender for those that not conform, so it follows that government should be responsible for any adverse effects caused by such vaccination. Majority of countries united in the OECD have adopted some form of compensation for individuals harmed by compulsory vaccines administration. In this presentation I would try to evaluate such programs with emphasis on the states of comparable size, similar legal systems and comparable level of public health systems and make proposition for such a program tailored for needs of the Czech Health care system.

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SPONTANEOUS ADVERSE DRUG REACTIONS REPORTING TO ORAL ANTICOAGULANTS IN THE CZECH REPUBLIC

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Shorter clinical use of direct oral anticoagulants (DOACs) leads to need for yet unknown risks monitoring. The aim of the study was the analysis of spontaneous adverse drug reactions (ADRs) reports to oral anticoagulants (OACs) in the Czech Republic.

Retrospective analysis of spontaneous reports of suspected ADRs to OACs from healthcare professionals and patients was conducted. Reports to warfarin, dabigatran, rivaroxaban and apixaban between 01/2005 and 11/2017 were identified in the State Institute for Drug Control (SÚKL) database and quantified as anonymized data in MS Excel 2019. The reports were evaluated by ADR character, seriousness, consequence, and patient's characteristics. The incidence of ADRs was calculated per exposure units expressed as million defined daily doses (mDDD). Drug utilization data come from the SÚKL database of quarterly reported drug supplies from distributors to healthcare facilities. Descriptive statistics and disproportionality analysis (DA), using reporting odds ratio (ROR) and 99% confidence interval (CI) was performed.

SÚKL received 297 reports to OACs with 672 ADRs. DOACs were related to 65% reports, resp. 64% ADRs. The most frequent ADRs were hemorrhagic and thromboembolic events, gastrointestinal and skin disorders. The DA showed that number of DOACs' ADRs exceeded warfarin (ROR = 10.77, 99% CI = 8.70–13.32) and number of ADRs of dabigatran exceeded rivaroxaban (ROR = 3.90, 99% CI = 2.88–5.29). In 96% of reports serious ADRs were noticed, of which 21 were fatal. In context with higher warfarin utilization, the ratio of ADR reports and mDDD was low; conversely to DOACs. In summary, the number of reports seems to be low, but most ADRs were serious. Higher reporting to DOAC could be a result of shorter time on the market, compared to warfarin long-term use and reduced susceptibility to the ADRs.[1]

The study was supported from the project of Specific Academic Research (SVV 260 417).

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FACTORS INFLUENCING ANTIBIOTIC PROPHYLAXIS FOR SURGICAL SITE INFECTION PREVENTION

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Antibiotic prophylaxis (AP) plays an important role in the reduction of surgical site infection (SSI). The aim of the presented study is to verify the use of the Risk Index as one of the tools for the individualization of the patient's AP in clinical practice. In February 2018, we conducted a cross-sectional study, in which we identified total length of AP as the main problematic area. Special tool – the Risk Index could be used as the basis for the indication and the total length of AP among the common principles (dirty-infected wound, the presence of foreign material, pharmacokinetics of antibiotic *etc*.). Before the surgical procedure, we could calculate the ACS (American College of Surgeons) Risk Index, which comprises risk factors (RFs), physical status and the type of surgery. ACS Risk Index estimates the chance of an unfavorable outcome such as SSI or the postoperative complications. After the surgical procedure, the NHSN (National Healthcare Safety Network) Risk Index could be calculated according to the ASA score (classification system that assesses the physical status of patient before surgery into 5 classes), the duration of operation and the wound classification. In 2019, we plan to perform cross-sectional study with an approximate number of 300 patients. In addition to the systematic literature review, we will collect patient and surgical procedure data before, during and after surgery. An AP individualization will be performed. Usability of ACS Risk Index and NHSN Risk Index

will be evaluated with the postoperative complication analysis. These will be monitored 30 days after the surgery if no foreign material was used or 1 year if patient obtains foreign material during the surgical procedure. According to these results, the interventions will be arranged to optimize and individualize use of AP.

The study was supported from the project of Specific Academic Research (SVV 260 417).

ANALYSIS AND MANAGEMENT OF NURSES' MEDICATION ERRORS DURING DRUG ADMINISTRATION

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During hospitalization, there is a frequent occurrence of various mistakes caused both by healthcare professionals and by patients. A major part of these comprises medication errors during inappropriate drug handling. The key role is played by the possibility of preventing these errors. The aim of the study is to analyze medication errors by direct observation of the nurses during drug preparation and administration.

The observation was conducted prospectively during 12 days in an inpatient rehabilitation facility. Three trained pharmacists monitored morning, noon and evening drug administration processes on seven wards. All errors were documented in real time, according to the predefined forms. The form always contained the name, strength, application form and dosage of all drugs prescribed to one patient. Each form was created for each patient individually, based on his or her regular medication. Within the form, the observed errors were further classified into 34 categories, including *e.g.* storage and labelling of the drug, administration route or drug strength. The obtained data were digitized and analyzed.

Nurses (n = 27) administered in total 4662 individual doses, on average 8.18 (SD ± 5.19) doses per patient day (n = 570). Of these doses, 92.8% were administered orally, 5.1% subcutaneously, 1.6% topically, and 0.5% other. Severe misconducts (drug name, or drug strength confusion, and drug omission) occurred in 21 (0.45%) cases, on average 0.037 error per patient day. Patients hospitalized for more than 20 days are more than 50% likely to experience severe nurses' medication error.

The analysis of the observed errors has revealed that nurses commit severe and less severe mistakes, which ultimately can lead to patient harm. It is therefore necessary to focus on their minimization.

The study was supported from the project of Specific Academic Research (SVV 260 417).

ANALYSIS OF PHARMACOTHERAPY AS ONE OF THE MAIN FALL-RELATED RISK FACTOR – A 12-MONTH PROSPECTIVE STUDY IN HOSPITALS OF SOUTH BOHEMIA REGION

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Pharmacotherapy belongs among one of the modifiable and predictive risk factors of falls. The aim of the study was to analyze the effect of pharmacotherapy and drug-related factors on falls at hospitalized patients with fall during 2017 at 16 hospital wards of four hospitals in South Bohemia region.

The results of this prospective study were collected through online instrument containing data about patients with falls. The data obtained from patient's medical records (*e.g.* drug and personal anamnesis, selected laboratory results) were completed with other information (*e.g.* circumstances of fall, internal and external risk factors). The data analysis was primarily focused on drugs, diagnoses and associated risk factors increasing risk of falls. Potential and individual risks were determined for each patient who experienced fall. The potential risk represented all drugs that showed an increased risk of falls described in current literature or according to its mechanism of action. If a clinical pharmacist could not exclude the drug influence on fall probability, the drug was reported as with individual risk. For data analysis, the descriptive and analytical methods were used. A p-value < 0.05 was considered statistically significant.

280 falls (51.1% women; mean age 77 ± 12), mean 8.8 drugs, and 4.1 drugs with potential or 1.8 individual risk per fall were identified. Drugs affecting the cardiovascular or the central nervous system demonstrated the highest potential risk (> 60%). Use of potential risk drugs were positively associated with increasing age ($p = 0.007$).

The application of pharmacotherapy-related predictive risk factors together with individual approach to the patient can be an effective preventive strategy in drug-induced falls.

The study was supported from the project of Specific Academic Research (SVV 260 417) and by the Ministry of Health of the Czech Republic (Project No. 16-33463A).

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SECTION OF BIOLOGICAL SCIENCES

AROMATIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AND THEIR BIOLOGICAL ACTIVITIES

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The plants of the Amaryllidaceae family are known to contain a specific type of compounds, namely the Amaryllidaceae alkaloids (AA). Among the many AA, galanthamine has been given a great amount of attention due to the fact that it possesses potent acetylcholinesterase inhibition activity, and is used worldwide for the treatment of Alzheimer's disease. Some of AA have shown remarkable cytotoxic and antiproliferative activity against diverse types of cancer cells.

One of the most interesting compounds is haemanthamine (HA), β-crinine-type of AA, which displays significant *in vitro* cytotoxic activity against several different types of cancer cell lines (*e.g.* MOLT-4, Jurkat, HeLa, MCF-7, SK-BR-3, A549, Caco-2, HT-29).[1,2]

HA was isolated from the bulbs of *Narcissus pseudonarcissus* cv. Dutch Master in large amounts as a start material for the preparation of its derivatives. Some of the previous prepared derivatives have shown promising cholinesterases inhibitory potencies and cytotoxic activities against wide spectrum of cancer cell lines. Based on these results, we decided to prepare further aromatic semi-synthetic analogues of HA with emphasis on optimizing the structure vs biological activity. Prepared haemanthamine derivatives were tested for their inhibition potency of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The cytotoxic activity against selected panel of cancer and healthy cells has been also studied. Derivative LC-90 is the most promissing haemanthamine analogue from the compounds prepared in this study.

The study was supported from the project of Specific Academic Research (SVV UK 260 412).

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INTERACTIONS OF TAMARIXETIN AND ISORHAMNETIN WITH COPPER

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Copper is an essential trace element involved in many vital metabolic processes. However, unbound copper ions cause damage to various biomolecules and lead to pathological conditions. Disorder of copper homeostasis can be treated with copper chelators.[1]

Tamarixetin and isorhamnetin are the main *O*-methylated metabolites of quercetin from the group of flavonoids. Flavonoids belong to plant secondary metabolites and have a positive impact on human health. Chelation of transient metal ions is one of their mechanisms of action.[1] Flavonoids may also have negative prooxidant effects, which have been related with their copper reducing activities and formation of free radicals through Fenton reaction.[2]

In this *in vitro* study, tamarixetin and isorhamnetin were tested for their interactions with both copper oxidation states at four (patho)physiologically relevant pH conditions (4.5, 5.5, 6.8 and 7.5) by two spectrophotometric methods. Apparently, the 2,3-double bond and the 3-hydroxy-4-keto group in flavonols were efficient for stable copper chelation. In conclusion, compounds showed very good ability to chelate and reduce copper ions, but in acidic condition the chelation effect was slightly lower.

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CENTAUREA CYANUS L. ALKALOIDS AND THEIR NEUROPROTECTIVE ACTIVITY

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Alzheimer's disease (AD) is the most common neurodegenerative disorder causing dementia. The current portfolio of drugs in use is very limited: there are two therapeutic approaches according to Evidence Based Medicine – cholinesterases inhibitors and *N*-methyl-D-aspartate blockers. In our screening of natural inhibitors of human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BChE), a summary alkaloidal extract from *Centaurea cyanus* L. seeds demonstrated promising selective inhibitory activity on *h*BChE with an IC_{50} value of 22.62 ± 3.62 μg/mL, but against *h*AChE was considered inactive (IC_{50} value > 200 μg/mL). The extract (4.67 g) was fractionated by column chromatography on neutral alumina and nine fractions (A–I) were obtained. Preparative TLC of fraction A (698 mg) on silica gel and C18-reversed phase silica gel led to the isolation of pure compound AD-1 (36 mg), which showed a positive reaction with Dragendorff's reagent. Based on LC-MS, HRMS, ^{1}H and ^{13}C NMR spectra, and optical rotation its structure was elucidated. The isolation of AD-1 is reported for the first time. The compound was screened for the ability to inhibit *h*AChE and *h*BChE and it was found that it is inactive (IC_{50} values > 100 μM). Furthermore, AD-1 will be tested on the inhibition of selected enzymes such as glycogen synthase kinase-3β, β-secretase, and prolyl oligopeptidase, which play an important role in pathophysiology of AD and represent promising targets for the treatment of this disorder.

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AROMATIC ESTERS OF AMARYLLIDACEAE ALKALOID GALANTHINE AS POTENTIAL DRUGS FOR TREATING ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an irreversible brain disorder that's progressive and slowly destroys the memory and cognitive skills. AD is the leading cause of dementia in adults and is ranked the third cause of death after cancer and heart disease. Several medications have been approved for treating the symptoms of AD and those that slow down the

progression of the disease. One of the available drugs on the market for treating AD is galanthamine sold commercially as Razadyne® or Reminyl® (galanthamine hydrobromide). Galanthamine is an alkaloid from the Amaryllidaceae plant family and other Amaryllidaceae alkaloids (AA) have been noted for their therapeutic activity. For instance, lycorine alkaloid derivatives showed dual cholinesterases inhibition activity and anticancer activity.

Galanthine is an AA of lycorine structural type and is distributed in *Narcissus* species. As a continuation of our study aimed at finding potential drugs for treating AD from AA a series of galanthine esters were synthesized. After synthesis the galanthine derivatives were identified by 1D and 2D NMR, GC-MS methods. The compounds are awaiting screening for their biological activity with mechanisms of action connected with he therapy of AD, which include the inhibition of cholinesterases, glycogen synthase kinase 3 (GSK-3) and prolyl oligopeptidase (POP) enzymes.

The study was supported from the project of Specific Academic Research (SVV UK 260 412).

GALLERIA MELLONELLA AS THE *IN VIVO* MODEL FOR THE STUDY OF CANDIDATE ANTIMICROBIAL DRUG vs. PATHOGEN INTERACTION

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This study focuses on the establishment of the *in vivo* invertebrate model *Galleria mellonella* primarily for the study of *in vivo* efficacy and toxicity of newly synthetized compounds with proved antiinfective potential. Recently, this model has increasingly been used as a surrogate to screen novel antimicrobial drug candidate especially due to noticeable advantages such as cost, ease of inoculation, performing of many replicates for production of statistically valid results and no ethical constrains.

The main part of our study is devoted to optimization of conditions for laboratory insect rearing, determination of lethal infectious doses after infection of *Galleria mellonella* larvae by methicillin resistant *Staphylococcus aureus*, implementation of methodical approach for assessing the relative acute toxicity of candidate antiinfective compounds, and establishment of laboratory manuals for routine histopathological studies.

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THE EFFECT OF PYRAZINE DERIVATES ON SECONDARY METABOLITES PRODUCTION IN *FAGOPYRUM ESCULENTUM* cv. BAMBY *IN VITRO* CULTURES

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The aim of this work was to investigate the effect of pyrazine derivative, 1-octyl-3-(pyrazin-2-yl) urea, as an abiotic elicitor on the production of flavonoid rutin in *in vitro* cultures of *Fagopyrum esculentum* Moench., cv. Bamby.

Suspension and callus cultures were cultivated on Murashige and Skoog nutrient medium (MS) with the addition of 2,4-dichlorophenoxyacetic acid (2,4-D) as growth regulator at a concentration of 1 mg/l. The ethanolic elicitor solution was added to the cultures at three concentrations: c_1 (100.0 mg/100 ml), c_2 (10.0 mg/100 ml) and c_3 (1.0 mg/100 ml). The elicitor was monitored at six time intervals: 6, 12, 24, 48, 72 and 168 hours. To control samples 1 ml of ethanol 96% was added instead of elicitor solution and samples were collected after 24 and 168 hours. Samples were taken at given time intervals and dried. Subsequently, the rutin content was monitored by HPLC. The rutin release into the nutrient medium was also tested.

During the experiment on the callus cultures no statistically significant increase in rutin production after elicitor treatment was observed. But elicitor increased rutin production in suspension cultures after treatment of all tested concentrations. The maximum rutin content (0.11 mg/g DW) was detected in the suspension culture after 12 hours of elicitor application at a concentration c_2 (10.0 mg/100 ml), related to a 24-hour control (0.06 mg/g DW). At 12 hours sampling, increased rutin content was always detected. A higher rutin production was also observed after 6, 48 and 72 hours of elicitor treatment at the concentration c_2. The rutin release into the nutrient medium was not found.

Elicitor-1-octyl-3-(pyrazin-2-yl)urea had the positive effect on rutin production only in suspension cultures of *Fagopyrum esculentum* Moench., cv. Bamby.

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OPTIMIZATION OF THE METHOD LEADING TO ROBUST STAPHYLOCOCCAL BIOFILM FORMATION *IN VITRO*

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Microbial biofilms are the cause of many chronic infections. They are known to increase antimicrobial resistance and improve microbial survival. In this capacity, infections

caused by biofilm-forming microbes increase the difficulties for the medical treatment resulting in higher morbidity and mortality. Staphylococci belong to the clinically important group of microbes causing so called biofilm-associated infections.[1]

The aim of this study included optimization of cultivation conditions leading to robust staphylococcal biofilm formation *in vitro* for the purpose of subsequent evaluation of antibiofilm activity of newly synthesized compounds. The analogous model corresponding to commercially available testing system, Calgary Biofilm Device[2,3], was applied. This approach included biofilm formation in 96-well panels and pegs incorporated into lids as well. For the study, two bacterial strains with proved biofilm phenotype and one without defined biofilm phenotype were used for the purpose of finding the most advisable *in vitro* conditions. The characterization of biofilm phenotype of 27 clinical isolates from the genus *Staphylococcus* was included in our study as well.

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TOPOISOMERASE II CATALYTIC INHIBITORS AND POISONS AND THEIR INFLUENCE ON DNA DAMAGE CAUSED BY ANTHRACYCLINES IN CANCER CELLS

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Anthracycline antibiotics (ANT) rank among the most widely used and potent anticancer drugs. They are commonly employed in the treatment regimens of various blood malignancies as well as solid tumours. However, their therapeutic value is lowered by several side effects including irreversible cardiotoxicity. Dexrazoxane (DEX) is the only clinically approved cardioprotective agent for treatment and prevention of the ANT-induced cardiotoxicity. In previous studies, it was suggested that DEX protects cardiomyocytes via its topoisomerase II (TOP2) catalytic inhibitory activity.[1] Furthermore, other TOP2 catalytic inhibitors were analysed in terms of their prospective cardioprotective properties.[2] But any possible cardioprotective agent would be therapeutically unusable if it protected the cancer cells from the DNA damage as well. This study is aimed at the differences of the anthracycline effects on cancer cells in respect to the DNA damage as the principal mechanism and the effect of selected TOP2 inhibitors on the HL-60 leukemic cell line.

DNA damage assessment was performed using two methods – Comet Assay (CA) and investigation of phosphorylated variant of histone H2AX. Each of them analyses the DNA

damage from different perspective. Unlike direct double strand breaks evaluation by CA, phosphorylation of histone H2AX indicates the initiation of DNA repair pathway and therefore it has been proved as a sensitive target for DNA double-stranded breaks assessment.[3]

The results show that in cancer cells, short-term daunorubicin treatment increases both phosphorylated form of H2AX and Comet signal. Other TOP2 inhibitors had different outcomes with several promising agents in a way that they do not interfere with ANT caused DNA damage in cancer cells. But there is a crucial need for studies using non-malignant cells (*e.g.* neonatal ventricular cardiomyocytes) to see if these compounds have a real impact on anthracycline cardiotoxicity.

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DEREGULATION OF microRNA IN SINONASAL CARCINOMA

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Head and neck cancer (including carcinoma of the sinonasal tract) is one of the most common type of malignancy in the world. The tumours usually arise from the epithelial cells of upper respiratory and digestive tract such as the oral and nasal cavity, sinuses and glands of the pharynx and larynx.[1,2] Stage of the tumour in time of diagnosis is a very important factor in treatment choice and efficiency. Patients diagnosed in early stages of the disease are often treated by combination of surgical intervention followed by radiotherapy; in case of advanced tumour chemotherapy with cytostatic agents is used.[3] MicroRNAs (miRNAs) are short (approx. 22–23 nt) non-coding RNA molecules participating in the regulation of gene expression. Their primary role lies in the inhibition of translation. In this study, we analysed relative expression of selected miRNAs in sinonasal carcinomas compared to non-malignant sinonasal control tissue. Relative expression of let-7a-5p, miR-146-5p and miR-196a-5p was analysed in set of sinonasal carcinoma samples fixed in the formalin, embedded in paraffin. The sample group consisted of 71 patients, of which 45 were males and 26 females. Relative expression was determined using the real-time PCR approach with specific TaqMan™ Advanced miRNA Assays and the data was evaluated using $2^{-\Delta\Delta Ct}$ method. Our results show statis-

tically significant upregulation of let-7a-5p and miR-196-5p in sinonasal cancer samples and some significant correlations with recorded follow-up data. The results of the study support the idea of potential future use of miRNAs as prognostic biomarkers.

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MODULATORY EFFECTS OF BILBERRY EXTRACT ON THE ACTIVITIES AND EXPRESSION OF SELECTED BIOTRANSFORMATION ENZYMES IN RAT

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Bilberry (*Vaccinium myrtillus* L.) is one of the richest sources of anthocyanins. These bioactive polyphenolic secondary metabolites give bilberry not only the characteristic blue/black colour, but they are also responsible for many health benefits. Therefore, bilberry has potential use in the treatment and prevention of conditions associated with visual disorders, inflammation, dyslipidemia, hyperglycemia or in some other disorders associated with increased oxidative stress.[1] The aim of this study was to evaluate the effect of bilberry extract, which was administrated for 29 and 58 days, on activity and expression of selected biotransformation enzymes in rat liver. Enzymatic activity and expression of mRNA was determined in the cytosols and microsomes of liver tissue from control and influenced rats. In this study, spectrophotometric or HPLC method was used to measure the activities of aldehyde reductase (AKR1A1), hydroxysteroid dehydrogenase (AKR1C), glutathione-*S*-transferase (GST), NAD(P)H quinone dehydrogenase (NQO1), carbonyl reductase (CBR), catechol-*O*-methyltransferase (COMT), UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) in subcellular fractions of rat liver tissue. Real-time quantitative PCR was used to determine the transcriptional levels of mRNA of NQO1, CBR, SULT and UGT. The results of measurement were statistically compared with the Student's paired t-test. The results have shown that bilberry extract does not have a significant effect on the activity and expression of selected biotransformation enzymes in rat liver and therefore the interactions of bilberries with co-administered drugs, metabolized via studied enzymes, are not expected.

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PISTACIA LENTISCUS ESSENTIAL OIL: CYTOTOXICITY AND ANTI-INFLAMMATORY ACTIVITY

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The aim of our work, which has been initiated in cooperation with University of Sassari (Italy), is to find out more details about biological activity of essential oil from *Pistacia lentiscus*. We want to contribute to the decision, if this essential oil can be used as a part of oral health products. The essential oil was prepared from leaves of wild occurring plants growing in North Sardinia. The cytotoxicity and anti-inflammatory activity of not only the pure essential oil but also of the lecithin nanoemulsion with *P. lentiscus* oil, and the two most abundant terpenes in the essential oil, (−)-α-pinene and (−)-terpinen-4-ol, were tested. The cytotoxicity was measured by the colorimetric assay using tetrazolium salt WST-1 on four oral cell lines (gingival fibroblasts, periodontal ligament fibroblasts, dysplastic oral keratinocytes, and primary gingival keratinocytes). The viability of human oral cells was not diminished by essential oil up to the concentration of 100 μg/ml medium. The anti-inflammatory activity was measured in a cell-free enzymatic *in vitro* assays using human recombinant cyclooxygenase 1 and 2 (COX-1, COX-2) and soybean 5-lipoxygenase (5-LOX). The activity of COX-1 and COX-2 has been determined by the colorimetric assay using ELISA kit. Tested essential oil showed the capacity to inhibit all enzymes, COX-1, COX-2, and 5-LOX. The content of the *Pistacia lentiscus* essential oil in oral health products could have some benefits for users as it is not cytotoxic for oral cells and shows an anti-inflammatory activity (the activities of COX-1, the constitutive form, COX-2, the concentration of which increases during the inflammation, and 5-LOX, that supports the inflammation too, were lowered). Based on our results, the essential oil can be a safety and beneficial part of oral health products.

The study was supported from the project of Specific Academic Research (SVV 260 416).

THE USE OF RNA INTERFERENCE FOR THE MODIFICATION OF DNA TOPOISOMERASE II LEVELS IN CANCER CELLS AND ITS INFLUENCE ON THE ANTINEOPLASTIC EFFECT OF ANTHRACYCLINES

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Topoisomerase II (TOP II) is an enzyme that alters the topological state of the DNA double helix during physiological processes through the formation of transient DNA double strand breaks.[1] Two TOP II isoforms are known: TOP IIα is essential for proper separation of chromosomes in mitotic cells, whereas TOP IIβ is primarily associated with gene transcription.[2] Anthracycline antibiotics (ANT) belong to the group of topoisomerase poisons that stabilize the complex between TOP II and DNA. This prevents the religation of the DNA double strand breaks and thus causes irreversible DNA damage leading to programmed cell death. Although ANT are frequently administered in various antineoplastic protocols (hemato-oncological malignancies, hormone-dependent tumors),[3] the therapy still possess a high risk of irreversible cardiotoxicity. The mechanism of cardiotoxicity remains unraveled. However, it has been previously discussed that TOP IIβ inhibition could play there a key role.[4]

The practical aim of this work was to optimize methodology of RNA interference using siRNA molecules targeting the TOP IIβ isoform in human tumor suspension cell line HL-60. The transfection was performed by the electroporation. Evaluation of TOP IIβ was accomplished both on mRNA level by RT-qPCR and protein level via immunoblotting. Furthermore, antiproliferative effect of daunorubicin as a model ANT was investigated in TOP IIβ depleted cells.

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CAN FENBENDAZOLE ENTER THE FOOD CHAIN?

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Drugs are potentially dangerous environmental contaminants, because they are designed to have biological effects at low concentrations. Especially anthelmintics, veterinary pharmaceuticals used in large amounts in modern husbandry for treatment and prevention of diseases in animals, enter the environment directly via animals excrements left in pastures, used in field fertilization or following direct application in aquaculture.[1] Depending on the increased production of soybean in agriculture and use of manure droppings, knowledge of biotransformation and uptake of anthelmintic drugs (*e.g.* fenbendazole) in soybean is very important.[2,3] Plant samples stressed with 10 μM fenbendazole were homogenized and subjected to liquid–liquid extraction. The samples were analysed using UHPLC/MS (QqQ) in positive-ion mode. The analysis of roots, leaves, pods and seeds confirmed the uptake of fenbendazole. Moreover, presence of metabolites substantiate that plant enzymatic systems were able to transform fenbendazole via several reactions.

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CHANGES IN EXPRESSION OF CYTOCHROMES P450 AND P-GLYCOPROTEINS IN *HAEMONCHUS CONTORTUS* AFTER EXPOSITION TO SUBLETHAL DOSES OF DRUGS

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The parasite, which causes significant losses of small ruminants through a disease called heamonchosis, is the abomasal nematode *Haemonchus contortus*.[1] The main problem with

this parasite is the presence of drug resistance to almost all administered anthelmintics. Ivermectin (IVM) is one of the anthelmintics used in veterinary medicine to eliminate this endoparasite. It is believed that xenobiotic-metabolizing enzymes such as cytochromes P-450 and membrane efflux transporters P-glycoproteins, play a role in resistance in *H. contortus*.[2,3] As the knowledge of the development and mechanism of drug resistance in nematodes is still insufficient, more research is needed. This study focuses on the effect of low concentrations of IVM, which could occur in environmental circulation, on expression of selected cytochromes P450 and P-glycoproteins. Females and males were separated before our experiment. After 4, 12 and 24-hour incubation of nematodes with three different IVM concentrations the levels of selected mRNAs were analyzed by qPCR. The results obtained in nematodes exposed to IVM were compared to the results in control nematodes (exposed to solvent only). Significant IVM-induced changes were found in expression of several genes. Significant sex-differences were observed in inducibility of tested genes. The induction effect of IVM was most pronounced in P-gp 1 genes, thus P-gp 1 might contribute to development of drug resistance in *H. contortus*.

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DNA METHYLATION CHANGES IN OROPHARYNGEAL CARCINOMA

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Oropharyngeal carcinoma (OP) is a type of head and neck cancer (HNC) emerging in the tissue of base of the tongue, tonsils, soft palate and pharynx. Traditional risk factors include excessive alcohol and tobacco consumption. Recently, human papillomavirus (HPV) has been identified as an additional independent risk factor for the development of these tumors.[1] Epigenetic alterations, such as DNA methylation, refer to heritable changes in gene expression that occur without changes in the underlying DNA sequence and can contribute to carcinogenesis.[2] The aim of this study was to investigate methylation levels

of selected tumor suppressor genes in oropharyngeal squamous cell carcinoma (OPSCC) and compare methylation levels with HPV status.

DNA methylation levels of selected tumor suppressor genes were analysed using Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) in metastatic tumor samples, metastases samples, non-metastatic tumor samples and control tissue samples (non-cancerous palatine tonsils). We considered sample to be methylated above cut-off limit 15%.

CADM1 methylation in OPSCC is increased by the presence of HPV infection, which is corresponding to methylation of the CADM1 gene in cervix carcinoma.[3] CADM1 gene methylation levels are probably not influenced by metastatic process, because our results show that corresponding metastases has the same methylation status as their primary tumors. Methylation of CADM1 could be used as potential OPSCC biomarker in the future.

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METABOLISM OF FLUBENDAZOL IN HUMAN LIVER SUBCELLULAR FRACTIONS

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Flubendazole (FLU), a benzimidazole anthelmintic drug with a broad spectrum of activity and low toxicity, has been used in veterinary as well as human medicine for a long time.[1] Owing its mechanism of action, based on the specific binding to tubulin, FLU is now considered a promising anti-cancer agent.[2] However, we do not have enough information about its biotransformation in human.[3] In our project, subcellular fractions from the liver of 12 human patients (6 males and 6 females) were used to study the stereospecificity, cellular localization, coenzyme preference and possible inter-individual or sex differences in FLU reduction. In addition, the risk of FLU interaction with other drugs was evaluated. Results showed that FLU is predominantly reduced in cytosol and the NADPH coenzyme is preferred. The strict stereospecificity of FLU carbonyl reduction was proven and carbonyl reductase 1 was identified as the main enzyme of FLU reduction in the human liver. A higher reduction of FLU and a higher level of carbonyl reductase 1 protein was found in males than in females, but overall inter-individual variability was relatively low. Hepatic

intrinsic clearance of FLU is very low, and FLU had no effect on doxorubicin carbonyl reduction in the liver and in cancer cells. For these reasons, interactions of FLU with other carbonyl bearing drugs are not expected. The obtained results demonstrate the safety of FLU use in human and support FLU repurposing for cancer treatment.

The study was supported from the project of Specific Academic Research (SVV 260 416).

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NEW MODEL OF MUTATED TGR5 AND ITS TREATMENT BY BILE ACIDS DERIVATIVES *IN SILICO*

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TGR5 is a member of the G-protein-coupled receptors family that represents a broad group of transmembrane receptors. It plays a role in regulation of cholesterol homeostasis, lipid, and carbohydrate metabolism. That makes TGR5 an interesting target in the pharmacotherapy of diabetes type 2 and obesity.

In this study, a homology model of TGR5[1] was used since no crystal structure has been hitherto available. All observed orientations of cholic acid derivatives in several studies[1,2] are consistent and very similar. The 3-α hydroxyl group on the A-ring is oriented towards a polar part of the cavity, forming a hydrogen bond with Glu169. The carboxylic group is facing another polar subdomain on the extracellular opening of the binding pocket probing for Ser270. These observations are in perfect line with our docking results received by employing Auto-Dock Vina software. Based on this we can conclude that Glu169 together with Ser270 are the crucial amino acids determining the proper orientation of a ligand in the pocket. Hydrophobic interaction between the rather nonpolar central part of the binding pocket and the polycyclic skeleton provides further stabilization. Another small nonpolar subdomain (Leu84, Leu87, Leu160, Phe125) hosts ethyl or ethylidene groups bound to the C_6. Macchiarulo *et a*l.[3] who propose orientation of ligands towards Tyr89 and Asn93 suggest a different model. This orientation is perpendicular to what we are observing and does not involve any connection of the ligand to Ser270.

The goal of this study is to shed light on this discrepancy by preparing series of mutants. Utilization of molecular docking or use of mutants should help us in further study of novel ligands.

The study was supported from the project of Specific Academic Research (SVV 260 401).

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HPLC ANALYSIS OF THE EFFECT OF SILYBIN ON THE FENTON REACTION

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Silymarin, the standardized extract of the milk thistle (*Silybum marianum*), is a widely used over-the-counter drug recommended for a number of liver diseases. Silymarin contains silybin as the main flavonolignan component. In general, flavonolignans, due to the presence of the flavonoid core functionalized with hydroxy and oxo groups, can interact with transition metals by forming complexes.[1] However, the biological behaviour of these complexes is unknown. Tight copper or iron chelation will hinder the redox activity of both metals, while reducing potency can be connected with pro-oxidative effect. Moreover, flavonolignans are direct scavengers of free radicals. The aim of this study was to test the influence of silybin on the iron- and copper-driven Fenton reaction, during which hydroxyl radical is produced and hence to determine if silybin acts as an anti- or pro-oxidant in the presence of these ions. Since compounds can behave differently depending on their ratio to metal, different ratios silybin : metal were tested.[2] Salicylic acid was used as an indicator of the hydroxyl radical production. An original method based on HPLC coupled to electrochemical (coulometric) and diode array detectors was developed and applied. In all tested ratios, silybin was able to decrease the production of the hydroxyl radical triggered by both metals and hence only the antioxidant activity was documented. In the case of iron, the highest inhibition was observed at the tested ratio of 1:1, silybin to iron, while progressive anti-oxidative effects of silybin were observed in the case of copper-driven Fenton reaction. Based on these results, the toxicity of silybin based on pro-oxidation via interaction with transition metals is unlikely.

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CHARACTERIZATION OF LIGAND BINDING TO M_1 MUSCARINIC ACETYLCHOLINE RECEPTOR USING FLUORESCENCE ANISOTROPY METHOD

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Methods with detection of fluorescence anisotropy are used in drug screening and kinetic studies of ligands binding to targets. Compared to gold standard radioactive binding, these present simpler, inexpensive and safer option.[1]

The aim of this work was to evaluate fluorescent anisotropy on M_1 muscarinic receptors expressed on surface of baculovirus particles. Apparent potencies expressed as IC_{50} of eleven classical muscarinic ligands, both agonists and antagonists and three new M_2-selective ligands were measured and compared to previously published K_i values based on radioactive binding assay. Furthermore, analysis with IQMTools for Matlab was performed to simulate the dynamic process of ligand binding to receptor.

IC_{50} values of muscarinic antagonists were mostly in good agreement with K_i from literature. However, agonists McN-A-343 and acetylcholine showed lower potency in comparison with published K_i. Apparent potency of carbachol could not be determined and UR-MK259 showed intriguing results.

Despite some discrepancy, fluorescence anisotropy assay presents highly valuable, homogenous and high throughput method in study of ligands binding to receptor.

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DETERMINATION OF RENAL TOXICITY IN BRAF INHIBITORS *IN VITRO*

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One of the most serious malignant skin disease nowadays is melanoma. Targeted treatment based on inhibition of oncogenic mutations of BRAF enzyme using their specific inhibitors, dabrafenib and vemurafenib, has been proved to be a new useful therapeutic procedure.[1] However, a renal damage associated with a dysfunction of podocyte cells in

the renal glomerulus has recently been observed.[2] The aim of this study was to verify the potential cytotoxic effect of dabrafenib and vemurafenib on human kidney cell lines *in vitro* and compare their quantitative cytotoxic parameters mutually and with comparative agents.

The *in vitro* experiments were performed using two human cell lines representing different types of kidney cells (HEK-293 and PODO/TERT256). The cells were incubated with the tested compounds at different incubation concentrations for 24 h and 48 h. The cellular toxicity of the BRAF inhibitors was compared with a positive control (kidney toxin amphotericin B) and a negative control (non-toxic paracetamol). A colorimetric method based on measurement of cell metabolic activity was employed. The IC_{50} (drug concentration inhibiting viability to 50%) values were determined by analysis of inhibition curves. The results showed clear toxic effects on kidney cells in both BRAF inhibitors. However, vemurafenib was significantly more toxic in comparison with dabrafenib (even its toxicity is higher than amphotericin B). The observed differences in toxicity of vemurafenib and dabrafenib towards both cell renal lines were not substantial, which might suggest that the renal damage by BRAF inhibitors might involve more types of kidney cells besides podocytes.

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DEVELOPMENT OF A METHOD FOR SCREENING OF COBALT CHELATORS

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Cobalt as the internal part of the vitamin B_{12} is an essential microelement for living organisms including humans. Its lack or excess is associated with pathological conditions. A deficiency of cobalt, which is not very common, could lead even to pernicious anaemia. Cobalt poisoning can be caused, for example, by exposure to cobalt metal dust during the production of tungsten carbide or follows the corrosion of metal hip prostheses. Patients intoxicated by cobalt can develop neurological damage, hypothyroidism and/or cardiomyopathy.

The aim of this work is to develop a standardized, rapid and cheap method for the screening of cobalt chelators. For this purpose, spectrophotometric detection using 1-nitroso-2-nafthol-3,6-disulfonic acid disodium salt as the indicator was used. Firstly, it was found that the addition of cobalt ions leads to a clear bathochromic shift of the maximum absorbance of the indicator. The relationship between the absorbance and cobalt concentra-

tion was highly linear from 470 to 560 nm at all four pH conditions tested (4.5 to 7.5). The sensitivity of the method was 500 nM at pH 4.5 and even lower at higher pH conditions. Currently, potential cobalt chelators are being tested.

In conclusion, a competitive method for screening of cobalt chelation, which as far as we know, has not been available previously, was developed.

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ROLE OF SELECTED ABC AND SLC TRANSPORTERS IN TRANSMEMBRANE PERMEABILITY OF MARAVIROC: EFFECT ON TRANSPORT IN PLACENTA

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Antiretroviral drug maraviroc is an inhibitor of CCR5-trophic HIV virus and belongs to the group of entry inhibitors. Nowadays, maraviroc is administered as a part of combination antiretroviral therapy (cART) primarily in adults and children over the age of two. In pregnant women, it is used to reduce the risk of transmission of HIV to the fetus. The knowledge of interactions of maraviroc with drug transporters in placenta is crucial for optimizing the therapy during pregnancy, in both terms of efficacy and potential adverse effects. Maraviroc is known substrate of ABCB1 transporter, which plays a protective role to the fetus by its efflux activity in the apical membrane of trophoblast. However, the results of our recent study suggest involvement of other transport mechanisms in the maraviroc transplacental pharmacokinetics, especially those operating in the opposite direction to ABCB1.

The aim of this study was to evaluate in *in vitro* studies whether, besides ABCB1, maraviroc interacts with other transplacental transporters. First, an accumulation study and bidirectional transport of maraviroc across the monolayer of placental BeWo b30 cells was performed. Significant reduction in maraviroc accumulation in the presence of verapamil (100 μM), ritonavir (10 μM) and elacridar (2 μM) suggests that some influx transporters might be involved. On the other hand, an increase of accumulation in the presence of inhibitor MK-571 (50 μM) suggests also involvement of some ABCCs efflux transporter(s). After the following evaluation of transport study, a significant transfer of maraviroc in B-A direction was observed sensitive to the presence of ritonavir and MK-571. In order to identify the interacting transport mechanisms, *in vitro* studies were performed using MDCKII cells overexpressing human ABCC1 transporter and A431 cells overexpressing human OATP2B1, 1A2 or 1B3 transporter. Substrate affinity of maraviroc to ABCC1, OATP1A2 and OATP1B3 was revealed, but not to OATP2B1.

Based on the obtained data we can suggest, that maraviroc interacts with several drug transporters in placenta that could partly reverse the limiting effect of apically localized ABCB1. Considering the fact that antiretroviral therapy is always administered as combination of drugs in developed countries, it can be assumed that maraviroc will be susceptible to drug–drug interactions and this newly obtained data could contribute to better understanding of the transplacental pharmacokinetics of maraviroc and optimization of the therapy.

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EFFECT OF CHELATORS ON ALCOHOL DEHYDROGENASE, A ZINC CONTAINING ENZYME

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Zinc is an ubiquitous metal present in food, water and air. It is also the second most abundant trace element in living organisms. Zinc is involved in many physiological processes. Among others, zinc is contained in alcohol dehydrogenase (ADH) and is needed for its catalytic role which lies in reversible oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD+ to NADH. Since many, even clinically used, metal chelators are non-selective, they can chelate zinc as well. The aim of this study was hence to test if different chelators can block enzymatic function of ADH. Shortly: different chelators dissolved in DMSO were mixed with ADH from *Saccharomyces cerevisiae* (0.04 UI/mL), ethanol (3% V/V) and NAD+ (7.2 mM) in a buffer of pH 8.8 at 25 °C. The reaction was monitored for 6 minutes measuring the absorbance at 340 nm, which corresponds to the formed NADH. Our preliminary results with 12 chelators have shown that most of them, in particular hydrophilic chelators, had very low or almost no effect on ADH activity. The most active chelators were nitro or halogen derivatives of 8-hydroxyquinolinole, but their activity at concentrations below 100 μM was quite low. In conclusion, it seems that metal chelators can interfere with ADH only at higher concentrations.

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EVALUATION OF NEWLY PREPARED INSECTICIDES *IN VITRO*

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Malaria is a widespread infection and one of the most dangerous diseases transmitted by insects. It threatens lives of millions of people all around the world, thus its regulation is necessary.[1] Most common malaria vectors are mosquitoes of genus *Anopheles*.[2] Novel structures of insecticides with selective inhibition of mosquito acetylcholinesterase (AgAChE) are subjects of research, with an intention to control this problem.[3]

The aim of this work was to test six newly prepared succinimide derivatives with insecticidal potential. The ability of these compounds to inhibit mosquito (*Anopheles gambiae*) and human (hAChE) acetylcholinesterase was evaluated. Lead structures of these compounds were also tested to find relations between chemical structure and biological activity. The modified Ellman's method was used to obtain half inhibitory concentration (IC_{50}) values for both enzymes.

Tested substances were able to inhibit only hAChE and none of them displayed activity against AgAChE. Compound LG-488 possesed IC_{50} value for human enzyme in the same range as tacrine. Further modifications could lead to finding new structures able to successfully fight pest insects.

The study was supported from the project of Specific Academic Research (SVV 260 414).

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STUDY OF THE EFFECT OF NOVEL ANTIVIRAL DRUGS ON CARNITINE TRANSPORT IN THE PLACENTA

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Nowadays, the antiretroviral treatment of HIV-positive pregnant women is the standard approach for restriction of transmission of HIV infection from mother to the fetus. In spite of necessity of this pharmacotherapy, it is important to know its safety and risks. For the correct fetal development and function of placenta it is (besides others) essential to

ensure the optimal supply of L-carnitine, the key factor for oxidation of fatty acids, from mother's blood to the placenta and fetal blood circulation.

The deficiency of L-carnitine generally leads to significant metabolic changes in the cells and it is usually demonstrated with cardiomyopathies and myopaties. Published studies indicate higher incidence of cardiovascular diseases and cardiomyopathies in children born to mothers treated with antiretroviral therapy during pregnancy. Optimal transport of carnitine into the placental cells is ensured due to the presence of functional transport protein OCTN2 in the apical membrane of trophoblast. The aim of this study was to evaluate, if antiretrovirals from groups of non-nucleoside reverse transcriptase inhibitors (rilpivirine and efavirenz), protease inhibitors (ritonavir, saquinavir, tipranavir, lopinavir and atazanavir) and integrase inhibitors (dolutegravir and elvitegravir) are able to inhibit OCTN2 transporter and thereby restrict the active transfer of carnitine into the cells. *In vitro* model of BeWo cell line derived from choriocarcinoma of the placenta and *ex vivo* model of microvillous plasma membrane vesicles isolated from human placenta obtained after the delivery were employed.

Our results demonstrate significant inhibitory effect of protease inhibitors ritonavir and saquinavir on the uptake of L-carnitine in both used models. The inhibitory effect of elvitegravir and rilpivirine on the OCTN2 was demonstrated only in BeWo cells but was not confirmed on isolated microvillous membranes. Our study indicates that possible carnitine defficit should be considered in therapeutic regimens involving protease inhibitors (mainly ritonavir and saquinavir). The other tested antiretroviral drugs seem to be safe from the perspective of L-carnitine availability for placenta.

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EFFECT OF FLAVONOIDS ON COPPER TRIGGERED RED BLOOD CELL LYSIS

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Flavonoids, a group of natural substances with variable substitutions of the basic core, are largely being tested for their potentially beneficial effects on human health. In particular, their anti-oxidant activity includes both direct scavenging activity and chelation of transition metals (iron and copper), since these metals can participate in the propagation of reactive oxygen species generation. Excessive level of free copper is causing hemolysis and this is a known complication in patients suffering from Wilson's disease, a genetic disorder associated with copper excess. The aim of the study was to test the ability of some flavonoids to protect red blood cells against copper triggered hemolysis. Lactate dehydrogenase (LDH) activity was used as the indicator for hemolysis. Human red blood cells were treated for 4 hours in the presence or absence of flavonoids with copper in a final concentration of 5mM. The LDH activity was measured thereafter and compared to total

LDH after complete erythrocyte lysis mediated by a lysis buffer. Preliminary results have shown that at least two flavonoids, namely 3- and 5-hydroxyflavone, were able to, concentration dependently, decrease the red blood cell lysis. Future experiments are needed to confirm these results.

The study was supported from the project of Specific Academic Research (SVV 260 414).

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SECTION OF CHEMICAL SCIENCES: SYNTHETIC PART

N-PYRIDINYLBENZAMIDES AS POTENTIAL ANTI(MYCO)BACTERIAL COMPOUNDS

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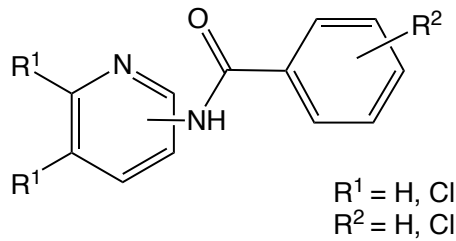
The derivatives of *N*-pyridinylbenzamide were designed and synthesized to be *in vitro* tested for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv*, M. avium, M. kansasii, M. smegmatis, M. aurum* and selected bacterial and fungal strains. These compounds are pyridinyl analogues of previously prepared *N*-pyrazinylbenzamides[1] that have shown a significant *in vitro* antimycobacterial activity. The title compounds were synthesized by acylation of aminopyridine or chloropyridine-2-amine by selected benzoyl chlorides. Biological results will be discussed in the presentation.



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STUDY OF INTRAMOLECULAR CHARGE TRANSFER IN AMINOPHTHALOCYANINES

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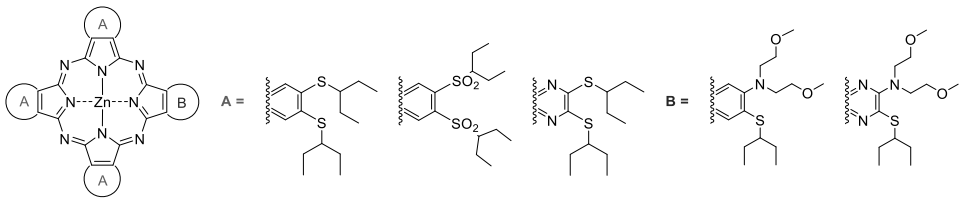
Phthalocyanines (Pcs) and their analogues have been studied for their ability to behave as fluorescence sensors due to effect known as intramolecular charge transfer (ICT). ICT, as an alternative pathway of energy dissipation, is competitive to fluorescence emission and requires peripheral substitution with electron donors, in this case tertiary amines.[1] The presence of the donor leads *via* ICT to fluorescence quenching and this phenomenon can be selectively enabled and blocked under specific conditions leading to usage in the sensing applications. Even though Pcs have been found so far less suitable as fluorescence sensors than azaphthalocyanines, the purpose of this study is to find substituents and/or modifications to Pc macrocycle with sufficient electron acceptor properties to allow the usage of Pcs as fluorescence sensors as well. For the synthesis of the Pc precursors, nucleophilic substitution and in two cases oxidation of the sulfide precursors were used. Oxidations of the sulfide are expected to result in better electron accepting properties of Pc macrocycle. Next step in the synthesis was cyclotetramerization from two precursors, which lead to mixture of different congeners from which the congener with one donor center was isolated by column chromatography. Fluorescence quantum yields for the final compounds were measured and efficiency of ICT evaluated.



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DESIGN, SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF HYBRID COMPOUNDS COMBINING PYRAZINAMIDE AND *p*-AMINOSALICYLIC ACID

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According to WHO, tuberculosis (TB) is the leading cause of death from a single infectious organism worldwide and the number of cases with drug resistant TB is still increasing, creating the need for new antituberculotics.[1] Therefore we report design, synthesis and antimicrobial evaluation of a series of hybrid compounds combining different pyrazinamide derivates and *p*-aminosalicylic acid as potential antituberculotic agents. The compounds were prepared by mixing different pyrazinecarboxylic acids, after activation by 1,1′-carbonyldiimidazole, with *p*-aminosalicylic acid in dimethylsulfoxide as a solvent. Obtained compounds were *in vitro* tested for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv and other four nontubercular mycobacterial strains. Prepared compounds were also *in vitro* screened for antibacterial, antifungal, and cytotoxic (HepG2) activity. Most compounds showed antimycobacterial activity in range of minimum inhibitory concentration (MIC) from 3.13–12.5 μg/mL against *M. tuberculosis* H37Rv and *M. kansasii* with no *in vitro* cytotoxicity. The most potent compound was 4-(6-chloropyrazine-2-carboxamido)-2-hydroxybenzoic acid with MIC against *M. tuberculosis* H37Rv = 3.13 μg/mL (11 μM). None of the prepared compounds exerted antibacterial or antifungal activity.

The study was supported from the project of Specific Academic Research (SVV 260 401), by the Grant Agency of Charles University (project C C3/1572317) and by the Czech Science Foundation (Project No. 17-27514Y).

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PREPARATION OF TRIPLE LABELED DNA-PROBES MODIFIED BY AZAPHTHALOCYANINE QUENCHERS

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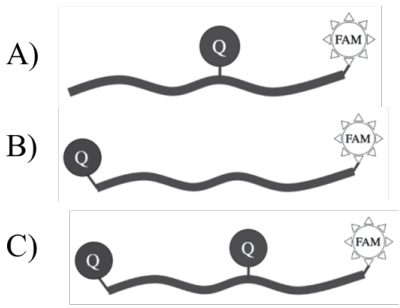
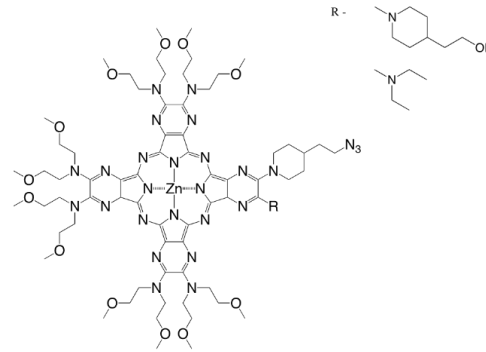
Unsymmetrical dialkylamino substituted azaphthalocyanines (AzaPcs) have unique photophysical properties – light absorption between 300 and 700 nm, almost no self-fluorescence and ability to quench fluorescence of other compounds. This makes AzaPcs

suitable candidates for universal dark quenchers which can be used in real time PCR.[1] In this work, we tried to evaluate differences in quenching between probes labeled in different positions of oligonucleotide (ODN) chain. Two quenchers bearing different functional groups suitable for different connection to ODN were synthetized. Following the synthesis of quencher, three different probes were prepared – one with quencher in the middle of ODN chain (Fig. A), second with quencher attached to the end of oligonucleotide chain (Fig. B), third with quencher in middle and at the end of ODN chain (Fig. C). All prepared ODN probes were purified on HPLC system. Subsequently, the quenching efficiency of these ODN probes was compared.





The study was supported by the Grant Agency of Charles University (Project. No. 994218) and from the project of Specific Academic Research (SVV 260 401).

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DERIVATIVES OF QUINOXALINE-2-CARBOXYLIC ACID AS POTENTIAL ANTIMICROBIAL COMPOUNDS

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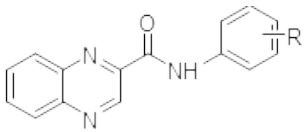
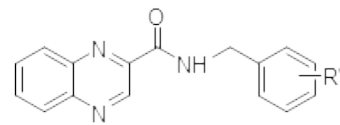
Despite the presence of well-established treatment plan, tuberculosis remains the number one killer of infections according to WHO.[1] One of the reasons behind this failure in eradicating this infection is drug resistance.[1] This fact potentiates worldwide efforts to develop new antituberculars. As part of our long-term research on pyrazine derivatives,

we prepared a series of *N*-substituted quinoxaline-2-carboxamides, refer to Fig. 1. below. Quinoxaline-2-carboxylic acid was activated by oxalyl chloride and reacted with different anilines in the presence of pyridine at room temperature, overnight with stirring, and then obtained crudes were purified with flash chromatography. Final products were evaluated for *in vitro* antimicrobial activities against five mycobacterial strains, eight fungal stems, along with four gram positive and four gram negative bacteria of clinical importance. Antimicrobial activity was not observed for any of the prepared compounds.



R = H, 3-CF_3, 3-OH, 4-OH, 3,5-diOCH_3



R' = H, 2-Cl, 2-CH_3, 3-Cl, 3-F, 3-CF_3, 4-CH_3, 4-OCH_3, 4-OH, 2,5- diOCH_3, 3,5-diOCH_3

Figure 1. The chemical structures of title compounds

The study was supported from the project of Specific Academic Research (SVV 260 401), as well as by the Grant Agency of Charles University (Project No. C-C3/1572317) and the Czech Science Foundation (Project No. 17-27514Y).

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1. World Health Organization. Global Tuberculosis Report 2018. Available from: https://apps.who.int/iris/handle/10665/274453.

SYNTHESIS AND EVALUATION OF POTENTIAL CHOLINESTERASES INHIBITORS

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Based on previous knowledge about salicylanilide derivatives [(thio)phosphates, carbamates] proposed as inhibitors of acetyl- (AChE) and butyrylcholinesterase (BChE),[1,2] a series of novel salicylanilide-organophosphorus derivatives was designed with this goal. The synthesis consists of MW preparation of salicylanilides followed by reaction with phosphorus reagents to provide esters, 2-substituted 3-(substituted phenyl)-3-hydrobenzo[*e*][1,3,2]oxazaphosphinin-4-one 2-oxides/sulfides. Their ability to inhibit both cholinester-

ases was evaluated using Ellman's method. AChE was inhibited with IC_{50} values within the range of 48–66 μM. 5-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl diethyl phosphite (Fig. 1) exhibited superior inhibition of BChE (IC_{50} = 2.37 μM). Additionally, this compound acts as a mixed inhibitor and now, it is under advanced evaluation.

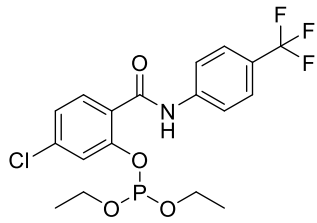

Figure 1. The most active BChE inhibitor

The study was supported by the Czech Science Foundation (Project No. 17-27514Y).

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INTERMOLECULAR INTERACTIONS STUDIED BY NMR SPECTROSCOPY

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We studied structural adaptations of modified nucleic acid bases induced by intermolecular interactions. Two rotamers depending on the orientation of methylamino group are present in substituted pyrimidine **1**. Separated signals for each rotamer can be observed in ^{1}H NMR spectra. The rotamer ratio can be influenced by interactions with compounds able

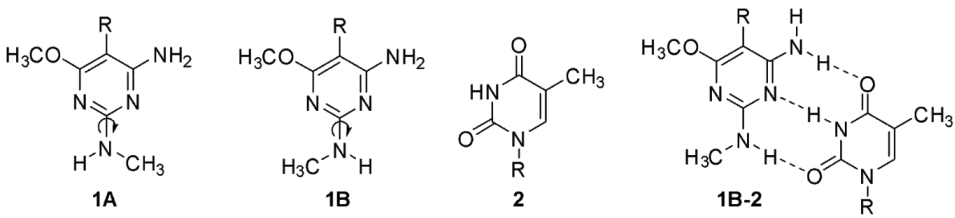

Figure 1. The two rotamers **1A** and **1B**, the binding partner **2** and formed complex **1B-2**

to form hydrogen bonds with one rotamer of **1** only. Compounds with the acceptor-donor-acceptor (ADA) structure can form three hydrogen bonds with rotamer **1B**, *i.e*. addition of substituted thymine (**2**) changes the ratio of rotamers in favour of rotamer B (dimer **1B**-**2**). NMR experiments with variable temperature and concentration of interacting partners may be used for the determination of free energy of complex formation. Similarly, intermolecular interactions change the tautomer equilibria of modified nucleobases significantly.

This work was supported by Czech Science Foundation (Project No. 18-11851S).

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PREPARATION OF DENDRALENES SUBSTITUTED WITH ELECTRON-WITHDRAWING GROUPS

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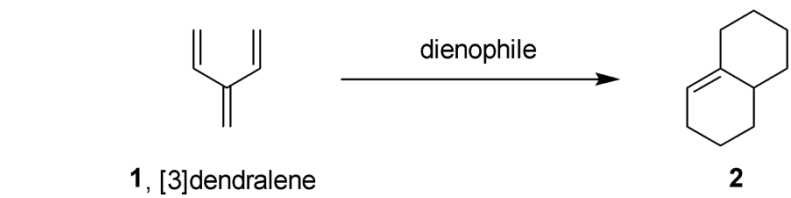
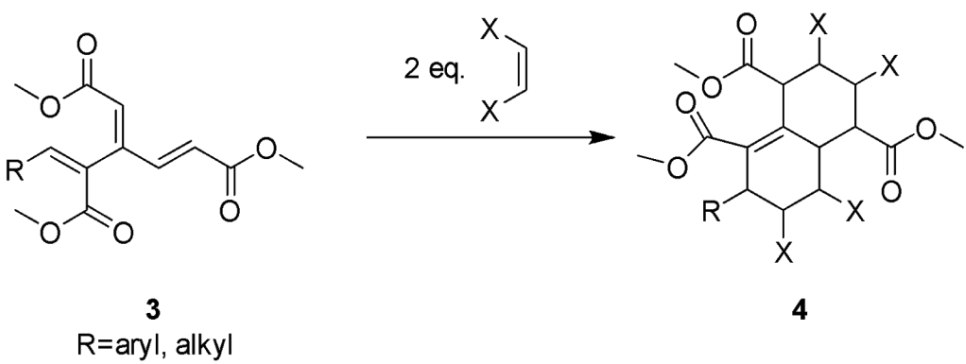


Scheme 1. Structure and reactivity of dendralenes



Scheme 2. Potential reactivity of EWG-[3]dendralenes

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**).

My goal was to prepare new dendralenes (Scheme 2, **3**), substituted with electron withdrawing groups (EWG), based on the procedures previously developed by our research group.[2] Furthermore, their ability to undergo Diels-Alder reaction was investigated (Scheme 2, **4**).

The study was supported by the Grant Agency of Charles University (Project No. 1054216), the Czech Science Foundation (Project No. 18 17868S) and from the project of Specific Academic Research (SVV 260 401).

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1. HOPF, H., SHERBURN, M. S.: Angew. Chem. Int. Ed., 51 (10), 2012, 2298–2338.
2. KRATOCHVÍL, J., NOVÁK, Z., GHAVRE, M. *et al*.: Org. Lett., 17 (3), 2015, 520–523.

SYNTHESIS OF NITROFURYL-SUBSTITUTED TETRAZOLES AND OXADIAZOLES AS POTENTIAL ANTITUBERCULAR AGENTS

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Tuberculosis (TB) is widespread infectious disease which is mainly caused by *Mycobacterium tuberculosis*. Referring to the World Health Organization, it is still among the top 10 causes of death in the world. Around 10 million people worldwide suffered from TB and 1.6 million died of TB only in 2017. TB is a leading-killer in the group of HIV-positive people.

This work is based on a previous successful discovery of new chemical structures with significant antitubercular activity, namely 1,5- and 2,5-disubstituted tetrazoles and 2,5-disubstituted oxadiazoles bearing 3,5-dinitrobenzylsulfanyl fragment, with minimal inhibitory concentration (MIC) values as low as 0.03 μM (*i.e.* lower MIC compared to

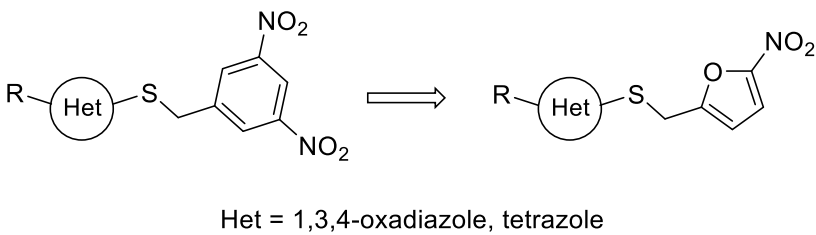

Figure 1. General formula of the compounds studied in this work

standard medicine used – isoniazid or rifampicin). In this study, we replaced 3,5-dinitrophenyl fragment with 5-nitrofuryl group, which has been identified as a pharmacophore in different types of anti-TB agents.

Hence, we prepared a series of nitrofuryl-substituted tetrazoles and oxadiazoles and studied their antimycobacterial activity against *Mycobacterium tuberculosis* and non-tuberculous *M. avium* and *M. kansasii*.

The study was supported from the project of Specific Academic Research (SVV 260 401).

TOTAL SYNTHESIS OF 6-HYDROXYCERAMIDES AND THEIR BEHAVIOR IN THE MODEL LIPID MEMBRANES

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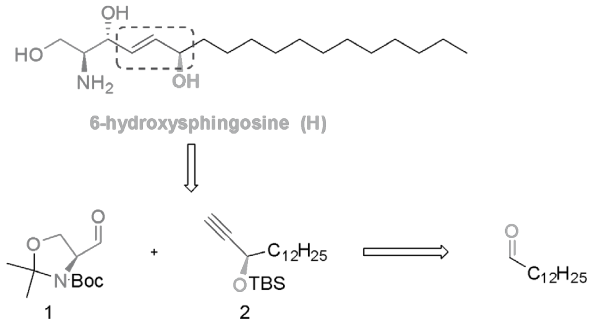
Ceramides (Cer), the members of sphingolipid family, occur in all human cells and play an important role in cell signalling. In high concentrations, Cer can also be found in the uppermost layer of epidermis called *stratum corneum*, along with free fatty acids and cholesterol (in equimolar ratio), where they form the intercellular multi-lamellar lipid matrix. The key function of *stratum corneum* is to ensure a permeability barrier, thus, to provide water and electrolyte homeostasis, and to prevent entry of harmful substances into the organism.[1]

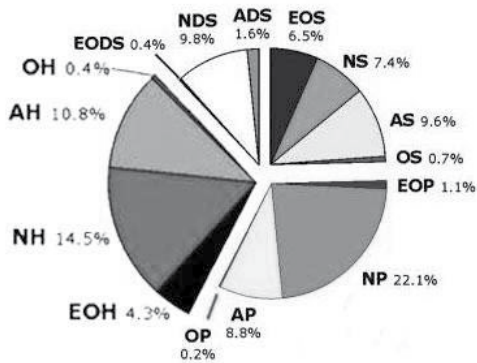
Cer are composed of a sphingoid base (*e.g.* sphingosine; C18) and an acyl part derived from long-chain fatty acid (*e.g.* lignoceric acid; C24). Cer based on 6-hydroxysphingosine (signed by Motta's nomenclature as **H**)[2] are one of the most unusual sphingolipids. In contrast to sphingosine-based Cer, 6-hydroxysphingosine-based Cer (**H-Cer**) are unique only for the epidermis and, in addition, **H-Cer** are not typical for all mammals.[1] Moreover, the function and biosynthesis of **H-Cer** in the skin is still not completely understood. Several dermatological studies showed that lower concentrations of **H-Cer** in skin accompany several skin diseases, such as atopic dermatitis.[1] The major limitation of understanding the importance and uniqueness of **H-Cer** is their commercial unavailability.

Therefore, the aim of this work was to explore the synthetic route towards **H** as a precursor of all known **H-Cer** subclasses. The total synthesis of **H** was based on the reaction of commercially available tridecanal with trimethylsilyl acetylene. The strategy for the synthesis of **H** involved an alkynylation of (*S*)-Garner's aldehyde (a protected L-serinal) (**1**) with protected (*R*)-pentadec-1-yn-3-ol (**2**) followed by a selective two-step reduction of triple bond to a *trans*-double bond. In this step, a mild and selective $[Cp^*Ru(CH_3CN)_3]PF_6$-catalyzed Trost's hydrosilylation followed by protodesilylation was used.[3]

In conclusion, physiological 6-hydroxylated sphingoid base has been prepared in seven reaction steps with overall yield 36.3%. This base was then used for the preparation of Cer



Scheme 1. Structure and retrosynthesis of physiological 6-hydroxysphingosine, *i.e.* (2*S*,3*R*,4*E*,6*R*)-2-aminooctadec-4-ene-1,3,6-triol



Scheme 2. (Pie Chart) Relative proportion of ceramides in healthy human skin[4]

NH (*N*-lignoceroyl 6-hydroxysphingosine), Cer AH (*N*-2-hydroxylignoceroyl 6-hydroxysphingosine) and Cer EOH (with omega ester-linked linolenic acid). Additionally, the phase behaviour and biophysical properties of Cer NH have been studied using model lipid membranes. In these experiments, we discovered the specific chain order, different phase transitions of CH2/CD2 chains, tight orthorhombic lateral packing and lowered miscibility of Cer NH with other skin lipids. Since sphingosine exhibits an antimicrobial effect, in the future, we plan to study the antimicrobial effect of **H** on different cell lines.

This work was supported by the Czech Science Foundation (Project No. 19-09135J) and from the project of Specific Academic Research (SVV 260 401).

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SYNTHESIS OF HETEROCYCLIC DENDRALENES

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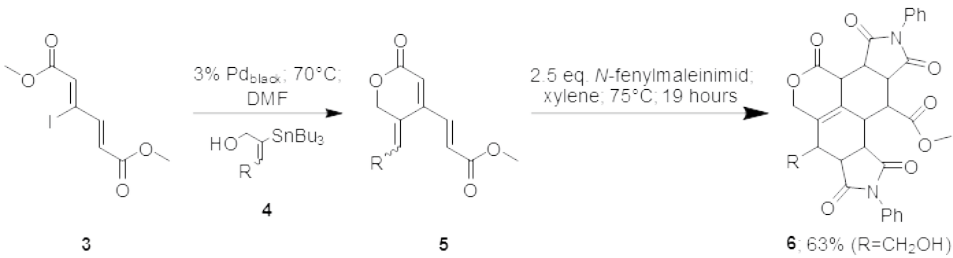
Dendralenes are acyclic, branched, cross-conjugated polyenes with a specific arrangement of double bonds (Scheme 1, **1**).[1] Unlike other polyconjugated systems, the structure of dendralenes requires at least three double bonds, where two of them are conjugated with the middle one but not with each other. The maximal number of double bonds in the molecule is unlimited (Scheme 1, **2**). These compounds have a great potential for further synthesis and their properties have not been thoroughly investigated yet.

The aim of my work was to prepare substituted heterocyclic dendralenes **5** (Scheme 2), through coupling of **3** with **4**, previously developed by our research group.[2] Another aim was to examine their reactivity, especially in Diels-Alder cycloaddition, in which complex, polycyclic compounds **6** are formed. Synthesis and reactivity will be discussed.

Scheme 1. Structure of dendralenes



Scheme 2. Synthesis of the target compounds

The study was supported by the Grant Agency of Charles University (Project No. 1054216), the Czech Science Foundation (Project No. 18 17868S) and from the project of Specific Academic Research (SVV 260 401).

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1. HOPF, H., SHERBURN, M. S.: Angew. Chem. Int. Ed., 51 (10), 2012, 2298–2338.
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BETA-SECRETASE-1 INHIBITORS

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Alzheimer's disease is a progressive neurodegenerative disease that remains incurable. One of the targets in the search for novel drugs is BACE-1 (β-secretase 1) whose blockage could help to stop the progression. Some of these inhibitors have already been tested in clinical trials. As BACE is a family of isoenzymes, the aim was to find selective BACE-1-inhibitors to minimalize adverse effects that may be caused by inhibition of BACE-2.

Molecular docking was used as a method to predict different interactions between the enzymes and inhibitors that had been already chosen by virtual screening. Appropriate complexes were chosen from RCSB Protein Data Bank and we prepared proteins and ligands for semi-flexible and flexible molecular docking using software Avogadro, Chimera and AutoDock Tools. The computational part was realized by AutoDock Vina. Suitability of the approach was verified by re-docking of ligands co-crystalized with the targets.Selective BACE-1-inhibitors were chosen based on different binding energy with the two enzymes and their subsequent comparison.

This study was supported from the project of Specific Academic Research (SVV 260 401).

SYNTHESIS OF LANSOPRAZOLE ANALOGUES AS POTENTIAL ANTITUBERCULAR AGENTS

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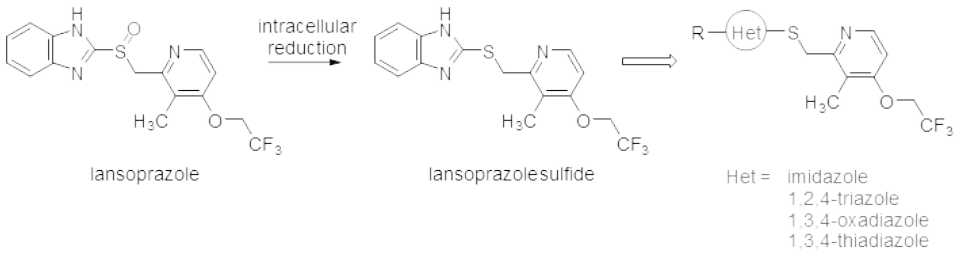
Mycobacterium tuberculosis is the bacterium causing serious infectious disease called tuberculosis (TB). Recent studies show an increase of the number of patients suffering from this disease which is the evidence of this bacterium's resistance to most antibiotics. The drug lansoprazole is well known as an inhibitor of gastric proton pump. However, recent study describes lansoprazole as a promising anti-TB drug candidate. The mechanism of the action is that lansoprazole kills *M. tuberculosis* by targeting its cytochrome bc_1 after intracellular reduction of sulfoxide to lansoprazole sulfide. This active metabolite does not inhibit human H^+K^+-ATPase thus providing an excellent lead compound for further structural optimization and structure-activity relationship study.[1]

In our work, we focused on modifying the lansoprazole structure by varying its benzimidazole fragment. Thus we prepared a series of imidazole, 1,2,4-triazole, 1,3,4-oxadiazole,

1,3,4-thiadiazole analogues of lansoprazole sulfide and evaluated their antimycobacterial activity against standard *M. tuberculosis* H37Rv and against non-tuberculous *M. avium* and *M. kansasii*.



The study was supported from the project of Specific Academic Research (SVV 260 401).

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TOTAL SYNTHESIS OF NOSTOTREBIN-6 – PROBLEMS AND CHALLENGES

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Nostotrebin-6 (Figure 1) is a polyphenolic secondary metabolite containing the cyclopentenedione moiety isolated from cyanobacteria *Nostoc* sp. The compound possesses

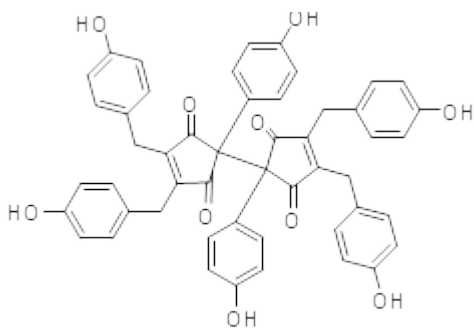

Figure 1. Nostotrebin-6

antimicrobial as well as acetylcholinesterase and butyrylcholinesterase inhibitory activity.[1] To the best of our knowledge, no total synthesis has been reported to date, and nostotrebin-6 can be obtained only using a specific method of isolation from natural sources. The aim of this work is to develop and optimize the synthesis of nostotrebin-6 and its analogues, and subject them to biological activity screening. Problems and challenges encountered during synthetic attempts toward the key intermediates and derivatives will be discussed.

The study was supported from the project of Specific Academic Research (SVV 260 401), the Grant Agency of Charles University (Project No. 1590119) and the Czech Science Foundation (Project No. 18-17868S).

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ALKALOIDS OF THE AMARYLLIDACEAE FAMILY AND THEIR BIOLOGICAL ACTIVITY I.

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The aim of this work was to isolate alkaloids from herbal extract, which was obtained from *Narcissus pseudonarcissus* cv. Dutch Master plant. The preparation and column chromatography of the extract were performed by PharmDr. Daniela Hulcová, Ph.D., as a part of her doctoral study. Using the preparative TLC method, 2 alkaloids marked as No. 2.1 and 2.2.2 were isolated from the fraction No.4. Their structure was determined using the NMR, GC-MS analysis and optical rotation. After comparing the data obtained with literature, the compounds were identified as (+)-homolycorine and (+)-masonine.

Both homolycorine and masonine were subsequently subjected to testing of inhibitory activity against acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), prolyloligopeptidase (POP) and glycogen synthase kinase 3β (GSK-3β). The activity was expressed as IC_{50} and was compared to IC_{50} values of the reference substances. Galanthamine ($IC_{50\,AChE} = 1.7 \pm 0.1$ μM, $IC_{50\,BuChE} = 42.3 \pm 1.3$ μM) and huperzine A ($IC_{50\,AChE} = 0.033 \pm 0{,}001$ μM, $IC_{50\,BuChE} > 500$ μM) were used as standards to compare the inhibitory activity against AChE and BuChE. While the inhibitory activity against POP was compared to *Z*-Pro-prolinal ($IC_{50\,POP} = 2.75 \times 10^{-3}$ μM) and berberine ($IC_{50\,POP} = 42 \pm 21$ μM), inhibition of GSK-3β was compared to SB-415286 compound ($IC_{50\,GSK\text{-}3\beta} = 70$ nM). Results for (+)-homolycorine: $IC_{50\,AChE} = 64 \pm 4$ μM, $IC_{50\,BuChE} = 151 \pm 20$ μM, $IC_{50\,POP} = 174 \pm 41$ μM and % inhibition of GSK-3β = 54 ± 1. Inhibitory activity of (+)-masonine: $IC_{50\,AChE} = 305 \pm 34$ μM, $IC_{50\,BuChE} = 229 \pm 24$ μM, $IC_{50\,POP} = 314 \pm 34$ μM,% inhibition of GSK-3β = 66 ± 4 and $IC_{50\,GSK\text{-}3\beta} = 27.9 \pm 0.8$ μM.

The results found indicate that homolycorine shows moderate inhibitory activity against AChE, and mild activity against BuChE and POP. On the other hand, the inhibition of

GSK-3β is relatively low. Concerning masonine, its inhibitory activity against all 4 tested enzymes is rather low in comparison with the reference substances.

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SYNTHESIS OF POTENTIAL CHOLINESTERASES INHIBITORS BASED ON PROPARGYLAMINE

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The contribution deals with the synthesis of potential acetylcholinesterase and butyrylcholinesterase inhibitors and testing their potential to affect the function of both cholinesterases. All the molecules contain the propargylamine structural motif. Propargylamine represents a proved scaffold for drugs targeting central nervous system and some of its derivatives are currently clinically used or investigated drugs for the treatment of neurodegenerative disorders including Alzheimer's disease.[1]

A total of 18 compounds (amides and imines derived predominantly from salicylic and cinnamic acid derivatives) were prepared in sufficient yields. All of them were investigated using Ellman's method to evaluate their biological activity against cholinesterases. Two compounds, *i.e.* 5-bromo-*N*-propargylsalicylamide and *N*-propargylbenzamide, showed the best IC_{50} values for acetylcholinesterase (8.05 and 23.16 μM, respectively). Two other compounds (5-bromo-2-(prop-2-yn-1-ylcarbamoyl)phenyl-(*N*-ethyl-*N*-methyl)carbamate and 3,5-dibromo-2-(prop-2-yn-1-ylcarbamoyl)phenyl-(*N*-ethyl-*N*-methyl)carbamate) showed the best inhibition of butyrylcholinesterase (IC_{50} of 25.10–26.09 μM).

Based on the presence of propargylamine moiety, the compounds are going to be evaluated also as potential inhibitors of monoamine oxidase B.

The study was supported by the Czech Science Foundation (Project No. 17-27514Y) and from the project of Specific Academic Research (SVV 260 401).

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SYNTHESIS OF 2-SUBSTITUTED 5-(3-NITRO-5-(TRIFLUOROMETHYL) PHENYL)-2*H*-TETRAZOLES AS POTENTIAL ANTITUBERCULAR AGENTS

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Tuberculosis (TB) is a widespread infectious disease predominantly caused by *Mycobacterium tuberculosis* (*M.tb*.). According to the World Health Organization, 10 million new TB cases were estimated and 1.6 million deaths from TB were registered worldwide in 2017.[1]

Previous work of our research group dealt with study of compounds with high antimycobacterial activity, high specificity to *M.tb.* and low toxicity. One group of such compounds were based on 2-substituted 5-(3,5-dinitrophenyl)-2*H*-tetrazoles. The antimycobacterial efficacy of compounds **1** reached MIC values of 0.03–0.5 μM against drug-susceptible and drug-resistant strains of *M.tb.*[2] Moreover, these substances were not active against other bacteria or fungi and showed low cytotoxicity.

In this study we focused on the preparation of novel derivatives in which one nitro group was replaced with trifluoromethyl group (**2,** Figure 1), because this group was successfully used as a replacement for nitro group in other nitro group anti-TB agent.

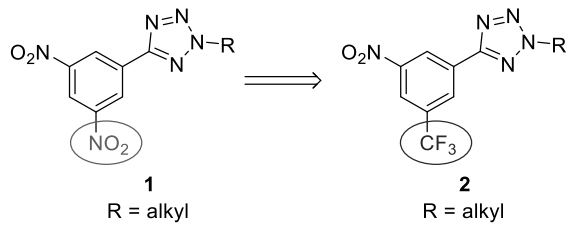

Figure 1. General formula of the substances studied in this work

Finally, three compounds of structure **2** were prepared and their antimycobacterial activity was evaluated using three mycobacterial strains.

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ALKALOIDS OF THE GENUS *NARCISSUS* AND THEIR BIOLOGICAL ACTIVITY

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Alkaloid extract obtained from bulbs of *Narcissus pseudonarcissus* L. cv. Dutch Master was extracted by ethanol and was purified by liquid-liquid extraction and fractionated by column chromatography to individual fractions. At the end, 11 pooled fractions were obtained, which were used to isolate pure alkaloids. The ND 3-5 / 7 fraction was processed by preparative thin layer chromatography followed by crystallization of pure substances. In total, 5 alkaloid substances ST1D2, ST1D3, ST2A, ST2B1 and ST3C were obtained from this fraction in various amounts. These substances were determined by GC-MS analysis, NMR analysis and optical rotation. Subsequently, the obtained data were compared with the NIST library spectra and the literature. Isolated substances have been identified as caranine, *O*-ethyllycorenine, narwedine, pluviine and *N*-demethylhomolycorine. The alkaloids obtained in sufficient amounts were subsequently subjected to tests to determine their biological activity against AChE, BuChE, POP and GSK-3β.

Cholinesterase inhibitory activity was determined *in vitro* by a modified Ellman's spectrophotometric method. POP inhibition was determined using Z-Gly-Pro-*p*-nitroanilide as substrate. GSK-3β inhibitory activity was determined by using the *in vitro* luminescence method of Baki *et al*.

Most of the isolated substances did not show significant inhibitory activity in the bioassays performed compared to the standards. The only interesting result was the inhibitory activity of caranine against GSK-3β (IC_{50} = 30.8 ± 0.3 μM).

The study was supported from the project of Specific Academic Research (SVV 260 412).

SYNTHETIC DERIVATES OF ALKALOIDS FROM AMARYLLIDACEAE FAMILY – *IN SILICO* STUDY

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Glycogen synthase kinase 3 beta, further abbreviated as GSK-3β, is an enzyme that acts as a negative regulator in glycogen synthesis. Besides metabolic function, it exhibits effect in the cell cycle, Wnt signaling pathway, regulates gene expression and interacts with many substances such as tau protein or mucin 1.[1,2] Recent studies show that inhibition of GSK-3β might be substantive in the treatment of Alzheimer's disease, stroke, various types of cancer and diabetes.[2]

Proceeding on modern trend in *in silico* studies, twenty selected derivates of alkaloids from Amaryllidaceae family were docked into GSK-3β, butyrylcholinesterase and prolyloligopeptidase using AutoDock Tools[3] and AutoDock Vina.[4] Based on simulation results, we have recommended substances with the best score for synthesis and *in vitro* study.

Computational resources were provided by the CESNET LM2015042 and the CERIT Scientific Cloud LM2015085, provided under the programme "Projects of Large Research, Development, and Innovations Infrastructures".

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TOWARD NEW ANALOGUES OF VITAMIN E: NEW POTENTIAL INHIBITORS OF 5-LIPOXYGENASE

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Many studies highlighted the biological potential of vitamin E, especially tocotrienols (T3), a vitamin E subfamily, particularly in the field of cardiovascular diseases and chronic inflammation.[1] A pharmacophore based virtual screening of these substances against various antiinflammatory targets showed that this class could be considered as potential inhibitors of 5-lipoxygenase, a key enzyme in the biosynthesis of chemoattractant and vasoactive leukotrienes. Consequently, this screening was confirmed by *in vitro* assays.[2]

However, usual natural sources of T3 provide complex mixtures involving particularly challenging purification processes. Thus, this work aims at designing and optimizing efficient semisynthesis towards pharmacologically relevant T3 derivatives from δ-tocotrienol, the main T3 isolated from *Bixa orellana* seeds, a renewable and easily available vegetal source from tropical regions, analysed mainly by HPLC chromatography. Verification of the most effective reaction conditions of semisynthesis and synthesis of other potential inhibitors of 5-LOX based on tocotrienols' structure are the following aims of the work.

During this study, the semisynthesis based on δ-tocotrienol was completely optimized and 3 new T3 derivatives were synthetized and fully characterized. Unfortunately, after several attempts, these most effective reaction conditions were not applicable to semisynthesis based on different analogues of T3 (*e.g.* δ-garcinoic acid).

The study was supported by Erasmus+ programme and from the project of Specific Academic Research (SVV 260 412).

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SECTION OF CHEMICAL SCIENCES: ANALYTICAL PART

IS SUPERCRITICAL FLUID CHROMATOGRAPHY SUITABLE FOR ANALYSIS OF NATURAL PRODUCTS?

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Supercritical fluid chromatography (SFC) has been known for several decades, but the most remarkable rebirth came in the last 20 years. Advanced version using sub-2 μm particles is ultra-high performance supercritical fluid chromatography (UHPSFC). Hyphenation to mass spectrometry (MS) opened the way to application fields such as bionalysis and natural product analysis, due to high selectivity and sensitivity. The purpose of this study is to verify the suitability of UHPSFC with PDA and MS detection for analysis of natural products, namely alkaloids from Amaryllidaceae family with effect in the therapy of Alzheimer's disease and in oncology.

We tested UHPSFC method as an alternative technique for analysis of 12 alkaloids of different structural types. The basic screening was performed on 10 stationary phases using 4 mobile phases. The most successful separation was obtained with BEH, Torus DIOL and Torus DEA columns, that became subject of following optimization. Efficient separation was a critical step due to the presence of many isomers. The analysis on a Torus DEA column with CO_2/MeOH + ACN (1:1) + 0.1% NH_4OH was the most suitable using gradient elution. These optimized conditions were used for analysis of plant extracts. Targeted and rapid analysis can be used to explain their metabolism and health effects on the human body.

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PROS AND CONS OF USING ELEVATED TEMPERATURE FOR PEPTIDE SEPARATION USING REVERSED PHASES

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Nowadays, powerful mass spectrometers are available for bottom-up LC-MS proteomic analyses. It has recently been demonstrated, however, that their capabilities cannot be fully exploited without significant improvements in peptide separation.[1] One of the easiest, but often neglected ways to enhance peptide separation efficiency is to increase column temperature. Elevated column temperature may contribute to more efficient separation by increasing low diffusivity of peptides, decreasing mobile phase viscosity and much more. On the other hand, stability of both a stationary phase and peptides limits increasing column temperature to values affording maximum effect on separation efficiency. Special care should always be taken to extended exposure of peptides to elevated temperatures during long proteomic gradients for complex peptide mixture analyses. After many experiments we explored the effect of column temperatures 30 °C, 45 °C, 60 °C, 75 °C and 90 °C on peptide separation efficiency using two columns packed either with superficial porous particles or totally porous particles. Elevated column temperature narrowed peaks for peptides eluted from totally porous particles whereas peak widths of peptides eluted from superficial porous particles did not changed remarkably. Subsequently, using a protein model we examined on-column thermal stability of peptide bonds. Surprisingly, combination of 0.1% formic acid in mobile phases and temperature 45 °C already led to detection of first degradation product. Lastly, we explored on-column chemical instability of amino acid residues based on elevated temperature. In conclusion, our results demonstrate that depending on type of particles temperature can improve peptide separation efficiency. However, it must be used wisely, particularly during long LC-MS analyses.[2]

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OPTIMIZATION OF WORKFLOW FOR METABOLOMIC ANALYSIS USING UHPLC-Q-TOF INSTRUMENTATION

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Polyethylene glycols (PEG) are widely used plasticizers, even in the equipment for buffer storage, sample manipulation *etc*. Thus, they are relatively common contaminants

along with phthalates and polypropylene glycols. These contaminants can interfere with analytes during ionization in the ion source of mass spectrometer, resulting in ion suppression of analytes. Therefore, elimination of contaminants from biological matrix is needed to accurately evaluate metabolomics data. Solid phase extraction pipette tips with porous titanium dioxide as sorbent were employed for PEG elimination.

Vaginal swabs were collected from patients suffering from vulvovaginal discomfort caused by *Candida albicans*. Samples were first extracted in phosphate buffer saline solution. The next step of sample preparation was solid phase extraction. The composition of binding solution, washing buffer, and elution agent and also number of aspiration/expelling repeats in each of these steps were optimized. The liquid chromatography separation was performed on Acquity BEH C18 column using gradient elution with 0.075% formic acid and 0.075% acetonitrile at flow rate of 0.5 mL/min in 13 minutes. Quadrupole time-of-flight high resolution tandem mass spectrometer Synapt G2-Si was employed for the data acquisition.

0.1% trifluoroacetic acid in 80% acetonitrile, 50 mM formic acid in 80% acetonitrile, 0.1% formic acid in water, 0.1% trifluoroacetic acid in water, and acetonitrile were tested as binding solutions. Water, dichloromethane, 0.1% formic acid in water, 0.1% formic acid in methanol and 0.1% trifluoroacetic acid in 5% acetonitrile were tested as wash buffers. Finally, 0.1% trifluoroacetic acid in 80% acetonitrile, 50 mM formic acid in 80% acetonitrile, 0.1% formic acid in 90% acetonitrile, 50% methanol, and 100 mM ammonium hydroxide were tested in elution step. The best combination was evaluated and used as the most appropriate sample preparation step in metabolomics workflow.

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STUDY OF α-BROMOPHENYLACETIC ACID SUITABILITY AS A MODEL ANALYTE FOR CHIRAL SEPARATIONS

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Rizvi and Shamsi reported the employment of α-bromophenylacetic acid (**I**) dissolved in water/methanol mixture (1:1, v/v) as a model analyte for chiral separations in capillary electrophoresis.[1] Recently, Kováčová proved the instability of **I** in the same solvent as well as in methanol.[2] This work was focused to determine the reaction order of the decomposition of 0.47 mM **I** by nucleophilic substitution in 50% aqueous methanol. The reaction kinetic study was performed by capillary electrophoresis (CE) in 50 μm (i.d.) polyvinyl alcohol capillary (30 cm/24.5 cm) with UV detection. The **I** and products of nucleophilic substitution (mandelic acid, α-methoxyphenylacetic acid, and bromide) were separated in 60 mM formate buffer (pH 3.0) at -30 kV; the detection λ was 200 nm. The first order reac-

tion kinetics was confirmed by linear and non-linear regression, yielding the rate constant $1.52 \times 10^{-4} \pm 2.76 \times 10^{-5}$ s^{-1} and $7.89 \times 10^{-5} \pm 5.02 \times 10^{-6}$ s^{-1}, respectively. Additionally, the identity of the degradation products was confirmed by CE coupled to mass spectrometric (MS) detection with electrospray ionization and triple quadrupole analyser. The CE-MS separations carried out in 60 mM formate buffer (pH 3.0) and in 60 mM acetate buffer (pH 5.0) confirmed the results obtained by CE with UV detection.[2] Our results provide strong evidence of the instability and fast degradation of **I** in 50% aqueous methanol indicating that **I** is not suitable as a model analyte for chiral separations in aqueous methanol medium.

The study was supported from the project of Specific Academic Research (SVV 260 412).

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DEVELOPMENT OF UHPSFC-PDA METHOD FOR THE DETERMINATION OF ATORVASTATIN AND ITS IMPURITIES

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The aim of this study was to develop ultra-high performance supercritical fluid chromatography method (UHPSFC) with PDA detection for the determination of atorvastatin and its potential impurities. Structure similarity of target analytes (stereoisomers, desfluoro and fluoro derivates) predicted their difficult separation. Simultaneously, it was necessary to achieve high sensitivity for the determination of impurities at low concentration levels (0.05% of API) as required by International Conference on Harmonisation (ICH) quality guideline Q3A.

After detailed optimization, the separation was finally performed on stationary phase HSS C18 SB. CO_2 modified with methanol/acetonitrile (2:1), 15 mM formic acid, 2.5 mM ammonium hydroxide, and 5% water was used as a mobile phase. Gradient was run from 10–18% of modifier in 3.5 min, followed by isocratic step at 18% of modifier until 9 min.

Method was validated for API (active pharmaceutical ingredient) and impurities according to the ICH quality guidelines Q2 including linearity, sensitivity, accuracy, precision, and interday accuracy and precision. Method was linear in the range of 0.1–100 µg/mL for atorvastatin and impurity C, 2–100 µg/mL for impurities A and B, 0.2–100 µg/mL for impurity lactone and 0.1–70 µg/mL for impurity D. The accuracy and precision of the method were determined at four different concentration levels for API and at three different concentration levels for impurities. The results for accuracy and precision were 96.6%–104.9%, RSD ≤ 3.7% for impurities and 101.5%–103.2%, RSD ≤ 1.6% for API. The interday accuracy and

precision were 96.0%–104.5%, RSD ≤ 3.1% for impurities and 101.1%–103.5%, RSD ≤ 1.3% for API.

In conclusion, this method was found to be suitable for determination of atorvastatin and its impurities in pharmaceutical quality control.

The study was supported from the project of Specific Academic Research (SVV 260 412) and the project STARSS (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

NOVEL AND FAST UHPLC-MS/MS ANALYSIS OF DEXRAZOXANE AND ITS POLAR METABOLITE

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Dexrazoxane (DEX), a bisdioxopiperazine derivative, is the only clinically used drug effective against anthracycline-induced cardiotoxicity. First studies indicated that DEX is a pro-drug bioactivated in cardiomyocytes by enzymatic hydrolysis of piperazine rings to its active metabolite – ADR-925. However, further research revealed that effective cardioprotection induced by bisdioxopiperazine compounds is more likely related to topoisomerase IIβ depletion induced by DEX itself.[1] The only bioanalytical method for simultaneous determination of DEX and its metabolite was developed using HPLC-MS/MS system.[2] Nevertheless, the analysis requires 30 min for each run, which does not accomplish requirements for modern bioanalysis. The aim of this project is to develop and validate a fast UHPLC-MS/MS method for determination of DEX and ADR-925 in plasma. The analyses were performed using an UHPLC system coupled to triple quadrupole mass spectrometer with ESI source in positive ion mode (both Shimadzu). Following stationary phases were tested: ZORBAX Bonus-RP (100 mm × 3.0 mm, 1.8 μm), Luna Omega Polar C18 (100 mm × 2.1 mm, 1.6 μm) and Kinetex F5 column (100 mm × 2.1 mm, 1.7 μm). Mixtures of acetonitrile or methanol with different concentrations of ammonium formate or formic acid were tested as mobile phases with various isocratic and gradient elutions. The best results were achieved on the column Kinetex F5 with 1mM ammonium formate and methanol as a mobile phase in a gradient mode. Method was partially validated within the concentration range from 0.5 to 100 μM for both compounds in plasma.

The work was supported from the project of Specific Academic Research (SVV 260 401).

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HPLC EVALUATION OF L-TRYPTOPHAN AND ITS METABOLITES IN BIOLOGICAL MATERIAL

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The purpose of this study was to develop optimized conditions for determination of L-tryptophan and its metabolites (L-kynurenine, kynurenic acid, serotonin, 5-hydroxyindole-3-acetic acid, melatonin) using high performance liquid chromatography.

Separation was achieved by the silica gel column Kinetex EVO C18 (100A, 150 × 3 mm, 5 μm) with guard column OPTI-GUARD 1 mm C18 using spectrophotometric and fluorimetric detection. Initial parameters of detection mentioned in the method were for kynurenine (absorbance at 369 nm, 227 nm and fluorescence detection Ex: 369 Em: 475). Detection and elution parameters of the method were further optimized for subsequently added analysed substances on the basis of their individual UV and fluorescence spectra.

Different types of mobile phase, different pH of buffer were examined. The final mobile phase consisted of two components:
– MF A: water + acetate buffer 0.1 M; pH 4.5; methanol in a ratio 97:3
– MF B: methanol.

The separation was performed by gradient elution. The flow rate was 0.5 ml/min. The column temperature was set at 30 °C. The injection volume was 100 μl. Total runtime was 30 min.

HPLC analysis was validated according to FDA guidelines. Vanillin was used as the internal standard. Selectivity, stability, linearity, accuracy, precision-repeatability and robustness were measured validation parameters and all found values were within acceptable ranges.

The study was supported from the project of Specific Academic Research (SVV 260 401).

HPLC METHOD FOR SEPARATION OF CHIRAL IMPURITIES OF DOLUTEGRAVIR

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Following ICH Guidelines Q3A(R2) and Q3B(R2), every new drug substance and every new drug product must be checked for pharmaceutical quality based on Good Manufacturing Practice (GMP).

In this study, three batches of a new drug substance and one batch of a new drug product (tablets) are tested by validated analytical method using chiral stationary phase Lux Cellulose-4. This HPLC-UV method is able to separate the main substance which is an antiviral drug – sodium salt of dolutegravir and its stereoisomeric impurities as well as some other related substances.

The main aim is to establish this method as a future monograph method in The International Pharmacopoeia for impurities testing of dolutegravir sodium.

Impurities in pharmaceutical products have potential carcinogenic, mutagenic, or teratogenic effects. Herein even low chiral contamination of enantiomer and also diastereomer developed through the synthesis of the substance can be detected and separated. Therefore, due to this analytical procedure it is possible to insure chiral purity, safety and quality of the drug dolutegravir.

The study was supported by Erasmus+, and from the project of Specific Academic Research (SVV 260 401).

IN VITRO TRANSDERMAL PERMEATION OF K1280

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K1280 (2-(hydroxymino)-*N*-[2-(pyrrolidin-1-yl)ethyl]ethanamide) is a new potential nerve agent antidote synthetized in the Department of Toxicology and Military Pharmacy (Faculty of Military Health Sciences). Compared to conventionally used oxime reactivators of acetylcholinesterase, K1280 is an uncharged non-quaternary compound. The absence of the permanently charged nitrogens is believed to improve oxime's ability to penetrate through biological barriers.[1]

Permeation of K1280 through skin barrier *in vitro* was determinated by the Franz diffusion cells using split thickness porcine skin as a model barrier. Full time of the experiment was 24 hours and receptor fluid was collected hourly. Samples of the receptor fluid were measured by optimized LC-MS/MS method. Atropine was utilized as the internal standard.

Permeation measures (steady state flux J_{ss}, lag time T_{lag} and permeation constant K_p) were calculated by SAMPA software. The permeation of K1280 was then compared with standard compound caffeine.

Based on the results obtained in this study, the K1280 oxime showed the potential to penetrate through skin *in vitro* indicating the possible use as a transdermal prophylactic against organophosphate intoxication.

The study was supported from the project of Specific Academic Research (SVV 260 401).

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UHPLC-MS/MS ANALYSIS OF JAS-2, THE NOVEL ANALOGUE OF DEXRAZOXANE

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Dexrazoxane (DEX) is a cardioprotective drug clinically used against anthracycline induced cardiotoxicity. 4,4′-(Butane-2,3-diyl)bis(piperazine-2,6-dione) (JAS-2) was synthetized as a novel, more effective analogue of DEX. It was reported that inhibition of topoisomerase II, which seems to be crucial for cardioprotective effect, is mediated by a meso form while the racemic JAS-2 is almost ineffective.[1] Nevertheless our *in vitro* experiments with racemic JAS-2 showed some cardioprotective effect. The aim of this work is 1) to examine the possible role of contamination of racemic JAS-2 with the meso form in the results of *in vitro* experiments and 2) to develop solid phase extraction (SPE) for JAS-2 and its metabolite (JAS-2_{met}) from plasma. The UHPLC coupled with a triple quadrupole mass spectrometer with ESI+ ion source, Bonus-RP column (100 × 3.0 mm, 1.8 μm) and formic acid (0.25%) with methanol as a mobile phase were used for separation of different forms of JAS-2. Analysis of JAS-2 and JAS-2_{met} was achieved on Luna Omega Polar column (100 × 3.0 mm, 2.5 μm) with a guard column. A mobile phase containing ammonium formate and acetonitrile were used. Four types of SPE columns (Discovery DSC-PH 100 mg/1 ml, Discovery DSC 18 100 mg/1 ml, Supel Select HLB 30 mg/1 ml, and Hypersep Verify AX 130 mg/1 ml), different types of washing solvents (H_2O, 5% MeOH, HCOOH) and elution solvents (ACN, MeOH, ACN + 10% HCOOH) were tested. In racemic JAS-2 we detected less than 0.1% of JAS-2 meso form. Hence, it seems unlikely that this low concentration can be responsible for the inhibition effect. The highest recovery of JAS-2 and JAS-2_{met} from plasma was achieved on Hypersep Verify AX column. Nevertheles, the presence of formic acid, which was necessary for elution of JAS-2_{met}, increased matrix effects and led to the high signal supression of JAS-2_{met}. Therefore, future extractions will be focused only on JAS-2, which is the active substance.

The study was supported from the project of Specific Academic Research (SVV 260 401) and by the Grant Agency of Charles University (Project No. 1550217).

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SECTION OF TECHNOLOGICAL SCIENCES

HOMOGENIZATION OF POWDER BLENDS USING A TURBULA MIXER

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Homogeneity is an important quality factor in the pharmaceutical industry because it directly influences the content uniformity and plays an essential role in the efficacy and safety of solid dosage forms.[1] In this experimental work, influencing the homogeneity of the powder mixture of acetylsalicylic acid (ASA) and microcrystalline cellulose (Avicel PH102) due to the rotational speed of the mixing vessel in a range of 23–101 rpm and the mixing time was studied using the Turbula shaker mixer. Within time interval 2–62 minutes, the content of ASA was measured by near-infrared spectrometry. The expression of standard deviation was used to evaluate the homogeneity of samples. The best results were detected at a rotational speed of 34 rpm.

The study was supported by the Grant Agency of Charles University (Project No. 1286218/2018).

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PREPARATION OF BIODEGRADABLE NANOPARTICLES FOR HYDROPHILIC MACROMOLECULAR DRUGS DELIVERY

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This study investigates the formulation of nanoparticles containing hydrophilic components (*e.g.* proteins). Selected material was PLGA based compounds synthesized at Department of Pharmaceutical Technology. Encapsulated compounds were Rhodamine B, FITC labelled dextran and FITC labelled albumin. Selected methods of nanoparticles formulation were double-emulsion technique and nanoprecipitation. Prepared nanoparticles were purified by three cycles of centrifugation and encapsulation efficacy and recovery yield were measured. Effect of different polymers and stabilizers was followed. More specifically, the principal objective was to explore the differences between parameters of the

created nanoparticles, such as size, zeta potential and efficacy of encapsulation. Changes in these characteristics were brought about by the chosen polymers, stabilizers, encapsulated compound, length of centrifugation period.

Prepared nanoparticles had size ranging between 150–474 nm and zeta potential approximately 30 mV. Even though the main goal of the study was to efficiently encapsulate protein, the amounts of encapsulated albumin were lower compared to rhodamine B or dextran. Main obstacles were presented by separation of nanoparticles from medium. The centrifugation time had a significant impact on the amount of collected nanoparticles. Nanoparticles tended to aggregate during the centrifugation. In case of smaller particles, centrifugation proved to be ineffective way of purification, therefore it was problematic to gain them. From the observation of the two methods working with the same substance (FITC labelled dextran) it is clear, that nanoprecipitation is more suitable for encapsulation of branched polymers, while double-emulsion is more appropriate for the linear PLGA polymer-based nanoparticles.

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A STUDY OF TABLETING MATERIALS AND TABLETS WITH THE COMBINATION OF MICROCRYSTALLINE CELLULOSE AND MANNITOL FOR ORALLY DISINTEGRATING TABLETS

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This work compares the co-processed dry binder Avicel® HFE-102 containing 90% microcrystalline cellulose and 10% mannitol with a physical mixture of related dry binders, microcrystalline cellulose (Avicel® PH-102) and mannitol (Pearlitol® 100SD) in the ratio of 9:1.

Flow properties, compressibility, lubricant sensitivity, tensile strength and disintegration time of tablets were evaluated. Compressibility was evaluated by means of the energy profile of compression process, and lubricant sensitivity by means of the lubricant sensitivity ratio. The results were also compared with the microcrystalline cellulose for direct compression Avicel® PH-102 alone.

The flow properties of the co-processed dry binder Avicel® HFE-102 alone and the physical mixture are comparable. Avicel® HFE-102 showes higher values of the energy of plastic deformation, tensile strength of tablets, and a markedly lower lubricant sensitivity than the physical mixture of dry binders. Tablets with the co-processed dry binder Avicel® HFE-102 show short disintegration. Avicel® HFE-102 is suitable for the use in orally dispersible tablets.

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STUDY OF MICROSTRUCTURE OF SKIN BARRIER MODEL USING DEUTERATED CERAMIDES

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Ceramides (Cer) are sphingolipids, which participate in various biological processes. In mammalian skin, Cer are localized in the uppermost layer of epidermis, the *stratum corneum* (SC). In this layer, Cer along with cholesterol (Chol) and free fatty acids form multilayer lamellae of intercellular lipid matrix.

The skin lipid arrangement in SC is still unclear. To evaluate the skin lipid arrangement, skin membrane models with labelled (deuterated) lipids have been used. Therefore, the aim of this work was to synthesize sphingosine with deuterated chain and Cer based on deuterated sphingosine, *i.e., N*-lignoceroyl sphingosine-d_{28} (with lignoceric acid acyl (C24) (*d*-CerNS) and *N*-lignoceroyl-d_{47} sphingosine-d_{28} (*dd*-CerNS) and to study their phase behaviour and arrangement in model membranes.

Synthesis of deuterated Cer started from elimination of 1-pentadecanol-d_{31} to obtain a deuterated terminal alkene. Next, vinylation of (*S*)-Garner's aldehyde led to an intermediate, which was treated in Grubbs' metathesis with terminal alkene. The product of Grubbs' metathesis (protected deuterated sphingosine) was then deprotected under acid conditions; free sphingoid base was acylated by protonated or deuterated lignoceric acid using water soluble carbodiimide.

Afterwards, synthesized *d*-CerNS and *dd*-CerNS were incorporated into SC model membranes. Model mixtures contained *d*-CerNS or *dd*-CerNS, (deuterated) lignoceric acid and Chol in 1:1:1 molar ratio with an addition of cholesteryl sulfate (5wt%). Overall, four types of model membranes with different representation of deuterated methylene (CD_2) chains, were studied by temperature depended infrared spectroscopy at temperature from 28 °C to 100 °C. A phase behaviour (conformation, lateral arrangement, and miscibility) of model lipid membranes was investigated. The results of this study could be helpful in explaining the (patho)physiological arrangement of SC lipids.

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THE STUDY OF INFLUENCE OF THE MEASUREMENT METHOD ON STATIC ANGLE OF REPOSE OF FREE-FLOWABLE EXCIPIENTS

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Powder flowability plays an essential role in their manufacturing into solid dosage forms.[1] Measuring of the static angle of repose (AOR) represents one out of the simple methods of flowability testing. This work is focused on AOR measurement for selected free flowable excipients for direct compression: sorbitol (Merisorb 200), lactose (Excipress GR 150), microcrystalline cellusose (Avicel 200) and dicalcium phosphate anhydrous (DI-CAFOS A150) or for dry powder inhalers: lactose (InhaLac 120). The results obtained using two different equipments for measuring the AOR: automatic tester Erweka and prototype tester for the orifice size 6 mm of a conical hopper were evaluated by analysis of variance (ANOVA, $\alpha = 0.05$, $P < 0.01$). Significant difference between methods was detected. According to generally accepted scale,[2] tested materials were classified as passable flowing using tester Erweka. On the contrary, good flow behaviour was detected using the protype.

The study was supported by the Grant Agency of Charles University (Project No. 1286218/2018) and from the project of Specific Academic Research (SVV 260 401).

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OPTIMIZATION OF DRUG-LOADED NANOPARTICLES PREPARATION

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Two methods of nanoparticles preparation from materials based on of poly(lactic-co-glycolic acid) were used. First method was emulsion-sonication, where the particles were formed using high frequency ultra-sound probe. The second method was spontaneous emulsification, where two solvents with different hydrophilicity were used.

Nanoparticles were prepared from linear PLGA and PLGA branched by polyacrylic acid. Nanoparticles prepared by emulsion-sonication method were stabilized by three different surfactants – poloxamer Pluronic F127, polysorbate Tween 20 and poly(vinyl) alcohol. Each surfactant was used in three different concentrations. Spontaneous emulsification nanoparticles prepared by spontaneous emulsification were stabilized by poly(vinyl) alcohol. Combination of solvents ethanol:aceton in various ratios was used throughout

experiments. Other solvents were also tested, however using ethanol:aceton leads to nanoparticles with the best properties. Rhodamine B, a fluorescent dye, was used as a model substance for encapsulation experiments.

Different properties of resulting nanoparticles were assayed – size, polydisperzity, encapsulation efficacy and reloaded yield. Using both methods nanoparticles of the size of 200 nm and less were produced. In case of spontaneous emulsification also with very good polydispersity index, ranging between 0.2 and 0.1. Encapsulation efficacy at the produced particles was ranging between 60% and 95%. Particles with size smaller than 100 nm, where the value of encapsulation efficacy decreased considerably owing to difficulties with purification. Recovery yield of the polymer moved by the prepared nanoparticles from 40% to 60%. Generally, emulsion-sonication method proved to be suitable for preparation of very small nanoparticles (down to 50 nm). Spontaneous emulsification is characterized by high recovery yields and very good polydisperzity of resulting nanoparticles.

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LIPOSOMES FOR (TRANS)DERMAL DELIVERY

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Delivery of active compounds through the (trans)dermal route has lately gained more attention over other conventional routes of administration due to its advantageous reduction of side effects, avoidance of first-pass metabolism and prolongation of short half-life drugs effect.[1] In the field of nanotechnology, liposomes seem to be one of the most efficient vehicles for drug delivery.[2] The aim of this study is to preparate, purify and characterize conventional imiquimod-loaded liposomes before testing their ability to efficiently deliver the drug to the epidermal layers.

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TERBINAFINE RELEASE FROM PLGA-BASED NANOPARTICLES

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Cutaneous fungal infections are a major cause of visit to dermatologist. The availability of several effective antifungal drugs and their therapeutic outcome is less than optimal due to limitations related to drug physicochemical properties and toxicity. Nanoparticles hold great promise to overcome these limitations due to their ability to enhance drug aqueous solubility, bioavailability and antifungal efficacy.[1] Biodegradable polymeric nanoparticles (NPs) based on poly(lactide-*co*-glycolide) branched on polyacrylic acid (PLGA/A) or dipentaerythritol (PLGA/D) loaded by terbinafine were prepared by the nanoprecipitation method. The particle size, polydispersity (PDI) and zeta-potential were measured. Nanosuspension was purified by centrifugation, and encapsulation efficiency (EE) was calculated. Dissolution tests were carried out at pH 3 or pH 5 at 37.0 °C. The amount of drug released was determined spectrophotometrically at 223 nm and ultra-high performance liquid chromatography. Permeation experiment was performed with the human skin using Franz cells. The standard *in vitro* procedure[2] was used to evaluate the antibacterial effect of the nanoparticles. NPs based on PLGA/A were smaller, possessed better PDI, and higher zeta potential in comparison to PLGA/D, also the EE was higher. Terbinafine release proceeded more rapidly from PLGA/D, and at pH 3.0. Permeation experiment showed no detectable amount of terbinafine in the acceptor phase, and its location in epidermis. The growth of *Candida* spp. was inhibited by terbinafine diffused through agar gel and achieved a concentration inhibiting growth of microorganisms. From the imaging documentation, the antimicrobial potential of all the evaluated samples was evident from the test preparations. The promising results of the performed experiments have shown that biodegradable polymeric nanoparticles based on PLGA/A loaded by terbinafine can be effective in the treatment of cutaneous fungal infections.

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CALCIUM PHOSPHATE BONE CEMENTS: SYNTHESIS, CHARACTERIZATION AND RELEASE PROPERTIES

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Non-healing bone traumas are currently a complication which may disable a patient from active life for a long period.[1] Since the bone mass consist mostly of hydroxyapatite, a derivative of calcium phosphate, using calcium phosphate cement (CPC) as an injectable bone substitute seems logical. In addition, the possibility to incorporate in the formulation a drug that would support the healing process would be beneficial.[2] The aim of this research was to synthetize a high quality α-tricalcium phosphate (α-TCP), to characterize its properties (X-ray diffraction, Raman spectra, compressive strength, injectability, washout resistance) and investigate its release properties after loading ibuprofen. The obtained data revealed that the conversion of αTCP to hydroxyapatite is finished in just one day. Additionally, a significant difference in compressive strength of pure CPC and ibuprofen loaded CPC was noticed while drug addition did not affect the crystalline structure of CPC. Furthermore, the release of ibuprofen lasted more than 21 days (reaching 80% of the initial amount). Finally, the injectability as well as the washout resistance appeared to be optimal. Our experimental results suggest that the CPC is a promising drug carrier.

The study was supported from the project of Specific Academic Research (SVV 260 401).

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ADHESIVE PROPERTIES OF THIN LAYERS BASED ON PLASTICIZED POLYESTERS

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Biodegradable branched polyesters found their use not only in surgery as sutures, but also in pharmacotherapy as drug carriers. Unlike linear polymers, the branched polyesters are endowed with continual degradation and with low degree of swelling, which are the advantageous characteristics for controlled drug release, decrease in frequency of dosage, reduction of side effects and improvement of patient compliance. This work

deals with rheological, adhesive, and dissolution properties of thin layers based on polyester of D,L-lactic acid and glycolic acid branched with 8% of dipentaerythritol (8D) and plasticized with 10%, 20% and 30% of methyl salicylate (MS). Additionaly 10% of salicylic acid was incorporated into the plasticized branched polyester and the salicylates release from thin layer were studied. The porcine gastric mucin was used as model substrate. Adhesive properties were established by equation of rheological synergism presented by Hassan and Gallo[1] based on viscosity of polymer, mucin and their mixtures measured at shear rate of 10 s^{-1}. Dissolution test was performed at 37 °C using phosphate buffer pH 7.4. Amount of salicylate released was determined spectrophotometrically at 298 nm. Results of the work showed good adhesive properties of tested biodegradable polymeric thin layers on model mucus substrate. The prolonged release of salicylates was influenced by the viscosity of the polymeric systems.

The study was supported from the project of Specific Academic Research (SVV 260 401).

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ACIDORESISTANT POLYMERIC NANOPARTICLES: PREPARATION AND ASSESSMENT

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Use of oral drug delivery nanosystems has a great potential in therapy of inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis. Nanosized delivery systems are more efficiently accumulated at the inflammatory site, targeting specifically macrophages to resolve inflammation locally and reduce systemic adverse effects.[1–3]

The aim of this research was to prepare pharmaceutical formulations based on polymeric nanoparticles. Three types of poly(lactide-co-glycolide) – two linear and one branched polymer – together with the acidoresistant polymer cellacefate (cellulose acetate phthalate, CAP) in various ratios were used to prepare nanoparticles by nanoprecipitation method. Rhodamine B was used as model active substance. The effect of acidoresistant component content on size and zeta potential of the nanoparticles was evaluated. Furthermore, dissolution tests were performed at both acidic and physiological pH.

It was found that CAP doesn't have any significant effect on size of the particles and their stability. Moreover, the release of rhodamine B in the acidic environment decreases with increasing proportion of CAP in the nanoparticles. Nevertheless, the nanoparticles consisting only from poly(lactide-co-glycolide) also showed acidoresistance that could be explained by their physicochemical properties determined by increased carboxyl content within branched polymer structure.

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EVALUATION OF TERBINAFINE ENCAPSULATION EFFICIENCY IN POLYESTER NANOPARTICLES

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The presented work is focused on evaluation of terbinafine encapsulation efficiency in biodegradable polymeric nanoparticles (PNPs) prepared employing nanoprecipitation method. In the experiment, the relation of used polymer and its concentration to the drug entrapment as well as the influence of cetrimide added to stabilize the nanosuspensions were assessed. To form the nanoparticles, linear p*oly*(*lactide-co-glycolide*) copolymer (PLGA), and its derivatives branched with 2% polyacrylic acid (A2), or 8% dipentaerythritol (D8) were chosen in two concentrations.[1] The amount of encapsulated drug was calculated using the results of terbinafine content in the samples obtained after centrifugation of the original nanosuspensions determined by high-performance liquid chromatography. Characteristics of prepared PNPs were also measured. Afterwards, the findings were compared with the data found in the literature to elucidate the phenomena observed.[2] There was an inverse correlation between the amount of surfactant and encapsulation efficiency and the polymer structure revealed to be very important. A2 provided the most promising results as it allowed to create nanoparticles of approximately 200 nm in size which were able to accommodate up to 24% terbinafine used.

The study was supported by the Czech Science Foundation (Project No. 17-06841S) and from the project of Specific Academic Research (SVV 260 401).

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SECTION OF SOCIAL AND CLINICAL PHARMACY

ANALYSIS OF CARDIOTOXICITY OF PULSE GLUCOCORTICOID THERAPY IN RHEUMATIC PATIENTS

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Pulse glucocorticoid (GC) therapy refers to the administration of ≥ 250 mg prednisone equivalent a day (usually intravenously) for one or few days. The immediate non-genomic effects produced by pulse GC could be additive to the genomic effects and contribute to the termination of acute exacerbations of immunologically mediated diseases.[1] Serious cardiovascular adverse events (AE) have been reported after pulse GC therapy, but its effect on QT interval is not well described. The aim of our retrospective, observational, cross-sectional study was to analyze the influence of pulse GC therapy and other known QTc-prolonging risk factors (RF) on ECG changes, in particular on QT interval, as prolonged QTc (QTc > 450 ms in men, QTc > 460 ms in women) interval may lead to potentially lethal ventricular arrhythmias.[2]

Data from medical records were used, 307 courses of pulse therapy were included (66.1% women). QT and QTc before (QT, QTc1) and after pulse therapy (QT, QTc2) and ΔQTc (QTc2 – QTc1) were determined. Average ΔQTc was 14.6 ms ($p < 0.05$). ΔQTc > 0 ms was found in 75.2% patients (n = 231 from 307), QTc > 450 ms in 7.7% men (n = 8 from 104) and QTc > 460 ms in 4.4% women (n = 9 from 203). Differences between ΔQTc in patients without RF and with ≥ 1 RF were not significant. Cardiac AE were observed in 5.9% (n = 18 from 307) courses, ΔQTc 15.7 ms ($p < 0.05$). 50.0% of them (n = 9 from 18) lead to premature cessation of pulse therapy, but ΔQTc was not significant and QTc above borderline was found in only one of these patients. Ventricular arrhythmia was not observed.

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FUNGEMIA IN THE UNIVERSITY HOSPITAL HRADEC KRÁLOVÉ IN THE PERIOD 2007–2018

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Fungemia, presence of fungi in the blood, is a life-threatening condition occurring especially in critically ill and immunocompromised patients. *Candida albicans* remains the most common cause, although contribution of non-albicans species has increased over past decade.[1]

Present study aims to describe current epidemiological situation in the University Hospital in Hradec Králové. Data on bloodstream isolates of the yeasts and patient characteristics were obtained from the laboratory information system and further analyzed.

From November 2007 to December 2018, fungemia was identified in 235 patients with overall incidence of 0.51 cases per 1000 admissions. Most of fungemic patients (64.3%) were hospitalized in intensive care units. *Candida albicans* accounted for half of the blood cultures (52.8%) followed by *C. glabrata* (14.5%), *C. tropicalis* (8.5%) and *C. parapsilosis* (6.8%). In general, *Candida* strains showed high antifungal susceptibility to echinocandins according to CLSI standard breakpoints. On the other hand, increased minimum inhibitory concentrations of azole drugs were observed in some of *C. glabrata* and *C. parapsilosis* isolates.

Incidence of fungemia, fungal spectrum and susceptibility to antimycotics in the fungi isolated in the University Hospital in Hradec Králové during the follow-up period corresponded to those of other hospitals in the Czech Republic.[2]

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COMPLEMENTARY AND ALTERNATIVE MEDICINE IN ENQUIRIES OF DRUG INFORMATION CENTER

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Drug Information Center (DIC) of the Faculty of Pharmacy in Hradec Králové, and University Hospital in Hradec Králové was established in 1994. Major activity of the

center is providing expert information about drugs – primarily in the form of answers on various medicines-related enquiries sent to the center by healthcare professionals. Aims of this study were to analyse enquiries related to complementary and alternative medicine (CAM) in the period 1994–2017 and to evaluate DIC's activity through feedback from questioners.

CAM enquiries were searched in the complete DIC database of enquiries. The analysis was focused on questioner's profession, region, urgency of enquiry, type of enquiry, professional CAM information resources used or time needed for resolving CAM enquiry. Moreover, the feedback of questioners, who sent the enquiry to DIC from 2015 to 2017, was obtained using an online questionnaire containing 18 items – sociodemographic data and awareness of DIC, quality of answers to enquiries and satisfaction with provided services, and lastly CAM related issues.

The total number of accepted CAM enquiries in the study period was 205 (of all 2204 enquiries), with the highest number in 2003 (26; 12.7%). Typical issues concerning CAM enquiries were related to interactions and indications or contraindications of CAM (58; 28.3%). Average time for resolving a CAM enquiry took 141 minutes. The feedback questionnaire was delivered to 94 health professionals with response rate of 40 (42.6%). Majority of respondents (35; 87.5%) were completely satisfied with services, especially because of detailed, comprehensive, clear and understandable answers. Answers were fully or partially used for a specific patient or for the needs of healthcare professionals. CAM related issues were mostly solved by pharmacists (24; 92.5%), especially community pharmacists. Database analysis showed the same pattern – 126 (53.7%) enquiries were sent to DIC by community pharmacists.

DIC's services received good feedback and all of respondents would use its services again including CAM related enquiries sent especially by community pharmacists.

The study was supported from the project of Specific Academic Research (SVV 260 417).

ANALYSIS OF NON-CARDIAC ADVERSE EVENTS IN RHEUMATIC PATIENTS WITH GLUCOCORTICOID PULSE THERAPY

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Intravenous glucocorticoid (GC) pulse therapy is effective in life threatening flares of rheumatic diseases and is considered to have low risk of adverse events (AE). However, it is not free of complications and frequency of AE in rheumatic patients is not completely clear, partly owing to additive non-genomic to genomic mechanism, which pulsed GC possess.[1]

The aim of this work is to analyze occurrence of non-cardiac AE in real-life setting, analyze its risk factors and find selected, more susceptible populations with underlying risk factors.

Patients were administered 1000 mg methylprednisolone in 3 to 5 doses on alternating days. Analysis includes 278 rheumatic patients with 326 pulse administrations. Majority of patients were women (67%) and median age was 55 years. Patients mostly suffered from connective tissue diseases (n = 191, 59%) and systemic vasculitis (n = 120, 37%). Data were collected retrospectively from their medical records.

Fifty-seven non-cardiac AE appeared during 42 (13%) pulse courses and 13 (4%) cases had to be terminated due to serious nature of occurred AE. Most common adverse event was hypertension in 4.6% (n = 15), metabolic disturbances in 4.3% (n = 14) and infections in 2.1% (n = 7) subjects. Common non-cardiac AE were also amylase increase and diarrhea, each appearing in 1.2% (n = 4) subjects. Occurrence of other AE was less common (< 1%).

Limitations of this study are based on its retrospective character, as we got to observe only what is being monitored in GC pulse regimen by default. Occurrence of serious AE was common (4%), but when assessing right risk management having all risk factors in mind, our study presents GC pulse therapy as relatively safe regarding non-cardiac AE.

The study was supported from the project of Specific Academic Research (SVV 260 417).

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ANALYSIS OF INDIVIDUAL COUNSELLING WITH PATIENTS IN COMMUNITY PHARMACY

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Pharmaceutical care in a community pharmacy can be provided as part of individual consultations. These are focused on drug-related problems (DRPs), monitoring risk factors and evaluating individual risk levels for various diseases and self-medication. The aim of this work was to analyze individual consultations realized in community pharmacy in period of 2009–2019.

Professional individual consultations were provided in the form of a discrete interview between patient and pharmacist in a consulting room of a basic type pharmacy with two dispensing places. Pharmacy is located in a municipality of up to 5,000 inhabitants, where no other pharmacy is available. A written record was derived from

each consultation. During the consultation, information from the patient's history was obtained: demographic data, personal history data, lifestyle, use of medicines and food supplements including dosage information. The identified DRPs were evaluated using the Pharmaceutical Care Network Europe V5.1 classification. The results were described by descriptive statistics.

A total of 346 consultations were performed in 148 patients over a defined period. Most patients (81; 55%) attended more than one consultation. In 113 (76%) cases they were women. The mean age of the patients was 68 ± 14.5 years. On average, patient used 7 drugs. Majority of DRPs involved problems with choice of drug (148; 32%) or administration of drugs (143; 31%).

The analysis indicated that providing individual consultations in the pharmacy can, among other things, strengthen the culture of safety in use of pharmacotherapy. Conclusions need to be verified on a larger sample of pharmacies.

The study was supported from the project of Specific Academic Research (SVV 260 417).

ATTITUDES AND BELIEFS ON HUMAN PAPILLOMAVIRUS INFECTION AND VACCINATION

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Human papillomavirus (HPV) infection is the most common sexually transmitted disease. Nearly 80% of world's population will get HPV infection at some point of their lives. This infection is associated with more than 99% cases of cervical cancer, which is the second most common malignancy of women. The most reliable protection against HPV is vaccination.[1,2]

The aim of the work was to find out knowledge of high school students about HPV infection and related diseases. In addition, to find out the vaccine coverage rate against HPV and attitudes of students to this vaccination. Data were obtained by survey, which took place at two high schools in the Ústí Region in 2018. Overall, 291 questionnaires were used.

Average age of the respondents was 17 years (ranging 15–19 years) and 73.5% of respondents were women. Only one of the students did not know the concept of cervical cancer or penile cancer. More than half of the students (56.7%) have already heard about the human papillomavirus vaccine. The vaccine coverage rate at these schools was 62.5% (60.5% women, 2% men). The most frequently reported reasons for not being vaccinated were related to the doubt about the vaccine efficacy, the fear of undesirable effects and distrust of the vaccination.

Compared to men, women had better knowledge about HPV infection, cervical cancer and vaccination. Equally, the knowledge was better with higher age of the respondents. The vaccine coverage rate could be enhanced by increasing awareness at target age groups.

Also, better knowledge about vaccination could positively change students' attitude to the HPV vaccination.

The study was supported from the project of Specific Academic Research (SVV 260 417).

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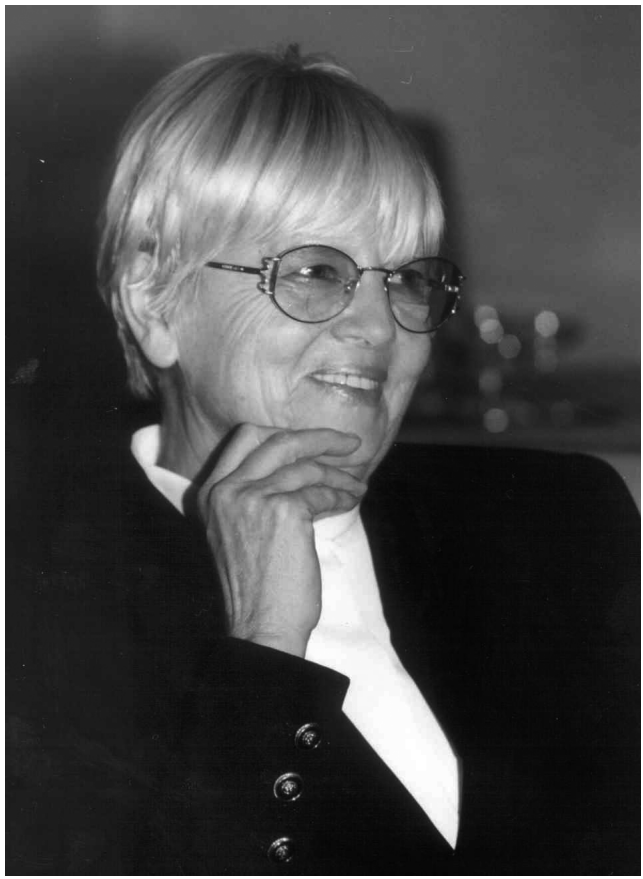
SOCIAL HAPPENINGS

JUBILEE OF Prof. RNDr. EVA KVASNIČKOVÁ, CSc.



In May 2019, Professor Emeritus of Charles University, former Vice-Rector of Charles University, former Dean of the Faculty of Pharmacy of Charles University in Hradec Králové Prof. RNDr. Eva Kvasničková, CSc., celebrated 85 years.

The native of Pardubice, she graduated from the Faculty of Pharmacy of Masaryk University in Brno in 1958. Then she worked for several years in the biochemical laboratories of the 1st Internal Clinic of the University Hospital in Hradec Králové. This work led her to specialize in biochemistry. She then spent most of her working life with the faculties of Charles University in Hradec Králové. From 1967 to 1971 she was an assistant professor at the Department of Medical Chemistry and Biochemistry of the Faculty of Medicine and in 1971 she moved to the then established Faculty of Pharmacy of Charles University. At the beginning of normalization, this faculty provided a relatively looser political atmosphere for the less politically reliable young teachers like Dr. Kvasničková. Moreover, within the Faculty of Pharmacy, the then small core of the Department of Biochemistry formed a rare friendly intellectual and research microclimate, which shaped its members for years, ideologically, intellectually and culturally, and to which Dr. Kvasničková made a significant contribution.

The Faculty of Pharmacy then remained her workplace for another more than forty years. Throughout these years, Professor Kvasničková's pedagogical work was a basic pillar of teaching biochemical disciplines at the Faculty of Pharmacy. For four decades, she was the main lecturer in General Biochemistry and moreover she established a separate special subject – Xenobiochemistry. At the same time she grew up, especially in the first years under the influence of Prof. Ivo Hais, to be a prominent scientific personality. In line with the goals of pharmaceutical research, she focused on studying the biotransformation of potential drugs and also published most of her scientific work in the field of xenobiochemistry. She received her habilitation in 1988 at the Faculty of Pharmacy in Bratislava, and was appointed professor of biochemistry in 1997 at Charles University.

The 1990s were a great impetus for her and finally enabled her to fully develop her organizational and managerial skills. In 1997–1999 she was the Dean of the Faculty of Pharmacy and then from 2000 she was the vice-rector of Charles University for two terms. She applied her important position in Czech drug research mainly as the leading researcher of the Research Centre of Structure and Mechanism of Action of Potential Drugs LN00B125, which has been successfully working under her leadership. She admirably managed the combination of the vice-rector's position in Prague with pedagogical and research work in Hradec Králové, including the management of graduates and doctoral students. She retired in 2013, but did not lose contact with either the Department of Biochemical Sciences or with the Faculty of Pharmacy.

Professor Kvasničková, together with her husband who is a professor of internal medicine, raised two children. Their daughter is a successful medical doctor and their son is a successful pharmaceutical manager.

In addition to her pedagogical and scientific achievements, Eva Kvasničková is also a personality with broad cultural interests. Since her youth, she has also been involved in sport activities. She loved skiing, playing tennis, and so far, in proportion to her current physical abilities, she enjoys golf.

On behalf of all my colleagues, I wish Professor Kvasničková many more years of health, optimism and much joy of life.

Jaroslav Držata

PharmDr. MAGDA VYTŘÍSALOVÁ, Ph.D., PASSED AWAY

PharmDr. Magda Vytřísalová, Ph.D., an assistant professor of the Department of Social and Clinical Pharmacy passed away on 31 December 2019 at the age of 40 years.

She came from the Moravian town Uničov. Having completed the high school she studied pharmacy at the Faculty of Pharmacy in Hradec Králové and graduated in 2003. Then she continued with the doctoral study of clinical pharmacy under the supervision of

Prof. RNDr. Jiří Vlček, CSc. and successfully defended her doctoral thesis in 2010. Her main research interests included compliance and persistence to treatment of various types of diseases, public health, osteoporosis in the primary care system and drug consumption. With her team, she has published about thirty professional original and summary works in domestic and foreign periodicals indexed not only in the Web of Science and SCOPUS databases. Her publishing activity also included the very successful monograph Clinical Pharmacy II, which together with prof. Jiří Vlček edited.

With the start of her doctoral studies, Magda began to engage in pedagogical activities and later as an assistant professor of the department she significantly participated in teaching Social Pharmacy, Professional Information on Medicines and guaranteed the subjects General Principles in Health Care, Pharmacoeconomics and Evaluation of Health Interventions, and Regulatory Affairs in Pharmacy. Since 2003, she has also worked as a pharmacist and especially as a professional editor of the journal Remedia (2006–2012).

Magda was a fighter in both her personal and professional life and was able to pursue the goal she had set out with immense tenacity and invention. She showed an innovative approach to teaching and was very close to habilitation. Classical music, playing the piano and dancing were her main personal interests.

Her early death hit us hard. Let us dedicate a silent memory to her in our minds.

Colleagues from the Department of Social and Clinical Pharmacy

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, center alignment)

<KoukalFig2.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, center alignment).

Results and discussion – 12, left alignment

Acknowledgements – 12 italic, left alignment, *e.g.*
This work was supported from the project of Specific Academic Research (SVV 260 183).

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.

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