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CONTENTS

ABSTRACTS

8th POSTGRADUAL AND 6th POSTDOCTORAL SCIENTIFIC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ, CHARLES UNIVERSITY, HRADEC KRÁLOVÉ, 24–25 JANUARY 2018

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

PYRAZINAMIDE: NOT THE END OF THE STORY

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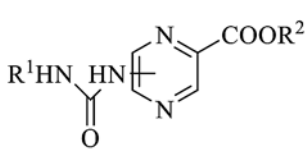
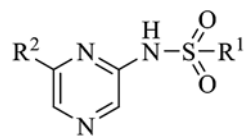
Despite being an old disease, tuberculosis remains the leading cause of death from infectious diseases at present time.[1] Among anti-tubercular agents, pyrazinamide particularly has captured research attention. Several new specific mechanisms have been recently identified by which pyrazinamide exerts its antimycobacterial effect. This achievement opened a window for possible structural modifications in order to improve its biological activity and overcome emerging resistance. We will discuss two derivatization approaches of pyrazinamide. In the first series **(1)**, urea moiety was introduced to the pyrazine core. Among all prepared compounds, propyl 5-(3-phenylureido)pyrazine-2-carboxylate (MIC_{Mtb} = 1.56 μg/mL, 5.19 μM) and propyl 5-(3-(4-methoxyphenyl)ureido)pyrazine-2-carboxylate (MIC_{Mtb} = 6.25 μg/mL, 18.91 μM) had high antimycobacterial activity against *Mtb* H37Rv with no *in vitro* cytotoxicity on HepG2 cell line up to the highest tested concentrations.[2]



R^1: Alkyl/Aryl Substituents
R^2: H/C_3H_5
(1)



R^1: Aryl Substituents
R^2: H/Cl
(2)

In the second series **(2)**, different pyrazine sulfonamides were prepared. Synthesized compounds are being evaluated for their biological activities, including anti-infective and any possible anti-cancer properties. Obtained results will be discussed in the presentation.

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 401) and (SVV 260 416), as well as by the Grant Agency of Charles University (project C-C3/1572317) and the Czech Science Foundation (project No. 17-27514Y).

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PREPARATION OF BENZODIAZINES WITH BRONCHODILATORY ACTIVITY

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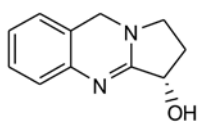
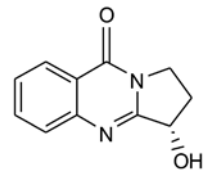
Asthma bronchiale, a chronic inflammatory disease, is becoming increasingly prevalent in most developed and many developing countries. Natural products have historically been an excellent source of new drugs for the pharmaceutical industry.

(-)Vasicine (**1**) and (-)vasicinone (**2**) are major alkaloids isolated from *Justicia adhatoda* L. known to have a moderate bronchodilatory effect.



1

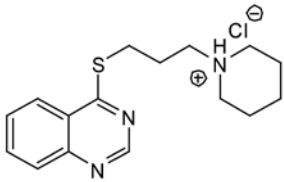
ED_{50} = 0,323 mmol/L



2

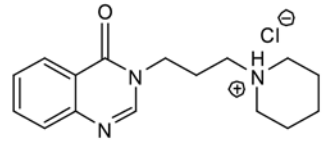
ED_{50} = 1,3 mmol/L

Several literature reports have described the modifications of the (-)vasicinone structure and showed that ring C is not necessary for a compound to possess bronchodilatory effect. So far, we have synthesized the first series of derivatives,[1] where the C-ring was replaced with alkyl chain terminated by a tertiary amine. The most active derivatives (**3, 4**) displayed bronchodilatory activity far exceeding the effect of theophylline (**5**) as a standard drug on isolated rat trachea.



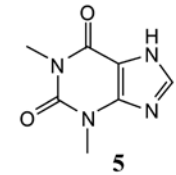
3

ED_{50} = 3,42 μmol/L



4

ED_{50} = 28,3 μmol/L



5

ED_{50} = 2090 μmol/L

Structures **3** and **4** were further modified, and another series of derivatives with substitution on ring A was synthesized and their bronchodilatory activity was evaluated.

The study was supported by Charles University in Prague (GAUK 398 315), SVV 260 401 and Czech Science Foundation (15-07332S).

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INTRAMOLECULAR TSUJI-TROST REACTION: NEW ROUTE TO HIGHLY SUBSTITUTED PYRANONES

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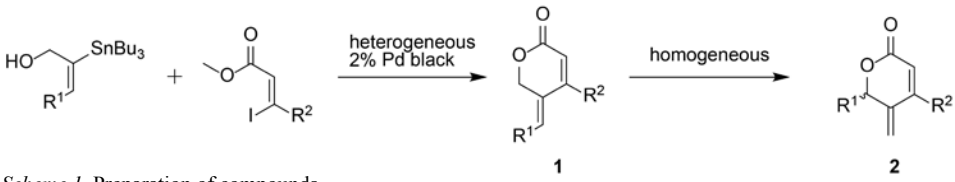
Lactones (Scheme 1, **1**) prepared from β-iodoacrylic esters and 2-tributylstannyl allyl alcohols under heterogeneous catalysis[1] conditions can isomerize to 5-methylene pyranones (Scheme 1, **2**). Interestingly homogeneous catalysis is required to trigger the isomerization of pyranones **1**. Following extensive optimization, a library of 18 compounds was obtained. Since the reaction generates a new stereogenic centre, chiral phosphines were employed to investigate the possibility of developing an enantioselective process.



Scheme 1. Preparation of compounds

The study was supported by the Grant Agency of Charles University (projects 1054216 and SVV 260 401) and the Czech Science Foundation (project No. 15-07332S).

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NEW AZAPHTHALOCYANINE DARK QUENCHER USABLE IN POLYMERASE CHAIN REACTION

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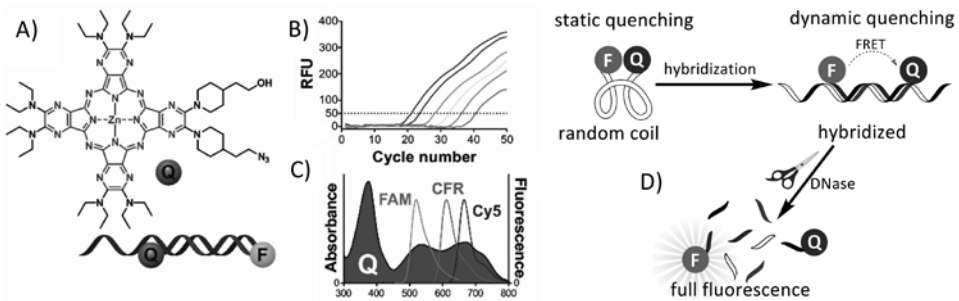
Unsymmetrical dialkylamino substituted azaphthalocyanines (AzaPc, Fig. A) have unique photophysical properties – *e.g.* light absorption between 300 and 700 nm (Fig. C), almost no self-fluorescence and ability to quench fluorescence of other compounds. This makes AzaPcs suitable candidates for universal dark quenchers.[1,2] To confirm the assumptions, series of oligodeoxyribonucleotide double-labeled probes were prepared with this AzaPc at 3′-end and with commercial fluorophores at 5′-end. Fluorophores were chosen with an emphasis to cover whole emission spectrum of fluorophores, which are used in polymerase chain reaction (PCR), *i.e.* fluorescein, CAL Fluor Red 610 and Cy5. A model of TaqMan assay was developed to enable direct photophysical comparison of different quenchers (Fig. D). AzaPc quencher had remarkably higher quenching efficiency in this hybridization assay than commercially used dark quencher such as BHQ-1, BHQ-2, BBQ-650. The real application (quantitative PCR) was tested for quantification of SLCO2B1 transporter gene (Fig. B). Resulting calibration curves indicated linearity in range from 10^2 to 10^7 of target copies.



The study was supported by the Grant Agency of Charles University (projects 1168217 and SVV 260 401).

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THE NOVEL LEAD CANDIDATES FOR THE TREATMENT OF ORGANOPHOSHOROUS INTOXICATION

GÓRECKI, L.,[1,2] KORÁBEČNÝ, J.,[1,2] MALIŇÁK, D.,[2] HEPNAROVÁ, V.,[1,2] HRABINOVÁ, M.,[1,2] SOUKUP, O.,[1,2] JUN, D.,[1,2] MUSÍLEK, K.,[1,2] KUČA, K.[1,2]

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Mono- and bis-pyridinium aldoximes are the only causal antidotes that are designated for the treatment of organophosphate (OP) poisoning. Intoxication by OP is caused either by pesticides or by nerve agents; the latter belong to group of chemical warfare agents. These compounds irreversibly inhibit essential enzyme acetylcholinesterase that is no more able to fulfil its physiological function. Mono- and bis-pyridinium aldoximes are able to cleave organophosphate acetylcholinesterase bond and restore enzyme's catalytic activity. The reactivating ability of aldoximes is hampered by several drawbacks like low blood-brain barrier permeation, low reactivation potency against specific nerve agents *etc*. In order to obtain efficient treatment of OP intoxications, the introduction of novel AChE reactivators is matter of importance. For over 60 years of intensive research, none of the new compounds reached sufficient activity. Herein, we present novel mono quaternary reactivators that abound with excellent *in vitro* activity to recover AChE activity after intoxication with different nerve agents as well as pesticides. The molecular docking simulations, total synthesis and biological evaluation will be discussed.

The study was supported by specific research of the University of Defence SV/FVZ201601 and MH CZ – DRO (University Hospital Hradec Králové, No. 17-32801A).

NONTOXIC COMBRETAFURANONE ANALOGUES WITH HIGH *IN VITRO* ANTIBACTERIAL ACTIVITY

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A library of thirtytwo 3,4-diphenylfuranones related to both combretastatin A-4 and antifungal 5-(acyloxymethyl)-3-(halophenyl)-2,5-dihydrofuran-2-ones was prepared. Cytotoxic effects on a panel of cancer and normal cell lines and anti-infective activity were evaluated, and the data were complemented with tests for the activation of caspase 3 and 7. High cytotoxicity was observed in some of the halogenated analogues, *e.g.* 3-(3,4-dichlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one with IC_{50}

0.12–0.23 mM, but the compounds were also highly toxic against non-malignant control cells. More importantly, notable antibacterial activity indicating G-positive selectivity has been found in the 3,4-diarylfuranone class of compounds for the first time. Hydroxymethylation of furanone C5 knocked out cytotoxic effects (up to 40 mM) while maintaining significant activity against Staphylococcus strains in some derivatives. MIC_{95} of the most promising compound, 3-(4-bromophenyl)-5,5-bis(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one against *S. aureus* strain ATCC 6538 was 0.98 mM (0.38 mg/mL) and 3.9 mM (1.52 mg/mL) after 24 and 48 h, respectively.[1]

This study was supported by the Czech Science Foundation (project No.15-07332S), by Charles University (projects No.1906214 and SVV 260 401) and by Ministry of Education, Youth and Sports of the Czech Republic (LO1220 and LM2015063).

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SYNTHESIS OF NEW SULFONATED AZAPHTALOCYANINE AND ITS PHOTOPHYSICAL AND PHOTODYNAMIC PROPERTIES

KOLLÁR, J.,[1] JANČÁROVÁ, A.,[1] MACHÁČEK, M.,[2] ZIMČÍK, P.[1]

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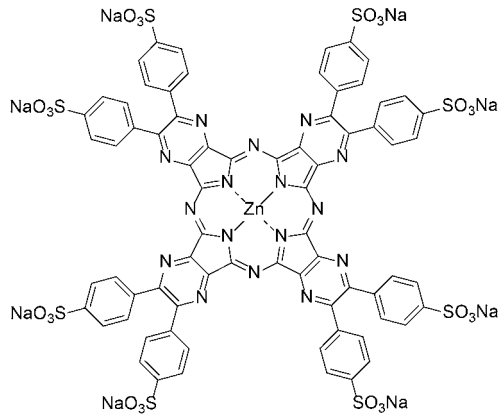
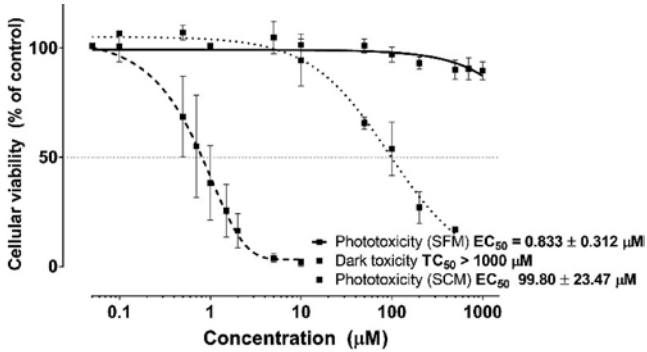
Azaphthalocyanines (AzaPc) are a group of organic dyes with promising photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) to be used as photosensitizers in PDT. The main drawbacks are, however, their low water solubility and strong tendency to aggregation that decrease their photodynamic activity.





The aim of this work was to synthesize an anionic derivative of AzaPc substituted with sulfonic groups on periphery which is characterised by good solubility in water and to evaluate its photodynamic properties. The first step in synthesis was condensation of diaminomaleonitrile and benzil giving 5,6-diphenylpyrazine-2,3-dicarbonitrile. Subsequently, the cyclotetramerisation with zinc acetate using 2-dimethylaminoethanol as a solvent was performed. The final product was obtained by sulfonation with chlorosulfonic acid followed by hydrolysis with sodium bicarbonate. The green coloured product was then purified by size-exclusion chromatography using Superdex® as stationary phase. Sulfonated AzaPc is soluble in water but according to absorption spectra it is partially aggregated. The final AzaPc was tested on photodynamic activity *in vitro* against HeLa cells using serum-free medium (phototoxicity $EC_{50} = 0.833 \pm 0.312$ μM) and was characterized also by low dark toxicity $TC_{50} > 1000$ μM, HeLa). It was practically inactive in serum-containing medium due to its strong binding to serum proteins. Photophysical properties of binding to serum proteins was studied.

The work was supported by GAUK 1060216 and SVV 260 401.

RHODANINE DERIVATIVES AS POTENTIAL AGENTS IN TREATMENT OF CHRONIC DIABETIC COMPLICATIONS

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In our continuous efforts to find new aldose reductase inhibitors,[1] a series of [(5*Z*)-(5-arylalkylidene-4-oxo-2-thioxo-1,3-thiazolidin-3yl)]acetic acids[2] was tested on inhibition of aldose reductase (ALR2). The enzyme plays a crucial role in the development of long-term diabetic complications.[3] Inhibitory activity of the compounds was determined on ALR2 isolated from rat eye lenses and compared to that of clinically used ALR2 inhibitor

epalrestat. Most of the compounds have shown IC_{50} in the submicromolar range and in addition, lower than epalrestat. Inhibitory activity on aldehyde reductase (ALR1) from rat kidneys has been measured in order to determine the selectivity. The obtained results were comparable to the selectivity index of epalrestat. The most potent inhibitor of ALR2 from the series showed mixed type of inhibition. Relationships between chemical structure, lipophilicity and biological activity have been derived. The work has been accompanied by a molecular docking study on 4JIR elucidating the intermolecular interactions.

The study was supported by the research program Development and Study of Drugs Progress Q42 (Charles University, Czech Republic).

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APPROACHES TO TOTAL SYNTHESIS OF NOSTOTREBIN 6

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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 an antimicrobial activity and is also an efficient inhibitor of both acetylcholinesterase and butyrylcholinesterase.[1] No total synthesis has not been reported to date. Possible synthetic approaches towards key intermediates and derivatives will be discussed.

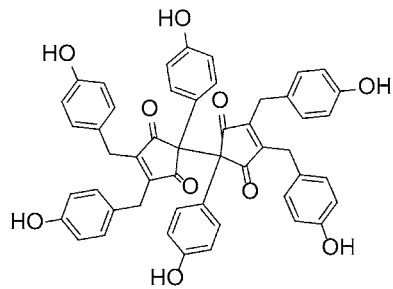

Figure 1. Nostotrebin 6

This work was supported by Charles University (SVV 260 401 and GAUK 262416) and Czech Science Foundation (Project No. 15-07332S).

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FIGHTING MICROBIAL RESISTANCE – WHO IS GOING TO WIN?

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Antimicrobial resistance is one of the most threatening as well as widespread global health problems. There can be found at least one resistant microbial strain in every country all over the world. It does not matter if you live in industrial country or developing country, or if you were born either in hot Sahara or beyond polar circle. Microbes are not choosy, and we need to find the way how to be more successful in this never-ending fight.

One way how to reach this goal is to find new compounds with novel mechanism of action, better pharmacological properties or simply new molecule that can kill these superbugs without killing us.

It is not only important to design and prepare new molecules but also, we need to reveal their properties that are essential in understanding the way they act. One of these properties is mechanism of action (MoA). The first step to study MoA is to determine minimal inhibition concentration according to EUCAST standards.[1] If the results are auspicious, we can focus on specification of possible MoA. Currently, 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 antimicrobial 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 (inhibition of all mentioned biosynthetic pathways).[2]

This approach will be used in future to determine possible MoA of antimycobacterial compounds as well. The first step will be to find specific biomolecules typical for mycobacterial biosynthetic pathways and their radioactively labelled equivalents, respectively, together with inhibitors of these pathways.

The study was supported by Research Program Development and Study of Drugs (Progress Q42).

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PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS SECTION

AMARYLLIDACEAE ALKALOIDS AS POTENT GSK 3β INHIBITORS

HULCOVÁ, D.,[1] BREITEROVÁ, K.,[1] HOŠŤÁLKOVÁ, A.,[1] ŠAFRATOVÁ, M.,[2] KUNEŠ, J.,[3] CAHLÍKOVÁ, L.[1]

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Alzheimer's disease (AD) is one of the most frequent causes of dementia in the world. During AD, pathological changes of some enzyme systems occur in the brain that result in loss of neurotransmitter acetylcholine and formation of amyloids plaques and neurofibrillary tangles (NFTs). The consequences are brain damage, development of dementia and loos of cognitive functions. NFTs consisting of paired helical filaments, with the main component being hyperphosphorylated τ-protein. Phosphorylation of τ-proteins is primarily dependent on glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5.[1] Glycogen synthase kinase-3 is an ubiquitous serine/threonine kinase that plays a crucial role in numerous cellular functions, including cell cycle regulation, differentiation and proliferation, and gene expression by regulating a wide variety of substrates like glycogen synthase or tau-protein.[2]

In our ongoing study focused on Amaryllidaceae alkaloids, we have investigated thirty previously isolated alkaloids from *Zephyranthes robusta*, *Nerine bowdenii*, *Chlidanthus fragrans* and various *Narcissus* cultivars for their GSK-3β inhibition potential. For all compounds, percentage inhibition at concentration 50 μM was measured. Inhibitory activity IC_{50} was determined for three compounds (masonine: 28.25 μM ± 4.05, 9-*O*-demethylhomolycorenine: 27.20 μM ± 10.80 and caranine: 31.54 μM ± 1.26). Since galanthamine is used in therapy of AD, Amaryllidaceae alkaloids are still promising goal in searching for new bioactive compounds.

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PHYTOCHEMICAL STUDY OF AMARYLLIDACEAE ALKALOIDS FROM *NARCISSUS* cv. CARLTON AND THEIR BIOLOGICAL ACTIVITY

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Plants of Amaryllidaceae family are crucial in natural product research for its structurally diverse group of compounds as long as broad range of therapeutic potentiality. The earliest evidence was found in fourth century B.C.E. when Hippocrates of Cos used *Narcissus poeticus* L. oil extract for the treatment of uterine tumors.[1] *Narcissus* cv. Carlton is ornamental plant under Amaryllidaceae family. Until now more than 600 Amaryllidaceae alkaloids signifying 18 skeletal types of alkaloids have been isolated, which comprise galanthamine, lycorine, haemanthamine, pancratistatine, pretazzetine, montanine, narciclassine and others. These active metabolites showed widespread therapeutic application as antitumor, antibacterial, antifungal, antimalarial, antiviral, cytotoxic, analgesic, acetylcholinesterase and butyrylcholinesterase inhibitors.[2] Among these active metabolites, galanthamine is most effective alkaloid and has been clinically used for the treatment of Alzheimer's disease (AD) since 2000. After this, searching of new alkaloids from Amaryllidaceae family have been accelerated. Haemathamine and haemanthidine are other important crinine type Amaryllidaceae alkaloids that demonstrated interesting anticancer activity against p53-mutated Caco-2 and HT-29 colorectal adenocarcinoma cells and human leukemic Jurkat cells,[3] and moderate anti-influenza virus activity against N5H1 cell lines.[4] GC/MS analysis of *Narcissus* cv. Carlton showed interesting number of compounds with typical spectra of Amaryllidaceae alkaloids. Some of them have been identified based on their mass-spectra. Unidentified peaks are assumed to be new structures of Amaryllidaceae alkaloids. So far, concentrated alkaloidal extract from 50 kg of fresh bulb of *Narcissus* cv. Carlton has been prepared and will be separated by column chromatography to isolate Amaryllidaceae alkaloids in pure form for biological tests and preparations of their derivatives.

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USE OF HPCCC FOR ISOLATING ASTAXANTHIN ESTERS FROM MICROALGAE *HAEMATOCOCCUS PLUVIALIS*

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Astaxanthin (AXT), the most powerful antioxidant found in nature, is a red pigment with noticeable and varied bio-functional properties of great significance in human health and nutrition. The microalgae *Haematococcus pluvialis* is the major AXT natural source, in which this compound occurs mainly in the form of monoesters (AXTme) and diesters (AXTde). These esters have been shown to exert better benefits than the non-esterified AXT.[1,2] Given that there is an increasing demand for AXT of natural origin in the cosmetic, food and pharmaceutical sectors, the development of an efficient method for isolating AXT esters from microalgae is necessary. For facing this challenge, a high performance countercurrent chromatography (HPCCC) method will be developed, optimized and applied to obtain individual AXTme and AXTde from the microalgae *Haematococcus pluvialis*. HPCCC is a liquid-liquid chromatography technique that uses a liquid stationary phase without any solid support. Two immiscible liquid phases are used for the separation of target compounds. One of the two liquid phases (the stationary phase) is retained in the column by centrifugal force, whereas the other (the mobile phase) is pumped through the column.[3] In HPCCC, the chromatographic separation is based on the difference in the partitioning of each target compound between these two immiscible phases. Given that HPCCC uses a liquid stationary phase without a solid support, the method offers many advantages over traditional solid-liquid chromatography, such as the absence of irreversible adsorption of target molecules, high sample loading capacity and recovery, low risk of sample denaturation, and low solvent consumption.[4] This chromatographic method is considered to be a cost-effective, high-throughput and scalable technology for the extraction of bioactive substances from natural sources. In the present proposal, the chemical identity of the isolated AXTme will be determined by high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (HPLC-APCI-MS/MS).

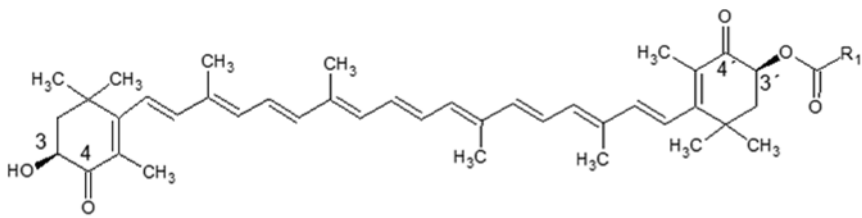

Figure 1. Representative chemical structure of AXTme

This work will provide a new chromatographic approach for the isolation of astaxanthins from microalgal biomass.

The study was supported by SVV 260 294 and by the Grant Agency of Charles University (project no. 1134217). The results of this investigation will be under patent application.

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ISOLATION OF AMARYLLIDACEAE ALKALOIDS FROM *ZEPHYRANTHES CITRINA*

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Zephyranthes is a genus of bulbous perennials belonging to family Amaryllidaceae. The plants of this family are used by native people of different countries for treating various diseases. The genus *Zephyranthes* is one amongst 75 genera under this family. It consists of about 90 species, out of which few have been studied for their chemical constituents. The phytochemical work on this genus revealed the diversity of compounds, especially alkaloids having various pharmacological activities as anticancer, anticholinesterase, antiviral, antifungal and antiinflammatory. To date, ten alkaloids have been reported in *Zephyranthes citrina* (lycorine, lycorenine, galanthine, haemanthamine, oxomaritidine, maritidine, haemanthidine, vittatine, galanthine, narcissidine).[1]

The summary ethanolic extract was prepared from the fresh bulbs of *Zephyranthes citrina*. More than six hundred fractions were collected by column chromatography (on Al_2O_3). Fractions were pooled into subfractions. So far, two pure alkaloids have been isolated. The isolated compounds were identified as haemanthamine and galanthine by comparison with the literature data and results of MS and NMR studies.

The study was supported by SVV 260 412.

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NMR ELUCIDATION OF ALKALOIDS ISOLATED FROM *MAGNOLIA × SOULANGEANA*

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Eight alkaloids were obtained from *Magnolia × soulangeana* Soul.-Bod at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové. The structure of two of them was predicted by MS analysis and confirmed by NMR analysis – liriogenine and *N*-methylcoclaurine. Six of isolated substances had to be characterized employing basic ^{1}H and ^{13}C NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC, gCOSY and NOESY. The identified structures were compared with available literature. All of these isolated compounds have already been described in literature as *N*-norarmepavine, armepavine, β-carboline, asimilobine, coclaurine, magnolamine.

This work was supported by the Czech Science Foundation (project 15-07332S) and SVV 260 401.

PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF SELECTED MEDICINAL PLANTS WITH A FOCUS ON TESTING THE IMMUNOMODULATORY AND TYROSINASE ACTIVITIES

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Given the early stage of research, this study has not provided valid data yet. The presentation will be focused on the introduction of selected plants and planed methods.

The first selected plant is *Scutelaria baicalensis* L. (Baical skullcap, family Lamiaceae) – one of medicinal herbs with a long history of usage in traditional Chinese medicine. Researches over the last decades revealed and confirmed a variety of important properties of content substances (over 40 flavonoids *etc*.), which contributed to the general awareness of this herb. *Scutellaria baicalensis* extracts are part of dietary supplements. Given the worldwide prevalence of deaths caused by cardiovascular diseases and the increasing challenge of infections, immunomodulatory and antiaggregation activity must be demonstrated and elucidated. Attention will be also put on antiparasitic and antityrosinase activities, whether the plant exhibits any, to this day untested, effects.

The second selected medicinal plant for this study is *Azorella compacta* Phil. (syn. *A. yareta*, Llareta, family Apiaceae). It is a cushion shrub grown at altitudes of the Andes

in South America's puna. Natives traditionally use the plant in a form of tea to treat cold, pain, rheumatism, diabetes, and also as a stomachicum and diureticum. Experiments with aqueous extracts proved the antioxidant and immunomodulatory effect of contained polyphenols, but it is still unknown which specific substances are responsible for the effects. In addition to the isolation of biologically active substances, evaluation of their antiaggregatory, antityrosinase and antiparasitic activities will be performed.

COPPER CHELATION ACTIVITIES OF ISOLATED COMPOUNDS FROM *SILYBUM MARIANUM* – STRUCTURE-ACTIVITY RELATIONSHIPS

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Silybum marianum (L.) Gaertn. is a medicinal herb from family Asteraceae. It is frequently used for its hepatoprotective properties in the form of a mixture known as silymarin. Silymarin is composed from flavonolignans including silybin A and B, isosilybin A and B, silychristin, isosilychristin and 2,3-dehydrosilybin and also flavanonol taxifolin. Pharmacokinetics of pure silybin diastereoisomers and identification of their metabolites in rat plasma was recently introduced.[1]

The aim of the study was to test four compounds isolated from silymarin, namely silybin A and B, silychristin and 2,3-dehydrosilybin, in order to assess their copper chelation properties at (patho)physiologically relevant pH conditions by use of our previously published approaches.[2,3]

All tested compounds chelated cupric ions under mildly competitive conditions (hematoxylin method). 2,3-Dehydrosilybin was shown as the most potent chelator in a more competitive assay (bathocuproin method) while other flavonolignans did not chelate either cupric or cuprous ion in this experiment. Evaluation of cupric reduction demonstrated that all tested flavonolignans were potent reducing agents.

In conclusion, 2,3-dehydrosilybin had the strongest copper chelating properties while silychristin was the most potent copper reductant.

The study was supported by Progress Q42.

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ALKALOIDS OF *VINCA MINOR* L. AND THEIR EFFECT ON ACTIVITY OF SELECTED ENZYMES AS BENEFIT TO PROGRESS OF ALZHEIMER'S DISEASE

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Vinca minor L. is an ornamental plant from Apocynaceae family commonly used in gardens, but it is also a source of alkaloids. So far, more than 45 alkaloids of indole type have been isolated from this plant. Indole type alkaloids are known as the source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease.[1]

In our study, 62 kg of dried aerial parts of *V. minor* were three times extracted with EtOH, the solvent was evaporated under reduced pressure, the extract was dissolved in hot water and filtered. The aqueous solution was adjusted to pH 9–9.5 with 25% NH_4OH, and alkaloids were five times extracted with $CHCl_3$. 454 g of crude extract was obtained after evaporation.

The mixture of the total alkaloids was divided by means of column chromatography into sixteen fractions containing alkaloids. Chromatography was performed on alumina using gradually enriched petrol-chloroform and chloroform-ethanol mixtures for elution. Subsequently combination of flash and repeated thin-layer chromatography led to the isolation of pure compounds.

So far, more than 8 alkaloids were isolated in our study. Chemical structures of compounds were elucidated by optical rotation, spectroscopic and spectrometric analysis (NMR, MS) and comparison with literature data. Next the human blood acetylcholinesterase (HuAChE) and human serum butyrylcholinesterase (HuBuChE) inhibitory activities of them were studied. In addition, selected compounds were tested for recombinant prolyl oligopeptidase (POP) inhibitory activity and in some cases the parallel artificial membrane permeability assay (PAMPA) was performed.

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PHARMACEUTICAL TECHNOLOGY SECTION

MONOLAYER STUDY OF GLUCOSYLCERAMIDE-TO-CERAMIDE PROCESSING DURING SKIN BARRIER FORMATION

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Ceramides (Cer) together with free fatty acids and cholesterol form the intercellular space of the uppermost skin layer, *stratum corneum* (SC). This lipid matrix represents the proper skin barrier, which protects mammalian organisms against environmental factors (exogenous substances, physical radiation) and prevents body from water loss. All Cer subtypes are synthesized from their polar precursor glucosylCer (GlcCer) by removing the polar part by hydrolytic enzyme β-glucocerebrosidase (GlcCer-ase). A lack of this enzyme leads to accumulation of precursors and a disturbed skin barrier function, *e.g.* in type 2 Gaucher disease.

The goal of this work was to study the processing of GlcCer to Cer by monolayer lipid models of SC. The control monolayers contained GlcCer, free fatty acids and cholesterol in equimolar ratio. In order to study the process of Cer formation, GlcCer were gradually (75, 50, 25, 10, 5%) replaced by Cer. As a liquid subphase under the monolayers, we tested phosphate (pH 7.4) and acetate (pH 5.0) buffer. At low surface pressure (1.5 mN m^{-1}), the lipids organize more spontaneously at pH 5.0 than at pH 7.4, apart from the mixtures with 25 and 50% of GlcCer, which are not influenced by pH. With increasing surface pressure (20 mN m^{-1}) there is similar trend at pH 7.4 like at lower pressure, however, at pH 5.0 the molecular area of the mixtures is lower compared to pH 7.4. Surprisingly low area per molecule at 20 mN m^{-1} (tighter organization of lipids) of mixture with 50% GlcCer corresponds with low permeability of multilayer model membranes. At pH 5.0, compressibility is lower in mixtures containing GlcCer than without GlcCer. By contrast, at pH 7.4, compressibility of mixtures containing GlcCer is higher than without it. Langmuir isotherms showed that the dependence of precursor concentration in monolayers on tight arrangement of lipid molecules is nonlinear and that pH slightly affects formation of monolayers. It seems that the structure of the polar head influences the mutual interactions between lipids during the formation of the SC lipid membranes.

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SILK FIBROIN AND ITS APPLICATION IN PHARMACEUTICAL TECHNOLOGY

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Silk fibers produced by silkworm *Bombyx mori* have been used in medicine for centuries as biocompatible and biodegradable surgical sutures. The native silk fibers after undergoing a process called degumming, during which water soluble protein sericin is removed, are composed of protein polymer called silk fibroin (SF).[1] Being naturally produced polymer, its renewable and biodegradable features make SF an attractive environmentally friendly polymer.

So far, the main applications of SF in drug delivery systems have been focused in tissue engineering. SF in crystalline form is insoluble in water and any common organic solvent.[1] These properties make the polymer suitable for sustained release drug delivery applications.

In our work we produced sustained release matrix tablets. Due to the inherent insolubility of SF in ethanol, these tablets are resistant to so called "alcohol-induced dose dumping". A phenomenon which leads to faster or immediate release of all drug content and potential overdosing when co-administered with ethanolic beverages.[2,3] The issue is especially relevant in case of opioid analgesics due to the well-known pharmacodynamic interaction and potentiating effect of ethanol on central nervous system.[3] Therefore oxycodone hydrochloride and tramadol hydrochloride have been selected as model compounds. The results show slower drug release with increasing ethanol concentration in dissolution medium and different swelling behaviour.

The study was supported by SVV 260 183.

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POLYMERIC NANOPARTICLES FOR INTRACELLULAR DELIVERY

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Multiple pathologies result in liver inflammation.[1] Inflammatory response is a complex process and intracellular receptors[2,3] in macrophages have been found to regulate

it. Appropriate carriers, carrying ligands to these receptors, at size ranging from 100 to 300 nm and negative surface charge can be passively targeted to liver and uptaken by macrophages.[4] Subcellular dimensions of nanocarriers enable their uptake by targeted cells and action on intracellular level. Poly(DL-lactic-co-glycolic acid) (PLGA) is a biocompatible polymer, its biodegradability results in a sustained release profile of the encapsulated drug.[5]

In our study, both linear and branched PLGA polymers were utilised. Screening through various types of stabilizers, in combination with different preparation methods was carried out. Nanoprecipitation method was used to encapsulate a fluorescent dye Rhodamine B also known as a ligand for aforementioned intracellular receptors. Particle size, polydispersity index and zeta potential confirmed stable negatively charged nanoparticles sized from 180 to 320 nm with polydispersity of acceptable values. Dissolution tests indicate desired sustained release profiles in range of days varying in respect to the utilized polymer. Such NP parameters together with promising relative encapsulation effiency up 90% meet the requirements for targeted drug delivery formulations.

The study was supported by SVV 260 401 and GACR 303/12/G163.

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STARLIKE POLYMERIC NANOPARTICULATE DRUG CARRIERS

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The aim of our work is to prepare polymeric nanoparticles from originally synthesized biodegradable branched polyesters with intent to achieve prolonged response and to reduce adverse effects of selected incorporated drugs (terbinafine hydrochloride, rifampicin, siRNA). We used aliphatic starlike polyesters branched on tripentaerythritol of average molecular weight (Mw 17400) and branched on poly(acrylic acid) with Mw 14400. Nanoparticles were prepared using modified nanoprecipitation method.[1] Firstly, we prepared organic phase consisting from solution of polyester in dimethyl sulfoxide, in which specific amount of drug was dissolved. Different concentration of surfactants in water represented aqueous phase. The aqueous phase at room temperature was placed on magnetic stirrer and stirred at medium speed. Then the organic phase was added dropwise into the aqueous phase, stirring proceeded for 30 minutes. We monitored multiple parameters such as particles size, polydispersity and zeta potential using Zetasizer Nano ZS by Malvern.

The size of the nanoparticles was 100–250 nm, and could be modified by concentration of the polyester and mixing technique of phases. For analysis, we used HPLC, fluorometer or spectrophotometer, according to analyzed drug. Dissolution tests showed prolonged release of incorporated drugs, which we attribute to the gradual swelling and degradation of the polyester in an aqueous medium.[2] Examined polyesters are perspective, original, and suitable for further observation.

The study was supported by SVV 260 401.

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THE COMPRESSIBILITY STUDY OF MICROCRYSTALLINE CELLULOSE PELLETS, MICROCRYSTALLINE CELLULOSE POWDER AND THEIR MIXTURES

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Pellets are very popular in pharmaceutical technology because they have spherical shape, smooth surface, narrow particle size distribution and excellent flow properties. They are very often used as drug carriers; a drug is either included into a pellet matrix or onto a pellet surface. However, according to our previous experiments, pellets themselves are not suitable to be used as a tablet filler and they need to be mixed with other additives to achieve a mixture with appropriate compaction properties.[1]

Hence, this study deals with the evaluation of compressibility of microcrystalline cellulose pellets Cellets® 100 (C100), powder microcrystalline cellulose Comprecel 102 (MCC) and their mixtures. Nine mixtures with different ratio of C100 and MCC were prepared and compacted under ten compaction forces in a range of 2–20 kN. The radial strength[2] and elasticity of prepared tablets were evaluated.[3,4]

The results showed that the radial strength of the tablets increased with the rising concentration of microcrystalline cellulose in the mixture. In accordance with the generally recommended optimal range of the tablet tensile strength 0.56–1.12 MPa, the mixtures containing 60 and 70% of pellets were the most promising for the tablet preparation. However, further studies involving tablet friability, disintegration and dissolution testing are necessary.

The study was supported by SVV 260 401.

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PRE-FORMULATION STUDIES OF LIQUISOLID SYSTEMS

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One of the trends of modern pharmaceutical technology is a formulation of dosage forms with enhanced dissolution rate and improved bioavailability of poorly soluble active ingredients. The scientific literature describes different methods improving bioavailability, however, the preparation of liquisolid systems (LSS) seems to be one of the most promising and innovative techniques among them. The basic principle of LSS formulation is a conversion of the drug in the liquid state into an apparently dry powder by its blending with specific carriers. The carrier particles are subsequently coated with a highly absorptive material giving the LSS the desirable flow and compression characteristics.[1]

Properties of LSS can be influenced by many factors, such as a solubility of the drug in the chosen solvent, properties of used excipients and calculations of the required amounts of carrier and coating material. Therefore, the presented work aimed at the determination of the optimal combination of the solvent, carrier and coating material for the preparation of LSS. The flowable liquid retention potentials of several carriers and coating materials for three non-volatile hydrophilic solvents were measured. According to the obtained results, the compressible liquid retention potential of the granulated form of magnesium aluminometasilicate (Neusilin® US2) for these solvents was evaluated. It was observed that the tablets containing 55% of polyethylene glycol 400 and 60 % of polyethylene glycol 200 fulfilled all requirements given by the Ph.Eur.. Subsequently, the processing properties of these mixtures, *e.g.* the optimal compression pressure and compaction behavior with the varying amounts and types of coating materials *etc*., were investigated for the possible use in dosage forms.

The study was supported by Progress Q42.

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PHARMACEUTICAL ANALYSIS AND BIOANALYTICAL CHEMISTRY SECTION

UHPLC-MS/MS METHOD FOR MONITORING OF ARGININE METABOLISM IN CHRONIC WOUNDS

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Wound healing presents very complex and dynamic process. Metabolism of amino acid arginine plays an important role in this process. It is a precursor for synthesis of nitric oxide, proline, hydroxyproline and polyamines. Arginine also stimulates the release of growth hormone, insulin-like growth factor 1 and other compounds that significantly influence wound healing.[1]

The aim of this study was to develop UHPLC-MS/MS method for the determination of L-arginine and its metabolites L-ornithine, L-citrulline and agmatine in a fluid obtained from non healing wounds. Several stationary phases were tested and compared for the optimal retention and separation of these polar analytes: HILIC, F5, two modifications of C18 and BEH Amide. The optimal conditions were applied for the determination of these molecules in real patient samples with simple sample pretreatment procedure. The study was performed using Nexera® UHPLC system with a Triple Quadrupole Mass Spectrometer LCMS 8030 (Shimadzu, Japan) operating in ESI positive mode.

In future, this method will be useful for the direct monitoring of arginine metabolism in chronic wound which can contribute to improve the therapy.

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DEVELOPMENT OF UHPLC METHOD FOR STABILITY STUDY OF OMEPRAZOLE SUSPENSIONS

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Omeprazole is one of the most widely used drugs from the group of proton-pump inhibitors (PPIs). Due to the instability of omeprazole in acidic conditions, enteric-coated tablets or extended-release capsules are commonly used dosage forms. This kind of drug administration is not convenient in pediatric and other patients having problems to swallow solid dosage form. Thus, individually prepared oral suspensions of omeprazole facilitate the administration and the dosing.[1,2]

A simple and fast ultra-high-performance liquid chromatography method with UV detection for the separation and quantification of omeprazole and its impurity and methylparaben (the internal standard) in six extemporaneous suspensions was developed and fully validated. Separation was performed using 1.7 μm porous shell particles (Kinetex™ C18, 50 × 2.1 mm) in combination with an isocratic elution of a phosphate buffer and acetonitrile. The separation of all compounds was achieved within 2 minutes. The method was successfully applied during a stability evaluation of the developed formulations, which are now being used in the therapy of acid-related disorders in paediatric patients. Results of stability study will be presented.

The study was supported by Project SVV 260 412.

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CHROMATOGRAPHIC METHOD FOR THE ASSESSMENT OF VITAMIN B_1 AND B_6 DERIVATIVES IN WHOLE BLOOD

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Derivatives of thiamine and pyridoxine have crucial role in cellular metabolism. Discrepancies in their status have serious deleterious effects. Manifestation is often vague and may be easily overlooked.[1] Therefore, monitoring of vitamin status has large importance especially in patients with intensive care. Methods for simultaneous determination of thiamine, its mono- and diphosphate derivatives, and with an active form of vitamin B_6 – pyridoxal-5-phosphate are still not widely established in diagnostics [2].

Novel HPLC-FLD method with pre-column derivatization was developed, optimized and validated for the simultaneous analysis of thiamine and its derivatives with pyridoxal-5-phosphate in whole blood. Separation was accomplished by Meteoric Core-BIO C-18 core-shell column (100 × 4.6 mm, YMC, Germany) protected with SecurityGuard C18-WP guard column (10 × 4.6 mm, Phenomenex, USA). During gradient elution all target compounds were eluted within 15 minutes. Limits of detection are below clinically important

values. Recoveries were in the range of 90 to 110% for all analytes. Bioanalytical method will be further implemented into routine practice and used primarily for the determination of thiamine and its derivatives in patients with supplementary nutrition therapy, where fluctuating level of metabolically active vitamins are associated with the occurrence of possible complications.

The study was supported by Project SVV 260 412 and by University Hospital in Hradec Králové, 00179906.

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MIXED-MODE STATIONARY PHASES: EFFECT OF MOBILE PHASE TYPE AND ITS IONIC STRENGTH ON SEPARATION

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The analysis of weak protonated bases by RP-HPLC has always been challenging when using acidic or neutral mobile phases due to significant peak tailing and subsequent decrease of chromatographic resolution and increase of detection limits. Charged Surface Hybrid (CSH) columns are mixed-mode stationary phases based on silica/ethylene hybrid particles (BEH). They are prepared by binding a controlled number of amino groups to these particles. Therefore, higher peak capacities for separations of positively charged analytes when using the low ionic strength acidic mobile phases, as well as symmetric peak shapes, and higher efficiency are possible due to positively charged pore surface with protonated amino groups at pH less than 3.

The screening was performed on three CSH columns (CSH C18, CSH Phenyl-Hexyl, CSH Fluoro-Phenyl) using gradient elution and pharmaceutically important compounds with basic, neutral, and also acidic properties. The study was carried out on UHPLC system Acquity UPLC with UV detection at 254 nm. The mobile phase consisted of acetonitrile and acidic aqueous solution or buffer with low ionic strength. The analyses with aqueous solutions (containing formic acid, hydrochloric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, or perchloric acid) and buffers with different pH (1.0–3.1) and concentrations (0.01–0.5%) were carried out. Thus, monovalent and multivalent mobile phases with different ionic strengths were tested. The ionic strength was the decisive parameter for retention time shifts of strongly basic compounds and was correlated as the logarithm of mobile phase ionic strength.

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ENRICHING OF PREPARED FLAT SHEET POLYSULFONE MEMBRANE FOR SEPARATION OF BIOMOLECULES WITH ANTIOXIDANT α-TOCOPHEROL AND α-LIPOIC ACID

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Patients suffering from chronic kidney disease, undergoing frequent haemodialysis (HD) treatment present significantly elevated level of oxidative stress (OS) and chronic inflammation caused by HD treatment itself, besides the disease conditions. The long-term contact of blood with artificial materials causes overstimulation of polymorphonuclear cells with subsequent inflammatory reaction and elevated OS leading to other severe complications of these patients. Polysulfone (PS) is nowadays the most used polymer for HD membranes due to its improved biocompatibility. Nevertheless, to minimize the negative effect of HD procedure, the bioactive hollow-fibre PS membranes modified with vitamin E are commercially used.[1]

In the present work, flat sheet PS membrane was prepared for laboratory purposes to mimic HD procedure, using spin coating technique, followed by phase inversion process. Developed PS membrane was optimized and tested to fulfil the removal characteristics required for HD. The antioxidant-enriched membranes were prepared by dissolving α-tocopherol or α-lipoic acid in *N*-methyl-2-pyrrolidone, during PS solubilisation process. The release of α-tocopherol or α-lipoic acid from the membrane during the phase inversion was quantified by fluorometry and UV spectrophotometry, respectively. Both types of enriched membranes were tested for separation characteristics and their antioxidant activity was evaluated by FRAP assay.

Obtained results show that membranes enriched with α-lipoic acid, compared to α-tocopherol, show better separation characteristics of biomolecules. However, the antioxidant activity was higher in the membranes coated with α-tocopherol.

To assess the capability of both types of enriched membranes to reduce OS, the *in vitro* tests with blood from HD patients should be further conducted.

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APPLICABILITY OF ULTRA-HIGH PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY IN PHARMACEUTICAL QUALITY CONTROL

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The interest in ultra-high performance supercritical fluid chromatography (UHPSFC) separations in the field of impurity profiling is continuously growing. About seventy different pharmaceuticals were analyzed by UHPSFC with UV and mass spectrometry detection in the first part of this study. Ten different quality control mixtures were selected and on a top of this, several beta blockers were added to this study in order to reflect the behavior of very basic compounds. Eight stationary phases (Torus diol, Torus diethylamine, Torus 2-picolylamine, Torus 1-aminoanthracene, BEH 2-ethylpyridine, BEH, CSH pentafluorophenyl and HSS C18 SB), 3 modifiers (methanol, ethanol, propan-2-ol), 3 modifier blends (methanol/acetonitrile, methanol/ethanol, ethanol/acetonitrile), and 5 additives in methanol (0.1% formic acid, 10 mmol/L ammonium formate, 10 mmol/L ammonium acetate, 0.1% ammonium hydroxide and 2% water) were tested in an attempt to find the most generic UHPSFC conditions. This approach resulted in UHPSFC methods for the determination of composition of 10 quality control mixtures. Their validation was necessary to prove their applicability in pharmaceutical quality control and for the determination of impurities at low concentrations. Using the best chromatographic conditions that emerged from the screening, four mixtures (atomoxetin, atorvastatin, estradiol, ticagrelor) were almost baseline separated. However, they required optimization, mostly due to limited resolution between active pharmaceutical ingredient and following impurity. Six mixtures (abiraterone, ezetimib, enzatulamid, agomelatine, vardenafil, dasatinib) were separated completely and generic methods used in the screening were validated either directly or after a fine tuning. The selected validation parameters followed the ICH guideline and included system suitability test, linearity, accuracy, and precision.

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NANOFIBROUS POLYMERS AS EXTRACTION SORBENTS IN A SAMPLE PREPARATION

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The nanofibers and their application are very popular topic of current research. Many papers about the nanofibrous polymers application in different fields of science have been

published. One of these is analytical chemistry, especially in a sample preparation procedures. This is an important part of analysis when interferences are removed from a sample and an analyte is pre-concentrated. At this time, new trends in the sample preparation methods are focused on using lower sample volumes, to achieve a higher specifity and selectivity, a lower consumption of organic solvents and fully automated methods. One of these trends is a search for new sorbent materials for solid phase extraction (SPE). SPE is the most used sample preparation method for liquid samples because of its simplicity and wide range of application.

The nanofibers have a good potential to be one of new sorbents for SPE through their high specific surface area. In this project, polyamide 6 nanofibers, prepared by electrospinning at the Technical University of Liberec, were tested. These nanofibers were packed into a SPE cartridge and their extraction efficiency was tested and evaluated for several pharmaceutical substances, namely parabens, steroids, flavonoids, and lipophilic insecticides fenoxycarb and permethrine. A selection of these substances was based on their different polarity, hydrophilic-lipophilic properties and molecular weight. The sample concentration and extraction conditions were optimized at the beginning of this experiment. HPLC coupled to spectrophotometric detection was utilized for the evaluation of the amounts of extracted analytes.

Some practical aspect of using the nanofibers (*e.g.* fabrication, packing and time of extraction) were also studied in this work.

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AUTOMATED CONTINUOUS IN-SYRINGE DISPERSIVE LIQUID-LIQUID EXTRACTION AND BACK-EXTRACTION FOR THE DETERMINATION OF NITROPHENOLS IN SURFACE WATER

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Continuous magnetic stirring-assisted dispersive liquid-liquid microextraction followed by dispersive analyte back-extraction to an aqueous acceptor phase is presented as a novel automated sample pretreatment method for pre-concentration of an analyte from a large volume of liquid sample. The procedure was automated using Lab-In-Syringe (LIS) technique. Mono-nitrophenols (*o-, m-,* and *p-*) were selected as model analytes known for their environmental impact.

The LIS flow technique uses the void of the syringe pump of a Sequential Injection Analysis (SIA) system as a size-adaptable extraction chamber. A continuous flow of sample provided by an additional pump is enabled by the secondary inlet in the syringe. First, the extraction solvent was aspirated into the syringe and the syringe was then filled with the sample for pre-concentration. The solvent was dispersed using a magnetic stirring bar inside the syringe that was driven by an external rotating magnetic field. During the ex-

traction, the sample was dispensed continuously using a low flow rate through the syringe void. After extraction, back-extraction to an aqueous acceptor phase was carried out and followed by spectrophotometric detection.

LIS operation mode, parameters of extraction including volume and type of the extraction solvent, flow rate and stirring rate, as well as parameters of back-extraction such as back-extraction solution type and concentration were optimized. The method was adopted for measurement of nitrophenols in surface water. Spectral analysis was applied for the quantification of the three isomers. The limits of detection for *o*-, *m*-, and *p*-nitrophenol ($\lambda = 400$ nm) were 0.14, 0.26, and 0.02 μmol/L, respectively. Three samples with a volume of 26.5 mL could be extracted in one hour.

The major advantages of this technique, in addition to typical characteristics of LIS, are continuous operation mode, which allows the use of large sample volumes, and consequent achievement of high pre-concentration factors exceeding 50.

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TESTING OF ELECTROSPUN NANOFIBERS FOR ON-LINE EXTRACTION IN CHROMATOGRAPHY SYSTEMS

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Solid phase extraction (SPE) is an important part of sample analysis. Recent trends in SPE based methods are focused on finding new sorbents and new approaches. Nanofibrous on-line SPE HPLC techniques fulfill both branches of modern trends in sample preparation. Nanofiber polymers have a great potential as sorbents due to their variability (chemical properties) and enhanced surface area (small fiber size), and on-line system ensures the repeatable and reproducible conditions of analysis with minimal demand on operator's skills.

Electrospun nanofibers are formed via electrospinning technology. Electrospinning, using strong electrostatic field for creation of polymer fibers from polymeric melt or solution, is elegant, widely used technique for obtaining nanofibers. It can be used alone (creation of base polymeric nanofibers) or in combination with other fiber producing technology (creation of composite materials).

In this project various types of electrospun nanofibers were tested as new undescribed sorbents for on-line SPE HPLC determination of some biologically active substances (*e.g.* bisphenol A, ochratoxin A, carbaryl and some pyrethroids). Materials included in this study were nylon 6, polyvinylidene difluoride (PVDF), polycaprolactone (PCL), polyethylene, and polystyrene nanofibers, and some composite materials, mixture of nanofibers and microfibers (PCL/PVDF, PCL/PCL).

New on-line SPE HPLC methods using nanofibers as sorbents were developed, validated and applied on real samples (river water, soil and beer).

The study was supported by Charles University SVV 260 412 and GAČR project no. 17-08738S.

SEPARATION BEHAVIOR OF SELECTED PHENOLIC ANALYTES

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Phenolic compounds in different fruit types represent a group of substances with wide range of physico-chemical properties. They contain at least one aromatic ring and more than one hydroxyl group. Phenolic compounds are mostly analyzed using reversed phase liquid chromatography with a gradient elution. The impact of different chromatographic conditions on separation efficiency was studied. Standard solution of main phenolic compounds including gallic acid, chlorogenic acid, epicatechin, rutin, phloridzin, quercetin, and phloretin, and methanolic apple extract were used in optimization.

The effects of mobile phase with pH values of aqueous component varying from 1.8 to 2.8, a column length of 100 and 150 mm, particle size of 3 and 5 μm, type of particles including fully porous, core-shell, and multilayered, and columns packed with specically modified stationary phases for the separation of polar substances were tested. Mobile phase composed of acetonitrile and acidified aqueous component under the flow rate of 1 mL min^{-1} with gradients of different profiles were eventually tested for the separation of standard mixture and apple extracts.

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DEVELOPMENT AND OPTIMIZATION OF UHPLC-MS/MS METHOD FOR ANALYSIS OF HCV ANTIVIRALS IN SAMPLES ARISED FROM CELL EXPERIMENTS

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Current clinical studies concerned with antiviral drugs show a great inter-individual variability of plasmatic concentrations even between patients with the same dosage. Antiviral drugs are known as substrates for some intestinal transporters important for their

absorption and treatment effectivity. However, the common therapeutic monitoring is based on monitoring of viral load, not on the monitoring of the antiviral drug levels in plasma. So far, only few antiviral compounds have been monitored for their membrane transport through intestinal barrier or their membrane transport was not evaluated at all. For this purpose, the Caco-2 cells serve as common absorption model for orally administered drugs. Samples originated from these experiments are complex mixtures with a high content of compounds including salts, glucose, vitamins, and albumin, and are limited in volume to 50–200 μL.

In this study, tenofovir disoproxil fumarate (TDF) and its two metabolites (tenofovir and tenofovir monoester), sofosbuvir, and ledipasvir, were analyzed. Metabolites of TDF were analyzed due to quite fast metabolism and pharmacologic properties. The sofosbuvir and ledipasvir are commonly used in HIV therapy in combination with other drugs for their complementary effect. Different chromatographic modes, such as RPxHILIC, mobile phase composition, additives, and stationary phases were tested during the optimization of method using the UHPLC-MS MS instrumentation. The compounds were detected in selected reaction monitoring mode with detailed optimization for each compound. Very important part of the analytical method was the sample preparation step. This step could not be accomplished via regular extraction due the small volume of the sample; therefore, use of microextraction techniques was needed. The liquid-liquid microextraction was selected with the different extraction solvents optimization as the method of choice for the sofosbuvir. The best conditions for extraction, chromatography, and detection will be used for biological experiments and the method will be validated.

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UHPLC-MS/MS STABILITY STUDY OF SOBUZOXANE AND ITS ACTIVE FORM ICRF-154 IN BIOLOGICAL MATRICES

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Inhibitor of topoisomerase II – sobuzoxane (SBZ) was synthesized as a prodrug of the anti-cancer agent ICRF-154 to improve its solubility and bioavailability. Furthermore, thanks to its structural similarity to dexrazoxane, the protective effect of SBZ on anthracycline-induced cardiotoxicity is under investigation. Although SBZ is approved as an anticancer drug in Japan, data on its bioactivation in biological matrices are scarce. Therefore, our aim was to conduct a bioactivation study of SBZ and ICRF-154 in several biological matrices and compare the results with our data collected on its close analogue – dexrazoxane (DEX).

SBZ (100 μM) was incubated with neonatal ventricular rat cardiomyocytes (NVCM), in the cell culture medium and in rabbit plasma for 24 hours. This experiment was followed by stability study of ICRF-154 and its open ring metabolite EDTA diamide in the cell culture medium and plasma. For chromatographic assay, NVCM cells and plasma were treated with protein precipitation, cell culture medium was simply diluted. All analyses were performed on reversed-phase Zorbax SB-Aq column using Nexera UHPLC system coupled with LCMS-8030 triple quadrupole mass spectrometer with ESI ion source (Shimadzu). Mobile phase composed of 1 mM ammonium formate and methanol in a gradient mode provided the best separation of all analytes and corresponding internal standards in 13 min.

Inside NVCM cells very low concentrations of SBZ along with high concentrations of ICRF-154 were determined. ICRF-154 was then gradually metabolized to EDTA diamide. The rapid degradation of SBZ was also observed in the cell medium without the NVCM and in rabbit plasma. The slower biodegradation of ICRF-154 was verified by an *in vitro* experiment. EDTA diamide was stable in all tested media for 24 hours.

The study was supported by Charles University (projects GAUK 344 615 and SVV 260 401).

IS SUPERCRITICAL FLUID EXTRACTION USEFUL TOOL FOR EXTRACTION OF BIOACTIVE COMPOUNDS?

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Sample preparation plays a key role in modern analytical methods. It usually takes more than 70% of time of the entire analytical process. Supercritical fluid extraction (SFE) is becoming very popular because it is environmentally friendly and can be used in a wide range of applications. The advantages of this methods result from the properties of CO_2 that is used as the preferred extraction solvent. It has low viscosity and high diffusivity facilitating passage of extraction fluid through the solid sample. This property permits fast extraction in mere tens of minutes. Moreover, the change in CO_2 temperature and pressure that affects the solvent strength allows extraction of compounds with various polarity in different fractions. Polarity of CO_2 can be increased via addition of polar organic solvent such as methanol and ethanol. Use of CO_2 with a small volume of organic solvents allows the effective pre-concentration of isolated compounds. SFE can be carried out in dynamic and static extraction operation modes where the flow-rate of solvent and time of extraction, both of which affect the mass transfer and the recovery of extraction, must be optimized. SFE can be readily automated and its on-line coupling with separation approaches represent the additional advantages. The contemporary SFE instrumentation allows the change of conditions during the extraction. Thus, the isolation of fractions containing biologically active compounds with various properties is possible to achieve in a single extraction run.

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UHPLC-MS/MS OF A NOVEL DEXRAZOXANE ANALOGUE JAS-2 AND ITS PRODRUG

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Dexrazoxane (DEX) is the only approved, clinically used drug effective in protection of myocardium against anthracycline-induced toxicity. 4,4′-(butane-2,3-diyl) bis(piperazine-2,6-dione) (JAS-2) was synthesized as a novel analogue of DEX. Pilot studies indicate that JAS-2 is more effective in protection of neonatal rat cardiomyocytes from toxic effect of anthracyclines as compared with DEX. Due to the poor solubility of JAS-2 in water, a prodrug with a code name GK-667 was prepared. Bioanalytical method for investigation of GK-667, its conversion to the active form as well as further metabolism of JAS-2 is needed. The aim of this project is 1) to develop and validate UHPLC-MS/MS method for determination of GK-667, JAS-2 and its metabolite ($JAS2_{met}$) in cell culture medium – DMEM and 2) to apply it for the stability/activation study. The UHPLC system coupled with a triple quadrupole mass spectrometer with ESI^+ ion source was used (both, Shimadzu, Japan). The analysis was achieved on Luna Omega Polar column (100 × 3.0 mm, 2.5 μm) protected with a guard column. A mixture of ammonium formate and acetonitrile in a gradient mode was used as a mobile phase. DMEM samples were treated by dilution using water ($JAS2_{met}$) or 2% formic acid (JAS-2, GK-667). Method was validated according to FDA guideline (GK-667 and $JAS2_{met}$: 1–100 μmol/l, JAS-2: 5–100 μmol/l, $R^2 \geq 0.991$). Stability study on GK-667 and JAS-2 (100 μmol/l) was conducted in DMEM at 37 °C *in vitro*. It has shown that GK-667 is rapidly converted to JAS-2, which is slowly degraded to $JAS2_{met}$. In comparison with the conversion of DEX to its metabolite – ADR-925, JAS-2 degraded faster to its open ring metabolite.

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TESTING OF NANOFIBERS AS NOVEL SORBENTS USING SEQUENTIAL INJECTION ANALYSIS SYSTEM

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Electrospun nanofibers (NF) have gained a lot of attention during the last years due to their unique features such as a large surface-to-volume ratio, possibility of using natural materials, and the possibility of customized chemical modification. Apart from their novel implementation, *e.g.* in medicine, NF are currently in the focus of analytical chemists for their great potential as a sorbent in solid phase extraction (SPE) techniques.

Sequential injection analysis (SIA) system comprising a piston pump, a switching valve, and a suitable detector is an advantageous tool to study the potential of NF as extraction sorbent offering simple operation, flow manipulation, and fast results evaluation. In this work, we studied handling, packing, and use of different geometries and devices to engage NF in a SIA system for the first time. Both planar arrangements and column format were studied. A specially designed 3D-printed holder allowing to house a single sheet of NF was chosen as the most suitable device due to the low amount of NF required, low backpressure, and minimized dead volume compared to the column format.

The extraction capacities of polyvinylidene fluoride, polyamide, polystyrene, polylactic acid, polycaprolacton and polyacrylonitrile NF were tested with model analytes differing in their physical-chemical properties. Retention of the molecules was evaluated from peak height measurements and the results will be presented.

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SEQUENTIAL INJECTION DETERMINATION OF THE HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID USING PRECONCENTRATION WITH A POLYMER INCLUSION MEMBRANE

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A sequential injection analysis (SIA) system for the determination of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in natural waters, which uses a polymer inclusion

membrane (PIM) with 20% Aliquat 336 as the carrier, 10% 1-tetradecanol as a modifier, and 70% poly(vinyl chloride) as the base polymer for on-line analyte extraction and pre-concentration has been developed. PIMs are extracting liquid membranes that offer improved stability compared to supported liquid membranes. They were used successfully in the past for on-line extraction in flow injection analysis.[1–3] After preliminary testing using batch-conditions, the SIA system parameters have been optimized and will be validated with the analysis of water samples.

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CANDIDA ALBICANS METABOLOMICS AND QUORUM SENSING IN HUMAN VAGINAL SWABS USING HIGH-RESOLUTION MASS SPECTROMETRY

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Candida albicans (CA) is a common part of human microbiota, typically present in oral cavity, gastrointestinal tract, and vagina. The quorum sensing is a system which regulates population density of the microorganisms and enables them to respond to changing physiological and environmental factors including human body. In the case of CA quorum, sensing molecules are represented with farnesol and tyrosol. In general, farnesol blocks the morphogenic switch from yeast to hyphae and tyrosol supports it.

Vaginal swabs were collected and diluted in phosphate buffer saline solution. Total of 68 patients and 14 healthy controls with and without vulvovaginal discomfort were analyzed. Presence of CA and its morphotypes (blastoconidia versus pseudo/hyphae) and culture positivity were also defined.

The study aimed at correlation of metabolomic data to microbiological evaluation of chronic vulvovaginal discomfort. Firstly, the MS full scan spectra were used for identification of features detected using the following parameters: minimum peak width 2 s, signal to noise ratio larger than 10, and < 5 ppm mass accuracy. Secondly, the principal component analysis (PCA) of individual patients, CA negative control, and pooling of samples were carried out. PCA of previously detected features and their dysregulation were distinguished as clusters, which correlated to microbiological parameters including pH, Nugent score, co-existence of different bacteria such as Gram-negative *Coccobacillus* and *Lactobacillus*, and presence of *Candida albicans.*

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DEVELOPMENT OF CAPILLARY ELECTROPHORESIS METHOD FOR THE CHARACTERIZATION OF PHARMACEUTICALS CONTAINING SILYMARIN COMPLEX

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The project is focused on the development and application of capillary electrophoretic (CE) method allowing separation of structurally similar *Silybum marianum* flavonolignans, namely silybin A (SBA), silybin B (SBB), isosilybin A (ISBA), isosilybin B (ISBB), silychristin (SCH), silydianin (SD) and their precursor taxifolin (TX) occurring in Silymarin complex.

The method development involved optimization of a number of experimental conditions such as concentration of boric acid, type and concentration of cyclodextrins, volume fraction of organic modifier, pH of the background electrolyte and length of capillary. Optimal background electrolyte was 100 mM boric acid of pH 9.0 (adjusted with 1 M NaOH), 5 mM heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and 10% (v/v) methanol. The separation was carried out in a fused silica capillary (internal diameter 50 μm, total length 80.5 cm and effective length 72 cm), with applied voltage 25.0 kV and UV detection at 200 and 320 nm. Base-line separation of all flavonolignans, including diastereomers SBA/SBB and ISBA/ISBB with resolution 1.73 and 2.59, respectively, was attained. The method was validated and subsequently applied to CE analysis of dietary supplements and a drug containing the silymarin complex.

The study was supported by SVV 260 412.

UHPLC-MS/MS ANALYSIS OF ANTIVIRAL AGENT SOFOSBUVIR FOR STUDY OF ITS TRANSPORT MECHANISM

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Antiviral agents are often administered in combination pharmacotherapy. Therefore, their administration increases the risk of drug-drug interactions on ABC transporters that

can potentially lead to failure of the treatment. For this reason, the understanding of the ABC transporters-mediated drug-drug interaction is important for safety and effectivity of the therapy. The aim of this project was the study of drug-drug interaction of sofosbuvir with other antiviral agents. Transport experiments were based on two buffer compartments, *i.e*. Hank's Balance Salt Solution, pH 6.5 or 7.4 with 1% albumin, separated by the Caco-2 cells monolayer, which served as the transport medium for the drug. The main analytical assignment was to develop and validate method for the determination of sofosbuvir in the buffer compartments.

Liquid-liquid extraction was used for the sample preparation with butyl acetate as the optimal extraction solution. Sofosbuvir was quantified using ultra high-performance liquid chromatography coupled with tandem mass spectrometry in the selected reaction monitoring (SRM) mode. The optimized chromatographic analysis in ACQUITY UPLC BEH HILIC 2.1 × 100 mm (1.7 μm) column was carried out in 3 min long isocratic HILIC elution with the mobile phase comprising 80:20 acetonitrile and 10 mmol/L ammonium acetate pH 6.0 at a flow rate of 0.3 mL min^{-1}. Our method was validated and subsequently applied for the samples obtained from the transport experiments. The results confirmed the presence of efflux transporters. In future, interactions of sofosbuvir with other antiviral drugs will be evaluated. The selectivity of UHPLC-MS/MS method will be improved by using isotopically labeled internal standards and by further optimization of separation to achieve separation of coeluting antiviral agents.

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PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

GLUTATHIONE PEROXIDASES, MicroRNA AND OBESITY ASSOCIATION

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Obesity is a serious health problem worldwide and is associated with increased risk of chronic diseases such as cardiovascular diseases, metabolic syndrome, and diabetes mellitus. The finding of the molecular mechanisms involved in adipogenesis could lead to the identification of novel biomarkers and therapeutic targets in treatment of obesity and potentially developing of anti-obesity drugs. Excessive cellular acummulation of reactive oxygen species (ROS) causes cell damage and is involved in a wide range of human diseases, including obesity and obesity-related pathologies. Cellular antioxidant system, that maintains the cellular balance of ROS, consists of non-enzymatic and enzymatic systems,

including glutathione peroxidases (GPxs). GPxs, catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols, and act also as sensors to transfer the message to its interacting proteins. Results of many studies showed that knockout of some GPx has fatal consequences, and changes in GPx expression are connected with severe pathologies, including obesity and diabetes. Therefore, findings of the regulatory mechanisms involved in GPxs expression are of a great importance.

One of significant ways in regulation of GPxs expression are microRNAs (miRNAs). MiRNAs are small non-coding, single-stranded RNA molecules consisting of about 22 nucleotides responsible for the negative posttranscriptional regulation of a variety human genes, hybridize mostly to 3′-untranslated region. Among others, the abberant expression of miRNAs may be associated with some disorders and diseases. In this study we decided to reveal the possible association between GPxs, microRNAs, and obesity, and observe the effect of excessive fructose in the diet. For our experiment, we used standard high fat diet obesity mice model with or without fructose administration. We observed changes in the expression of GPxs and selected miRNAs in liver and three types of adipose tissue (brown, visceral, and subcutaneous).

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ACUTE TOXICITY OF *R*-PULEGONE AND *R*-MENTHOFURAN IN HUMAN LIVER SLICES

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Monoterpenes *R*-pulegone (PUL) and *R*-menthofuran (MF) are major constituents of several plants and essential oils (*e.g.* peppermint, pennyroyal) used for flavoring foods and drinks, for herbal medicinal products and cosmetics. MF is the major metabolite of PUL in the body and they both display similar hepatotoxicity in rodents. Exposure to PUL and MF is primarily through food products and beverages flavored with spearmint oil, peppermint oil, or synthetic PUL. Serious/lethal cases of intoxication from pennyroyal oil with a high content of PUL indicated first that it is a potent hepatotoxin. Tolerable daily intake for PUL and MF has been set for food at 0.1 mg/kg of body weight, however doses exceeding that are commonly encountered in herbal medicinal products.[1] Despite a large number of PUL and MF toxicity and metabolism studies, vast majority of them are limited to rodents, making it difficult for regulatory authorities to apply the information to humans. In our experiments, 5 human liver samples received from surgery were used to gain precision-cut liver tissue slices, which were cultivated for 24 hours in the presence of PUL and MF

to determine acute toxicity in humans *ex vivo*. The half maximal inhibitory concentration (IC_{50}) for PUL and MF was determined to be 293 μM and 418 μM, respectively. We are planning to determine also influence of these hepatotoxicants on expression of liver enriched miRNAs (*e.g.* 122-5p, 885-5p, 192-5p, 125b-5p), since there is, to the best of our knowledge, no such a study validating liver slices on miRNA level.

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IN VITRO STUDY OF DEXRAZOXANE ANALOG JAS-2 AND ITS WATER-SOLUBLE PRODRUGS

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Anthracyclines (ANT) such as daunorubicin or doxorubicin are among the most effective anticancer drugs, but their use is hampered by their irreversible cardiotoxic side effects. 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.[1] Latest studies suggest that its activity is caused by catalytic inhibition of topoisomerase II.[2] However, its use in clinical practice is limited due to its high price and potential to increase some side effects of ANT (*e.g.* myelotoxicity). Therefore, in this work we prepared and studied DEX analog JAS-2 and, due to its poor solubility in water, also soluble JAS-2 prodrugs.

JAS-2 as well as its prodrugs significantly protected isolated neonatal rat cardiomyocytes against toxicity induced by daunorubicin. Especially in lower concentrations, their effectiveness was significantly higher than the effect of DEX. Whereas they are topoisomerase II inhibitors and act on the same enzyme as ANT, we also studied their impact on cell proliferation and ANT antiproliferative activity. All studied compounds showed significant antiproliferative activity in HL-60 leukemic cell line, also better than DEX. None of studied compounds compromised the antiproliferative effect of daunorubicin, thus they can be further developed as potential cardioprotective agents against ANT-induced cardiotoxicity.

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ROLE OF HEXOSE-6-PHOSPHATE DEHYDROGENASE IN PROLIFERATION AND MIGRATION OF CANCER CELLS

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Hexose-6-phosphate dehydrogenase (H6PD) produces reduced NADPH in the endoplasmic reticulum (ER) lumen. NADPH constitutes a cofactor for many reducing enzymes. The H6PD gene is amplified in several types of malignancies, and earlier work pointed toward a potential involvement of the enzyme in cancer cell growth. In the present study, a pivotal role of H6PD in proliferation and migratory potential of 3 human breast cancer cell lines was demonstrated. Knockdown of H6PD decreased proliferation and migration in SUM159, MCF7, and MDA-MB-453 cells. To understand the mechanism through which H6PD exerts its effects, the cellular changes after H6PD silencing in SUM159 cells were investigated. Knockdown of H6PD resulted in an increase in ER lumen oxidation, and down-regulation of many components of the unfolded protein response, including the transcription factors activating transcription factor-4, activating transcription factor-6, split X-box binding protein-1, and CCAAT/enhancer binding protein homologous protein. This effect was accompanied by an increase in sarco/endoplasmic reticulum Ca^{2+}-ATPase-2 pump expression and a decrease in inositol trisphosphate receptor-III, which led to augmented levels of calcium in the ER.

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EVALUATION OF DRUG UPTAKE AND DEACTIVATION IN PLANT

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Veterinary pharmaceuticals are used in large amounts in modern husbandry for treatment and prevention of diseases in animals. These drugs represent important source of environmental pollution as they can reach environment through the treatment processes, inappropriate disposal of used containers, unused medicine or livestock feed, and manufacturing processes. Plants are exposed to veterinary pharmaceuticals in pastures with treated animals, in fields fertilized with dung from treated animals or in aquatic ecosystems. Several reviews have been published regarding the potential impact of pharmaceuticals on plants. Pharmaceuticals as well as other xenobiotics enter plant body and can induce stress and consequent response. Anyway, in plants as well as in all other organisms, each xenobiotic represents a potential risk. Therefore, a sophisticated defence system in the form of xenobiotic-metabolizing enzymes exist. Owing these enzymes, plants are able to transform xenobiotics into various metabolites and store them in the vacuoles and cell walls.[1] Generally, the metabolites are non-toxic or less-toxic, but some metabolites can be similar or even more toxic than their parent compound.[2] For this reason, more detailed information about the metabolic pathways of each xenobiotic is necessary for a complex evaluation of eco-toxicological risks.

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PHARMACOLOGY AND TOXICOLOGY SECTION

OXYSTEROLS AFFECT ENDOGLIN EXPRESSION, INFLAMMATION AND DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN HUMAN AORTIC ENDOTHELIAL CELLS

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Endoglin (CD105, TGF-βRIII receptor), acts as auxiliary partner protein in TGF-β receptor complex. This protein is essential for endothelial NO synthesis and proper endothelial function. On the other hand, increased expression of endoglin was shown to participate in inflammatory infiltration of leukocytes and development of endothelial dysfunction. In addition, previous *in vivo* studies showed that hypercholesterolemia affects membrane and soluble endoglin expression/levels, however the precise importance/consequence of this effect is unknown. In this study, we aimed at elucidating the effects of 7-ketocholesterol (oxysterol with non-enzymatic origins, 7K) and 22-hydroxycholesterol (oxysterol with enzymatic origins, 22OH) on the development of endothelial dysfunction and expression of endoglin with respect to role of endoglin in the development of endothelial dysfunction in human aortic endothelial cells (HAEC).

HAECs passage 5 were exposed to 7K or 22OH (5.10 μg/mL) for 12 hours. Gene activity was evaluated using qRT-PCR and protein expression using flow cytometry (direct, indirect or intracellular). We demonstrated that only oxysterol with non-enzymatic origins – 7K was able to significantly increase expression of cell adhesion molecules and endoglin on gene, but also protein levels. These results suggest potential involvement of endoglin in endothelial dysfunction development after 7K treatment. Therefore, we focused on regulation of endoglin expression via 3 main transcription factors – KLF6, LXR and NFκB pathway. We have found, that inhibition of either KLF6 or LXR pathway is able to prevent 7K induced increase in endoglin expression.

We demonstrated that 7K is able to affect endoglin expression, its signaling, and inflammation in endothelial cells. Precise importance of endoglin and its regulation with respect to potential protective or harmful effects on endothelium is further investigated in our lab.

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HIGH SOLUBLE ENDOGLIN LEVELS ACCOMPANIED BY MILD HYPERCHOLESTEROLEMIA AGGRAVATE ENDOTHELIAL DYSFUNCTION IN MOUSE AORTA

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Soluble endoglin (sEng) is generated by the cleavage of the extracellular domain from membrane-bound endoglin in endothelial dysfunction-related pathologies, such as atherosclerosis. With respect to hypercholesterolemia as a risk factor of endothelial dysfunction, we hypothesized that combination of high sEng levels and hypercholesterolemia will results in the development/aggravation of endothelial dysfunction.

Three month old female transgenic mice on CBAxC57BL/6J background with high levels of sEng (Sol-Eng$^+$ *high*) and their littermates with low levels of sEng (Sol-Eng$^+$ *low*) were fed high fat diet for six months. We analyzed sEng levels (ELISA), total cholesterol levels and inflammatory markers (LUMINEX) in blood. Functional parameters of vascular reactivity were measured by wire myograph. Western Blot analysis of protein expression in aorta was performed.

Functional analysis of aorta showed impaired KCl induced vasoconstriction, endothelial-dependent relaxation after administration of acetylcholine as well as endothelial-independent relaxation induced by sodium nitroprusside in *Sol-Eng$^+$ high* group compared to *Sol-Eng$^+$ low* group. The expressions of membrane endoglin, p-eNOS/eNOS, pSmad2/3/Smad2/3 signaling pathway affecting vascular properties of aorta were significantly lower in *Sol-Eng$^+$ high* group compared to *Sol-Eng$^+$ low* group without significant effect on vascular inflammation markers (VCAM-1, ICAM-1, P-selectin and NFκB).

The results indicate that long-term hypercholesterolemia combined with high levels of sEng leads to the aggravation of endothelial dysfunction with possible alteration of membrane endoglin/eNOS signaling suggesting that high levels of sEng might be considered as a risk factor of cardiovascular diseases.

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PHARMACOKINETIC INTERACTIONS OF NOVEL ANTICANCER DRUGS WITH ABC DRUG EFFLUX TRANSPORTERS AND CYTOCHROMES P450

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Cancer treatment often fails due to multidrug resistance (MDR) of cancer cells. Possible causes of pharmacokinetic MDR include increased efflux of cytostatic drugs from the cell by ATP-binding cassette (ABC) drug efflux transporters and/or deactivation by drug metabolizing enzymes such as cytochromes P450 (CYP450s). Both of these mechanisms can lead to decreased concentrations of a cytostatic below the cytotoxic level. In the present work, we investigated interactions of nine new small molecule targeted drugs (alisertib, talazoparib, entinostat, entrectinib, onalespib, sapanisertib, tepotinib, ensartinib and vistusertib) with ABC transporters and CYP450s that are recognized perpetrators of pharmacokinetic MDR, *i.e.* with ABCB1, ABCG2, ABCC1 and CYP3A4, CYP3A5, CYP2C8, respectively. Tested compounds represent promising candidates from several pharmacological groups currently undergoing late phases (II/III) of clinical evaluation for lung and/or breast cancers, the unshakeable leading killers within oncological diseases. Inhibitory properties of selected drugs toward examined ABC transporters were tested using Hoechst 33342 and calcein AM accumulation/efflux methods in MDCKII-ABCB1, MDCKII-ABCG2 and MDCKII-ABCC1 cell lines transduced with respective human transporters. We observed significant dual inhibition of ABCB1 and ABCG2 by onalespib, tepotinib and ensartinib. In addition, tepotinib as well as alisertib were demonstrated to be also ABCC1 inhibitors. CYP450 inhibition was assessed using commercial Vivid CYP450 screening kits. In these experiments, we found entinostat as the multiple inhibitor of CYP3A4, CYP3A5 and CYP2C8 while entrectinib inhibited tested CYP450 isoforms with the exception of CYP2C8. Obtained screening results will be confirmed with other appropriate methods. Verified inhibitors will be further evaluated in combination experiments and their ability to potentiate the cytotoxic effect of MDR-victim cytostatics will be determined. In conclusion, our results serve as an important starting point for future studies, which might reveal possible beneficial therapeutic strategy.

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THE *IN VITRO* AND *EX VIVO* TESTING OF ANTIVIRAL DRUGS EFFECT ON RHODAMINE123 INTESTINAL ABSOPTION

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ATP-binding cassette (ABC) efflux transporters including highly expressed P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) are known to reduce intestinal absorption of many orally administered drugs. Drug-drug interactions (DDIs) on these transporters can result in changes in levels of plasma concentrations associated with decreased efficacy or safety of therapy.

As DDIs on ABCB1 or ABCG2 transporters cannot be investigated directly in humans, surrogate techniques have to be used. In our project we employed *in vitro* bidirectional transport experiments in colorectal adenocarcinoma cells Caco-2 cell line and *ex vivo* precision cut intestinal slices (PCIS) from rat ileum and we tested potency of selected antiviral drugs (lopinavir, ritonavir, abacavir, zidovudine, tenofovir disoproxil fumarate, rilpivirine, saquinavir, atazanavir, maraviroc, etravirine, ledipasvir, daclatasvir, sofosbuvir) to inhibit ABCB1/ABCG2-mediated transport of fluorescent ABCB1/ABCG2 model substrate rhodamine123 (RHD123).

Lopinavir, ritonavir, and saquinavir significantly abolished transport of RHD123 across monolayers of Caco-2 cells and RHD123 efflux from PCIS while atazanavir and daclatasvir revealed significant effect only in one model used; in Caco-2 cells and PCIS, respectively.

In conclusion, we further confirmed that reported inhibitors lopinavir, ritonavir, and saquinavir have potential to increase intestinal absorption of ABCB1/ABCG2 substrates. Moreover, method of accumulation in PCIS that combines advantages of experiments *in vivo* with high throughput capability of *in vitro* systems was shown as suitable model for screening/verifying the DDIs on ABCB1/ABCG2 transporters, however, interspecies differences should be always considered.

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ENTECAVIR TRANSPORT ACROSS PLACENTA AND THE ROLE OF NUCLEOSIDE TRANSPORTERS

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Entecavir is a highly used hydrophilic nucleoside derived antiviral drug exhibiting high efficacy against hepatitis B virus (HBV). As there is lack of safety data and information

about mechanism involved in entecavir placental transfer, its use during pregnancy is limited. Entecavir is suggested substrate of nucleoside transporters (NTs) that are categorized in two subfamilies – equillibrative nucleoside transporters (ENTs) and concentrative Na^+ dependent nucleoside transporters (CNTs). ENTs subtypes can be further distinguished by the sensitivity to *S*-(4-nitrobenzyl)-6-thioinosin (NBMPR).

We tried to determine whether NTs participate in transplacental passage of entecavir. For this purpose, we employed an *in vitro* uptake experiment in BeWo cells at 37 °C and 4 °C and an *in situ* dually perfused rat term placenta model (open-circuit), analyzing materno-fetal (M-F) and feto-maternal (F-M) transplacental clearances of entecavir on the organ level. Using BeWo cell line model we observed the effect of all inhibitors with significant decrease of [^{3}H]entecavir intracellular concentration in presence of 100 μM NBMPR, 5 mM uridine and 1 mM adenosine. [^{3}H]entecavir M-F and F-M clearances showed low level of its transplacental permeation, with negligible placental accumulation after the perfusion (≤ 3% of the [^{3}H]entecavir dose). We observed significant discrepancy between M-F and F-M compartments. NBMPR (100 μM) decreased entecavir total clearance significantly in both directions, whereas in M-F direction the significance appeared also in presence of 0.1 μM NBMPR and similarly in presence 5 mM uridine.

In conclusion, our data suggest involvement of ENTs and CNTs in transplacental permeation of entecavir. Further studies have to be performed to specify the subtypes of ENTs or CNTs involved in entecavir placental transfer and propensity of entecavir to drug-drug interactions on these carriers.

The study was supported by GAUK 324215/C/2015 and SVV 260 414.

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THE INHIBITORY EFFECT OF ANTIRETROVIRAL DRUGS ON THE TRANSPORT OF L-CARNITINE IN HUMAN PLACENTA

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Fetal L-carnitine levels, a cofactor in fatty acid beta-oxidation, are predominantly maintained through the materno-fetal placental transport. The uptake of L-carnitine to the placental trophoblast layer is ensured through the Na^+-dependent, high-capacity organic cation/carnitine transporter 2 (OCTN2), largely expressed in the apical plasma membrane of syncytiotrophoblasts. Affected transplacental transfer of carnitine can cause carnitine deficiency in fetus that is believed to be associated with pathological conditions, such as cardiomyopathy, skeletal myopathy, hypoketotic hypoglycemia *etc*.

The aim of our study was to investigate the inhibitory potential of a broad range of antiretroviral drugs on the OCTN2-mediated uptake of L-carnitine in human placenta. This was achieved by employing the *in vitro* approach in choriocarcinoma cell line BeWo and *ex vivo* techniques of L-carnitine uptake into the fresh villous fragments and microvillous plasma membrane vesicles (MVM), both isolated from human term placentas. The drugs included in the study comprised those targeting the human immunodeficiency virus enzymes, specifically: reverse transcriptase, proteases (PIs) and integrase; as well as viral entry inhibitors.

The initial screenings in BeWo cells revealed all tested PIs as inhibitors of L-carnitine uptake. Subsequent uptake experiments in *ex vivo* models confirmed the significant inhibitory potency of the protease inhibitors ritonavir (10 μM) and saquinavir (10 μM), causing decrease of the L-carnitine uptake by 31% and 38% in MVM vesicles and 44% and 35% in placental fresh fragments, respectively. Further evaluation will be needed to confirm the impaired transplacental carnitine transport caused by protease inhibitors in clinical settings and verify its impact on the fetal health in order to help optimize pharmacotherapy in pregnancy.

The study was supported by the Grant Agency of Charles University (Grant No. GAUK 1574217/C/2017).

NUCLEOSIDE TRANSPORTERS: ROLE IN PHARMACOKINETICS AND MECHANISMS OF REGULATION

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Nucleoside transporters (NTs) are ubiquitously occurring proteins categorized into two subfamilies: equilibrative nucleoside transporters (ENT1, ENT2) and concentrative nucleoside transporters (CNT2, CNT3). They are predominantly needed for maintaining nucleoside homeostasis and exhibit significant role in pharmacokinetics of nucleoside-derived drugs. In our research, we intended to elucidate expression profile of NTs in the placental tissues, mechanisms of gene expression regulation and to investigate whether they contribute to placental pharmacokinetics of nucleoside-derived antivirals and to pharmacoresistance of pancreatic cancer. We found that ENT1 and CNT2 are dominant subtypes of ENTs and CNTs, respectively, in first-/third- trimester human placenta, rat term placenta, and BeWo cells. Both subfamilies revealed considerable inter-individual differences in all types of tested tissues and CNT2 exhibited intraindividual variability in human placentas,

increasing its expression in the course of gestation. In the follow-up study, we showed that this increase might be associated with induced activity of cAMP/protein kinase A pathway. Concerning placental pharmacokinetics, we observed that NTs significantly contribute to placental transfer of anti-HBV entecavir, anti-HCV ribavirin, and anti-HIV abacavir. As ENT1 was suggested as predictive biomarker of susceptibility of patients with pancreatic cancer to gemcitabine, we performed retrospective study that, however, did not confirm the reported hypothesis.

In conclusion, our findings give significantly broadened knowledge about placental expression of NTs, their regulation, and role in drug pharmacokinetics, in particular in distribution of nucleoside-derived antivirals into fetal circulation and pharmacoresistance of pancreatic cancer.

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RADIOLABELED MONOCLONAL ANTIBODY RAMUCIRUMAB: *IN VITRO* AND *IN VIVO* BINDING TO THE TARGET

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Ramucirumab (RAM) is a fully humanized monoclonal antibody targeted against the extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2) which serves as a key receptor of angiogenesis, including tumour angiogenesis. RAM binds to a human VEGFR2 with much greater affinity than its natural ligands and selectively inhibits its function. Several types of cancer are well known for their overexpressing of VEGFR2. Therefore, RAM with proper radiolabeling could be potentially used for diagnostic imaging or targeted radiotherapy. The aim of this work was to compare selected methods of radiolabeling in terms of binding ability to VEGFR2.

Several radiolabeling methods (direct and indirect ^{99m}Tc labeling,^{131}I direct labeling according to chloramine T protocol and indirect ^{177}Lu labeling) were employed and the prepared radiopharmaceuticals were subsequently tested *in vitro* for receptor-ligand binding affinity with real-time radioimmunoassay. Two VEGFR2 expressing human cancer cell lines (PC3, SKOV3) were used in the binding study. A pilot *in vivo* experiment using mice bearing PC3-positive tumors was performed on the basis of *in vitro* experiment results. Regarding the ^{177}Lu labeling results we have determined radiopharmaceutical parameters following the conjugation of RAM with three selected macrocyclic chelators.

All introduced labeling methods demonstrated preserved affinity to the VEGFR2 receptor. However, both direct labeling methods augmented non-specific binding ability of RAM. Based on the results from the above stated experiments, the indirectly labeled

^{99m}Tc-HYNIC-RAM was selected for the animal experiments. According to the lutetium labeling experiment results, we have selected the p-SCN-Bn-CHX-A″-DTPA chelator as the most promising agent for the further experiments *in vitro* and in vivo.

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INVOLVEMENT OF FARNESOID X RECEPTOR IN NOVEL HUMAN CELLULAR MODEL OF STEATOHEPATITIS

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Non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide, encompasses liver damage initiating from simple steatosis to superimposed lobular inflammation (steatohepatitis, NASH) leading eventually to liver cirrhosis and hepatocellular carcinoma.[1,2] Up-to-date, treatment for NAFLD is limited to lifestyle modification, but recently a farnesoid X receptor (FXR) agonist, obeticholic acid has been shown to suppress liver inflammation suggesting the importance of FXR pathway in inflammatory hepatopathologies.[3] Currently, there is no established cellular model of human hepatocytes with hepatic immune cells with respect to NASH. Therefore, we aimed to introduce and validate human cellular model of steatohepatitis to investigate involvement of FXR pathway during this condition. For this purpose, we co-cultured hepatic HepaRG cells or primary human hepatocytes together with human macrophages differentiated from monocytic THP-1 cell line. The induced steatosis was measured by colorimetric triglyceride assay and visualized by BODIPY staining. Subsequently, we performed PCR to analyze expression of genes involved in lipid metabolism. Secreted inflammatory cytokines were determined by ELISA. Finally, expression of FXR sensitive genes was assessed by PCR. Here, we introduce a novel human *in vitro* model for steatohepatitis where we manage to induce significant steatosis in hepatocytes mimicking NAFLD without affecting cellular viability and subsequently we set up inflammatory conditions. We show that in our model, FXR pathway is inducible which allow us to investigate its involvement in NAFLD.

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ASSESSMENT OF THE STOICHIOMETRY OF IRON AND COPPER WITH DEHYDROSILYBIN A AND B

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Silymarin, the standardized extract from milk thistle (*Silybum marianum*) is approved in the EU for supportive treatment of alcoholic liver disease. Other effects of silymarin, which is a mixture of different flavolignans, on human health are suggested. The aim of this study was to analyse the interaction of pure isolated isomers of dehydrosilybin with copper and iron which can be relevant mainly in the gastrointestinal tract due to low biovailability of unmodified components of silymarin. Stoichiometry of the iron/copper complex with 2,3-dehydrosilybin A and B (DHSs) isomers was assessed using two independent methods (Job's[1] and complementary method[2]) in four (patho)physiologically-relevant pH values (4.5., 5.5, 6.8, and 7.5). The addition of ferrous, ferric and cupric ions to dehydrosilybin at pH 5.5, 6.8 and 7.5 resulted in clear bathochromic shifts of the absorbance maxima. This confirmed the formation of complexes between metal ions and both DHSs under these conditions. On the contrary, addition of Cu^{+} ions did not modify the absorbance spectrum of DHS pointing out to inability or low affinity of this substance to chelate cuprous ions. At pH 4.5, DHS formed a complex with Cu^{2+} and Fe^{3+} ions, but not with Fe^{2+}. In general, under all above mentioned conditions in which the metal complex was formed, DHSs were able to chelate metal ions at two different chelation ratios (2:1 and 3:1, DHS to metal, respectively). Due to different interactions of individual components of silymarin with iron and copper, the biological effect needs to be traced in a more complex assay.

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THE INFLUENCE OF FLAVONOID METABOLITES ON PLATELET AGGREGATION

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Flavonoids seem to have beneficial effects on the cardiovascular system. These include also antiplatelet activity.[1] The pharmacokinetic of flavonoids is very complex and the bioavailability of parent flavonoids is minimal. Most of them are metabolized by human gastrointestinal bacteria to smaller phenolic compounds, which are subsequently absorbed into systemic circulation, where they reach higher concentrations than parent flavonoids.[2] Therefore, the contribution of these metabolites in antiplatelet effect cannot be excluded.

The available phenolic compounds were tested at different levels of platelet aggregation in whole human blood. The initial screening of 30 metabolites has shown minimal or small inhibition effect of the most tested compounds on platelet aggregation induced by arachidonic acid, with the exception of four compounds. These were also able to block platelet aggregation induced by collagen. The mechanisms of action of these compounds include inhibition of platelet cyclooxygenase 1 and partly the effect on thromboxane A_2 synthase. Other potential targets of these metabolites will be investigated in further experiments.

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INTERGENDER DIFFERENCES IN THE VASOACTIVE EFFECT OF SELECTED ISOFLAVONOIDS AND THE THEIR COLONIC METABOLITES

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Isoflavonoids are well known phytoestrogens which can positively affect the human cardiovascular system.[1] Although they are poorly bioavailable after oral administration, isoflavonoids might be converted by some intestinal bacteria into absorbable metabolites.[2] Five biologically active isoflavonoids (daidzein, genistein, glycitein, biochanin A and formononetin), as well as four of their human gut metabolites (*S*-equol, *O*-desmethylangolensin, 4-ethylphenol and 2-(4-hydroxyphenyl)propionic acid) were tested in this study. Their vasodilatory action was measured *ex vivo*, on thoracic rat aorta, isolated from both male and female animals. The aortic rings were precontracted by norepinephrine (10 μM) and then the tested compounds were administered in a cumulative way, in concentrations ranging from 100 nM to 1 mM.

Dose-dependent vasodilation was evoked by most of isoflavonoids and their metabolites in biologically relevant concentrations, however their maximum effect was achieved only by relatively high doses. For some compounds a significant difference between EC_{50} values, obtained from male and female rat aorta, was observed. Interestingly, *O*-desmethylangolensin, an intestinal metabolite of daidzein, possessed a higher potency than its precursor on male aorta, but not on the female one. We can conclude that the effect of vasoactive isoflavonoids might be influenced not only by gut metabolism, but also by gender variation.

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INVOLVEMENT OF DRUG TRANSPORTERS IN MARAVIROC TRANSPORT ACROSS PLACENTAL BARRIER

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The chemokine receptor 5 (CCR5) antagonist maraviroc is an HIV entry inhibitor used in combination antiretroviral therapy (cART) including prevention of mother-to-child transmission in HIV positive pregnant women. Up to date, there are sparse data about maraviroc administration during pregnancy. Some case reports suggest limited passage of maraviroc across the placenta as indicated by the low umbilical-cord/maternal blood ratio reaching 0.33–0.37. This imbalance could be caused by activity of drug efflux transporter ABCB1 since maraviroc is assumed as ABCB1 substrate. However, this hypothesis has not

yet been verified directly in placental tissue. Therefore, the aim of this study was to clarify the mechanisms involved in transport of maraviroc across the placental barrier.

Employing dually perfused rat term placenta, a significant asymmetry in maraviroc transplacental clearances was revealed, showing accelerated transport in the fetus-to-mother direction, when compared to the mother-to-fetus direction. This transport of maraviroc from fetal to the maternal compartment was saturable and reduced in the presence of Abcb1 inhibitor elacridar. On the other hand, it did not change after addition of Abcg2 inhibitor fumitremorgin C, suggesting involvement of Abcb1- but not Abcg2-mediated efflux of maraviroc to the maternal circulation. The non-specific inhibitor ritonavir caused even more efficient reduction of maraviroc transfer to the maternal compartment indicating involvement of another transport system, except for Abcb1 in the transplacental kinetic of maraviroc.

In vitro transport assay across monolayer of human Caco-2 cell line confirmed maraviroc as a substrate of human ABCB1. Maraviroc transport in the presence of more or less specific ABCB1 inhibitors, such as elacridar, zosuquidar, verapamil, ritonavir, however, suggested an involvement of another human transporter(s) in maraviroc transfer. This hypothesis was further verified on human choriocarcinoma BeWo cell line (clone b30), which lacks human ABCB1. Significant decrease in maraviroc accumulation into the BeWo cells was caused by polyspecific transporter inhibitors (ritonavir, verapamil and dexamethasone) indicating involvement of uptake transporter. To evaluate the interplay of ABCB1 and uptake transporters in the feto-maternal transfer of maraviroc, the perfusion study in human placental cotyledon is currently being performed and analysis proceeds. To conclude, maraviroc is a substrate of ABCB1. The transporter accelerates transfer of maraviroc from fetus to mother. Nevertheless, some other transport mechanism seems to contribute to this process as well.

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OVERCOMING DAUNORUBICIN RESISTANCE VIA ABCC1 AND AKR1C3 INHIBITION BY CYCLIN-DEPENDENT KINASE INHIBITORS

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Pharmacokinetic mechanisms contribute to development of multidrug resistance (MDR) to anticancer drugs. These include efflux activity of ATP-binding cassette (ABC) transporters as well as degradation of active drug by drug-metabolizing enzymes. Daunorubicin (DNR) is an anthracycline cytostatic drug transported by various ABC pumps

including ABCC1 transporter with confirmed role in MDR. It is also a well-established substrate of aldo-keto reductase (AKR) 1C3, carbonyl reducing enzyme involved in anthracycline resistance in various tumors. We investigated two cyclin-dependent kinase inhibitors, AZD5438 and R547, and their ability to modulate DNR resistance on the level of ABCC1 and AKR1C3.

We identified AZD5438, in contrast to R547, as potent inhibitor of ABCC1 enhancing significantly DNR accumulation in MDCKII-ABCC1 cells. Respecting the drugs high toxicity, resistance reversal studies were conducted. However, we observed no difference between synergism in ABCC1-expressing and parental cell line when calculated by Chou-Talalay combination index method. Both compounds were also shown to inhibit AKR1C3 in transfected HCT-116 cells. Even though less potent, only R547 exhibited synergism in AKR1C3 transfected cells compared to parental ones, and thus revealed AKR1C3 inhibition as DNR resistance modulation option.

Even though we identified interactions of studied drugs with both ABCC1 and AKR1C3, the limited outcome of the reversal studies, taking into the account inhibitory data, may contribute to high intrinsic toxicity of both studied compounds. AZD5438 still shows potential to interact with multiple structures crucial in DNR resistance development, which could be conveniently exploited in heterogeneous resistant tumor tissue.

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NOVEL RAPID BUT PRECISE SPECTROPHOTOMETRIC SCREEENING METHOD FOR DETERMINATION OF ZINC CHELATION

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Zinc is an essential and one of the most important trace elements.[1] Both its lack and excess are associated with pathological states. Deficiency of zinc is more common and can also result from the treatment with approved iron/copper chelators.[2] Therefore, it was desirable to elaborate a new methodology for screening of zinc chelation.

The aim of this work was to prepare a reliable, rapid and cheap method for the screening of zinc chelation. Spectrophotometric assessment, using a known zinc indicator dithizone, was selected.

Initial screening performed by comparison of spectra of dithizone and its complex with zinc suggested 530 and 570 nm as suitable wavelengths for determination of zinc at pH 4.5 while 540 and 590 nm for pH 5.5–7.5. Additional research showed the lower wavelengths to be more suitable for this methodology. The sensitivity of the method was always bellow 1 μM with good linearity relationship between absorbance and zinc con-

centration. The method suitability was confirmed by use of two known zinc chelators, ethylenediaminetetraacetic acid (EDTA) and tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN). This method represents a sufficiently precise method for zinc chelation screening usable at (patho)physiologically relevant pH conditions. Such method can be employed for both screening of novel zinc chelators and for testing affinity of other metal chelators for zinc.

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CONSTITUTIVE ANDROSTANE RECEPTOR IN THE REGULATION OF XENOBIOTIC METABOLISM

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Constitutive androstane receptor (CAR) represents the superfamily of Nuclear Receptors (NR), that belong to the group of ligand-activated transcriptional factors that influence the expression of the target genes. CAR is coded by the gene NR1I3 and is primarily present in hepatocytes. This receptor belongs to the group of nuclear receptors that have no known endogenous ligands and therefore are called "orphan receptors". CAR is constitutively active and transcriptionally regulates its target genes independently of the presence of the ligands. CAR may play significant role not only in the xenobiotic metabolism, but also in intermedial metabolism.[1] CAR is not adequately expressed in standard human cell lines and it quickly loses its activity in isolated human hepatocytes. The only exception is the HepaRG cell line, that expresses functional CAR after one month differentiation.[2] In this project, we have used genetically modified CAR Knockout HepaRG cell line with deleted CAR and parent HepaRG cell line, to study the role of CAR in the regulation of xenobiotic metabolism genes. We have focused on the expression of two most important phase I enzymes, CYP3A4 and CYP2B6, that are controlled mainly by PXR (pregnane X receptor) or CAR, respectively. Based on our data, CAR Knockout HepaRG cell line seems to be functional and potential good model for the study of CAR-mediated regulation.

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ASPECTS OF POST-TRANSCRIPTIONAL REGULATION OF PREGNANE X RECEPTOR

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Pregnane X receptor (PXR) is an important transcription factor playing a critical role in regulation of xenobiotic and endobiotic metabolism. Although PXR was broadly studied regarding its function, less is known about mechanisms controlling its own expression. MiRNAs are short (~22 nt) nucleotides, which target MiRNAs responsive elements (MREs) predominantly within 3′-untranslated region (3′-UTR) of mRNA leading to suppression of gene expression. Up to date, only several MREs were described within 3′-UTR of PXR spanning 1272 nt. In our study, we tried to uncover mechanisms standing behind post-transcriptional regulation of PXR.

At first, gene reporter study revealed a strong suppressive role of 3′-UTR of PXR in hepatoblastoma-derived HepG2 cells. Since miR-18a-5p responsive element was predicted *in silico* and recently confirmed within 3′-UTR of PXR, we tested whether miR-18a-5p could be responsible for inhibition of PXR expression. As shown by RT-qPCR, miR-18a-5p is substantially expressed in HepG2 cells. However, activity of reporter vector including MRE did not differ from that of control vector comprising reverse sequence of MRE. In the same line, antisense oligonucleotide against miR-18a-5p did not lead to increased activity of either MRE or 3′-UTR PXR vectors. Forced expression of miR-18a-5p caused decrease in activity of both MRE or 3′-UTR PXR vectors suggesting that MRE for miR-18a-5p is functional.

In conclusion, 3′-UTR of PXR appears to contribute to regulation of PXR expression. More other aspects, *e.g.* feedback regulation of PXR will be discussed during presentation.

INTERACTIONS OF ANTIRETROVIRALS WITH DRUG TRANSPORTERS: ROLE IN THE PHARMACOKINETICS

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The efficacy of anti-HIV therapy depends strongly on maintaining sufficient levels of antiretroviral drugs in plasma and body tissues and on the ability to prevent the development of resistant viral strains. Co-administration of two or three antiretrovirals, so called combination antiretroviral therapy (cART), is therefore recommended in most therapeutic regimens. However, it bears the risk of drug-drug interactions (DDI). In particular, DDI on membrane drug transporters can significantly affect absorption, distribution and

elimination of co-administered therapeutic compounds. Here we aimed at studying interactions of several antiretrovirals with selected ABC and SLC drug transporters using *in vitro* accumulation and transport assays on different cell lines expressing selected human ATP-dependent (ABC) transporters or solute carriers (SLC) transporters, *in situ* method of dually perfused rat placenta or *in vivo* pharmacokinetic study on Wistar male rats. We revealed emtricitabine as substrate of MATE1 but not OCT1, OCT2, P-gp, BCRP or MRP2 membrane transporters. Further, we proved that etravirine is inhibitor of BCRP but not P-gp and is able to increase transport of tenofovir disoproxil fumarate (TDF) across placenta from mother to foetus. We also described rilpivirine as an inhibitor of P-gp and BCRP but not MRP2, OCT1, OCT2 or MATE1 and its ability to enhance bioavailability of perorally administered abacavir. We further discovered, that efavirenz is inhibitor of OCT1, OCT2, MATE1 and MRP2 but is not a substrate of P-gp, BCRP or MRP2. Its DDI on OCT and MATE1 transporters significantly decreases renal clearance of lamivudine, prolongs its elimination half-life and leads to increased lamivudine accumulation in renal tissue. Our results also show, that lamivudine transplacental passage is probably not influenced by P-gp, MRP2 nor BCRP, but it seems to be influenced by MATE1.

In conclusion, our results clearly show high potential of several antiretrovirals to cause transporter-mediated DDI. These data should help to optimize the therapy of HIV positive patients.

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CLINICAL AND SOCIAL PHARMACY SECTION

COLD, BUT RISING STAR OF VALUE ADDED MEDICINES IN EUROPE

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Drug therapy today is moving from a one-fits-all patients approach towards drug therapies in conjunction with disease specific diagnostics to "personalized medicines" as well as patient centric therapies for different patient populations towards "individualized medicines".[1] The current pool of existing molecules potentially re-positioned, re-formulated or combined with new technological platforms and services might offer therapeutic alternatives and opportunities for patients and healthcare systems. Even if this concept has been known for many years and despite their significance, refined pharmaceuticals are still described in a rather confusing manner.[2] There is still no agreed terminology to name properly the pool of those re-innovated products, although Medicines for Europe[3] established one single terminology known as Value Added Medicines.

Comparing utilization of such an innovation, significant difference has been spotted between US and EU. Based on selected case studies (*e.g.* risperidone thin film), it is per-

ceived, that the bottleneck in better utilization of such a concept seems to be market access and non-harmonized pricing conditions in the various European countries, thus preventing its broader use.

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THE BIOIMPEDANCE SPECTROSCOPY APPLICATION IN PREGNANT WOMEN WITH PRETERM PREMATURE RUPTURE OF MEMBRANES

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Many pregnancies are complicated by preterm premature rupture of membranes (pPROM) that causes approximately one third of preterm deliveries. It can lead to significant perinatal morbidity and risk of fetal death. The most patients deliver within one day of pPROM, whereas especially patients in lower gestation week give birth within 1 to 4 weeks. Unfortunately, a tool that could determine the term of delivery appropriately is still lacking. During pregnancy, there is an increase in the volume of maternal body fluids, which culminates before labor. These changes are measurable by bioimpedance spectroscopy (BIS). Therefore, the hypothesis of this study was to evaluate the BIS application for delivery term prediction. In this study, 97 pregnant women after pPROM were examined by BIS on the day of diagnosis and compared with 173 examinations of healthy pregnant women in different period during pregnancy including the delivery day. Our results show that during pregnancy together with increasing total body fluids, the resistance measured by BIS decreases with the lowest value on the delivery day. No significant difference between this value in healthy labouring women and labouring women after pPROM ($p = 0.920$) was demonstrated. What is important, resistance is significantly different from mother with pPROM who is giving child and from pregnant woman delivering ≥ 7 days of diagnosis ($p < 0.0001$). Confirmation of this finding gives this simple method for the term delivery prediction that could improve care of pregnant women with pPROM.

The study was supported by Charles University (SVV 260 417, GAUK 772216, PROGRESS Q42) and MH CZ – DRO (University Hospital Hradec Králové, 00179906).

ADJUSTMENTS IN ENERGY EXPENDITURE AND SUBSTRATE OXIDATION DURING PREGNANCY

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Metabolic adjustments occur during pregnancy to support fetal growth; however, there are only a few longitudinal studies describing these changes over the time of pregnancy. By indirect calorimetry (IC), we measured resting energy expenditure (REE) and substrate oxidation in healthy, active, non-smoking, pregnant women after 12 h of fasting in 3 different periods – 2nd trimester (23.39 ± 2.16 weeks of gestation); 3rd trimester (31.04 ± 1.13 weeks of gestation) and late 3rd trimester close to delivery (37.41 ± 0.72 weeks of gestation). With increasing gestational age, O_2 consumption ($r = 0.364$; $p = 3.000 \times 10^{-5}$), CO_2 production ($r = 0.463$; $p = 4.695 \times 10^{-8}$), respiratory quotient ($r = 0.249$; $p = 0.005$), REE ($r = 0.402$; $p = 3.000 \times 10^{-6}$) and carbohydrate oxidation ($r = 0.249$; $p = 0.005$) were increasing. Only protein oxidation was decreasing ($r = -0.222$; $p = 0.013$). Very similar correlations were found between all mentioned parameters and the number of days from examination until delivery. What is more, protein oxidation in the third period inversely correlated with the newborns birth weight ($r = -0.464$; $p = 0.019$). These results indicate increasing REE and higher use of carbohydrates as a source of energy with increasing length of pregnancy caused probably by the alteration in maternal tissue and metabolism to ensure foetal growth and development. Protein utilization is conversely decreased to retain protein for tissue synthesis. Metabolic examination done by IC during pregnancy could be potentially useful since it allows recommendations on energy and macronutrients for achieving a balanced intake.

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POTENTIALLY INAPPROPRIATE MEDICATION USE IN NURSING HOME RESIDENTS IN THE CZECH REPUBLIC: RESULTS FROM THE EU SHELTER PROJECT

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Potentially inappropriate medication use (PIM use) presents a frequent problem of potentially inappropriate prescribing that may lead to higher risk of adverse drug events

in older patients, often suffering from multiple disorders, polypharmacy and presenting higher degree of frailty. Analyses of the EU SHELTER project (Services and Health in the Elderly in Long-Term care, 7th FP, 2009–2014) focused on description of comprehensive clinical characteristics and prescribing practices in 4156 long term care residents in 7 EU countries (Czech Republic, Italy, Germany, Netherlands, Finland, UK, France) and Israel. This work presents findings of PIM prescribing in nursing home residents in the Czech Republic after application of Beers 2012 criteria, Czech national 2012 consensus on PIMs and STOPP/START criteria.

490 nursing home older patients (65+) residing in 10 Czech long-term care facilities in geographically different areas were prospectively assessed during the baseline period of the EU SHELTER project. In this prospective assessment, RAI-LTCF comprehensive geriatric tool was applied including different characteristics (*e.g.* demographic characteristics, functional status and mobility, clinical characteristics and medication use) and various functional scales. Prevalence of PIM use was determined using 3 sets of standard explicit criteria: Beers 2012 criteria, Czech national 2012 consensus on PIMs and STOPP/START criteria. Descriptive statistical methods were applied using SPSS Software v.12.

The highest prevalence of potentially inappropriate medication use (62.3%) was determined by Czech national consensus, then by Beers 2012 criteria (60.2%) and STOPP/START criteria (44.5%/52.9%). The most prevalent prescribing problems according to Czech national consensus were: long-term use of benzodiazepines (BZDs) in depressive patients (7.8% in the total sample), untreated constipation caused by opioid analgesics (7.4%), long-term use of NSAIDs, indication of ACE-I without clinical monitoring (6.1%), use of verapamil in patients with chronic constipation (3.9%) and use of doxazosin in older patients having urinary incontinence (2.9%). The most prevalent problems according to Beers 2012 criteria were: long-term use of BZDs in patients with the history of falls (6.3%) and in cognitively impaired older residents (4.3%), long-term use of zolpidem in cognitively impaired patients (4.3%) and long-term use of ASA or clopidogrel and NSAIDs without gastroprotection (3.7%). Among problems of undertreatment according to START criteria were identified the most frequently: no anticoagulation treatment in atrial fibrillation (7.1%), no ACE-I or sartans in patients suffering from chronic heart failure (4.5%) and no antidepressive treatment in patients having diagnosed depression (3.9%).

Application of Czech national consensus of PIMs yielded the highest prevalence of prescribing problems compared to Beers 2012 and STOPP/START criteria in Czech nursing home residents. The Czech national consensus is a specific tool summarized from different, until now published explicit criteria, including also Beers and STOPP/START criteria. This tool reflects specific availability of PIMs on the Czech pharmaceutical market and national prescribing habits.

The study was supported by SVV 260 417.

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DRUG UTILIZATION STUDY OF ANTICOAGULANTS

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Anticoagulants are used in the prevention and treatment of thromboembolic events. Mainly warfarin had been used in the area of oral anticoagulants (OACs) until the direct oral anticoagulants (DOACs) were approved, therefore some changes in OACs utilization are expectable. The aim of the study was to explore drug utilization patterns of anticoagulants in the Czech Republic (CR) during the period 2007–2016. A retrospective analysis was conducted using the data from the State Institute of Drug Control database. This database contains reports of drug supplies from distributors to pharmacies, health care facilities, vendors of selected pharmaceuticals, and veterinarians. All anticoagulants, approved in the CR during the study period, were included in the study. Drug utilization was calculated as a ratio of number of defined daily doses per thousands of inhabitants per day (DDD/TID). Also the anticoagulants expenditures analysis was performed. Descriptive statistical analysis was provided to describe drug utilization patterns. Cross-correlation was applied to assess NOACs and warfarin utilisation relationship. The utilization of all anticoagulants increased during 2007–2016 from 14.15 DDD/TID to 26.42 DDD/TID. The DOACs utilization increased from 0.002 DDD/TID in 2008 to 5.26 DDD/TID in 2016. On the contrary, warfarin utilization decreased after DOACs approval from 10.03 DDD/TID in 2007 to 8.36 DDD/TID in 2008. However, its current utilization almost stagnates. Increase in parenteral anticoagulants utilization was also apparent at low molecular weight heparins, nevertheless the use of unfractionated heparin and fondaparinux was low. The financial expenditures analysis revealed that the OACs expenditures increased obviously, due to the higher cost of DOACs. In conclusion, the results showed increasing utilization of anticoagulants and that increasing use of warfarin was stopped by rising use of DOACs significantly. This trend, also notified in other studies, can contribute to further research and enhance anticoagulants practical use manners.

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ANALYSIS OF FACTORS INFLUENCING THE RISK OF FALLS: PILOT RESULTS

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In hospitalized patients, the risk of falling is increased due to the changes of environmental factors and very often by modification or changes in pharmacotherapy. The goal of the project was to analyze the impact of pharmacotherapy and other factors associated with falls in patients who fell during the first 6 months of 2017 within their hospitalization in 16 selected departments of South Bohemia Hospitals.

The results of this prospective case-control study were gained from a web application containing data about patients who fell down. The obtained data originated from patient's medical documentation (*e.g.* drug and personal anamnesis, selected laboratory results) and were completed with other information (*e.g.* associated risk factors *etc*.). Each fall was matched with 10 control patients who did not fall during hospitalization and had similarities in some defined parameters (hospital department, sex, age, duration of hospitalization, number of drugs). The analysis was mainly aimed to identify risk drugs, risk diagnosis and other risk factors, which could have led to falls. The analysis of risk diagnosis was conducted by literature review and it considered 30 risk diagnoses. Potential and individual risks were determined for each patient who fell down. The potential risk represented all drugs that showed an increased risk of falls described in current literature or it was possible to assume risk according to mechanism of action. If a clinical pharmacist could not exclude drug influence on the fall probability, then the drug was marked as the individual risk. The overall influence of pharmacotherapy on falls was classified by the Likert scale. The obtained pilot results were described by methods of descriptive statistics.

157 patients were analysed with the representation of 52% men and the median age reached 79 ± 12.2 years old. Total number of drugs was 1271 for all patients and the potential risk was reported in 45% of them. Clinical pharmacist marked the individual risk in 42% of all potential risk drugs. The average number of diagnoses with a fall risk was 2.7 per patient.

The influence of administered drugs on the chance of fall was detected in our study. One of the elimination ways is to engage the clinical pharmacist into the multidisciplinary team that takes care of patients in health-care facilities.

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POTENTIALLY INAPPROPRIATE MEDICATION USE (PIM USE) IN OLDER PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL IN SOUTH INDIA: PREVALENCE AND RISK FACTORS

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Even if the quality and safety of drug prescribing in older population is a major global health care concern, Potentially Inappropriate Medications (PIMs) are still widely prescribed in older adults both outside the hospitals as well as in acute care. Strategies supporting the medication safety in older population emphasize nowadays to avoid unnecessary use of PIMs because of frequent drug-related problems. Older adults tend to use multiple drugs and frequent age-related physiological changes, multiple disorders and clinical problems also contribute to potential inappropriateness of some medications. The objectives of this study were to determine the prevalence and risk factors of PIM use in older patients admitted to a tertiary care teaching hospital in Warangal, India and to describe the most frequently documented PIMs on different acute care wards.

A prospective observational study was carried out among elderly patients admitted to a 1000 bedded tertiary care teaching hospital, Warangal, South India from November 2016 to June 2017. Data on age, gender, diagnoses, duration of hospital stay, treatment and therapeutic outcomes were collected. Prescriptions were assessed using potentially inappropriate medications defined by American Geriatric Society 2012 Beer's criteria. Patients were counselled regarding disease conditions and medication use. Data has been analysed using SPSS.

The prevalence of PIM use (total prevalence on admission and differences between clinical wards) and risk factors have been analyzed in older patients admitted to acute care.

A total of 1050 geriatric patients (75.04% males and 24.96% females) were admitted to acute tertiary care hospital in India during the study period. The average age of geriatric patients was 71.69 ± 4 years. The prevalence of polypharmacy (measured as 5 and more medications) was 74% and users of PIMs were mostly admitted to Cardiology (38%), Department of Internal Medicine (24%) and Surgery (12%). According to the Beers 2012 Criteria, a total of 798 admitted patients (76%) were prescribed at least one PIM. Out of all the medications used, 39.5% were PIMs. In 25.8% of older patients these PIMs should have been avoided independently of disease conditions, in 6.1% were inappropriate in the presence of certain illnesses or symptoms and in 4.6% PIM users could be prescribed with a special caution. In the multivariate regressive analytical model, variables of polypharmacy ($p = 0.0187$), psychiatric disorders ($p \leq 0.0001$) and cerebrovascular diseases ($p = 0.0036$) were significantly associated with PIM use.

Usage of PIMs is highly prevalent in older patients, particularly outside the hospitals. The associated factors of PIM use in acutely admitted older adults were mainly poly-

pharmacy and psychiatric or cerebrovascular morbidity. Health care professionals outside hospitals are nowadays recommended to follow geriatric guidelines in order to significantly reduce unnecessary and risky use of PIMs. These professionals are requested to intervene through proper knowledge in order to reduce frequent adverse drug events and high hospitalisation rates in geriatric population. In this effort, clinical pharmacy services and interdisciplinary cooperation with clinical pharmacists are very important.

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KNOWLEDGE OF DIFFERENT PHARMACY PROGRAM STUDENTS ON RATIONAL GERIATRIC PHARMACOTHERAPY

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Knowledge of rational geriatric pharmacotherapy and appropriate geriatric care becomes an important issue with aging of the world population and increasing proportion of geriatric patients. The aim of the study was to assess and compare the level of knowledge in rational geriatric pharmacotherapy and geriatric care among students of different pharmacy programs in Telengana, India.

A multicentre, cross-sectional design was used to collect the data for this study. The study was conducted between 1st November and 31st December 2014 in the state Telangana, India. Self-administered questionnaire was used to collect the data by using a convenient sampling technique from the final-year students of three different pharmacy programs enrolled at four colleges of pharmacy in Telangana state, India (number of responders were 438 out of 720 students). A 31 item open-ended questionnaire containing questions related to sociodemographic features (7 items), and remaining 24 questions related to geriatric care: aging (5 items), physical activity (6 items), pharmacotherapy (10 items) and nutrition (3 items). The geriatric pharmacotherapy knowledge scores ranging from 1 to 25.

A total of 438 pharmacy students with different pharmacy degree levels (109 diploma in pharmacy students, 198 bachelor of pharmacy students, and 131 Doctor of pharmacy students) completed the survey. The overall mean score of knowledge of geriatric issues was 7.99 ± 2.96 among pharmacy students. The PharmD students recorded significantly

the highest mean score of knowledge (mean score = 8.88) than the D. Pharm and B. Pharm students (7.55 and 7.72, respectively; $p < 0.001$). The level of knowledge related to rational geriatric pharmacotherapy and geriatric care was low among pharmacy students in India. Significant differences were found among the three pharmacy program students. An optimal plan of education should be established to begin more geriatric-focused courses and training in future pharmacy curriculum in order to enhance the knowledge of pharmacy students and their preparedness for their clinical practice after graduation.

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CARDIOVASCULAR COMORBIDITIES AND RISK FACTORS IN ACCORDANCE WITH PHARMACOGENETIC OF METHOTREXATE IN RHEUMATOID ARTHRITIS PATIENTS: PRELIMINARY DATA

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Patients with rheumatoid arthritis (RA) have a higher occurrence of cardiovascular risk factors and thus cardiovascular diseases (CVD) are by about 50% more frequent than in the general population. Despite modern approach in RA therapy, methotrexate (MTX) is still considered as an anchor drug. Due to its anti-inflammatory activity, MTX works favourably on the risk of CVD development.

The aim of our study is to determine whether folate pathway related single nucleotide polymorphisms – SNPs (rs2298383, rs3761422, rs2267076, rs2236624, rs17602729, rs2372536, rs1127354, rs2236225, rs1801131, rs1801133, rs4149056) might be predictive of increased cardiovascular comorbidities in RA patients treated with oral MTX.

Data from genotyped patients were collected at the University Hospital in Hradec Králové from the 1st September 2016 to the 31st May 2017. Personal and drug anamnesis were obtained from medical documentation. Moreover, other data such as blood pressure, height, weight, waist circumference and ECG were collected. Blood and urine samples for biochemical and haematological analysis were also gathered during patients visit.

115 patients were enrolled (34 men and 81 women) with 24 CVD and 219 cardiovascular risk factors in total. The average age was 60.6 ± 11.8. ΔDAS reflecting the change in RA disease activity was 2.07 and the average number of drugs used by these patients was 7.8.

Treatment with MTX is connected with many adverse reactions, which could lead to serious side effects. On the other hand, in some patients, the usual dose of MTX is without therapeutic effect. We suppose that these situations might be caused by different genetic predisposition in the genes of the folate pathway. If we find a correlation between higher prevalence of CVD and those SNPs it would help us to individualize dosing depending of patient's genotype.

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PREVALENCE OF DIETARY SUPPLEMENT USE IN PATIENTS IN PRE-OPERATIVE PERIOD

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The prevalence of complementary and alternative medicine (CAM) use by presurgical patients ranges from 7.2% to 49.8%.[1,2] Use of some dietary supplements (DS), mostly herbal remedies, may lead to an increase of adverse drug effects during or after the surgery.[3] In the Czech Republic, the prevalence of vitamins/minerals and herbal remedies use is 54.6% and 47.8% in general population, respectively.[4] No data on DS use in presurgical patients exist, therefore the aim of this study was to determine the prevalence rate of DS use in presurgical patients. A self-administered questionnaire was distributed among 180 presurgical patients at nine hospital departments of the University Hospital Hradec Králové from July 2017 to January 2018. A total of 108 respondents participated in the study (response rate 60%). Fifty five percent of respondents used some form of CAM less than 30 days before surgery, 89.8% of these respondents used at least one DS. The most commonly used DS were herbals (64.4%), non-herbal DS (49.2%) and vitamins and minerals (37.3%). A total of 31 respondents consumed herbs with a daily diet. There is a relatively high prevalence rate of DS use in presurgical patients. Further analysis is needed to identify potential risks associated with DS use prior surgery as well as patients' awareness of such risks.

The study was supported by Charles University SVV 260 417.

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SECTION OF BIOLOGICAL SCIENCES

THE EFFECT OF SPINAL ANESTHESIA ON THE EXTENT OF DNA DAMAGE

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Anesthesia can impact the organism in several ways. Both general and spinal anesthesia are used during joint and hip surgery, but which technique offers a better outcome remains controversial.[1,2,3,4] There are no well documented data on the DNA damage during spinal anesthesia. Our aim was to monitor DNA damage in the human body through spinal anesthesia in a pilot study.

21 patients undergoing orthopedic surgery on limbs and/or big joints were included. Blood samples were obtained from patients after informed consent with the study. The blood was collected before and right after the surgery and was subjected to DNA damage analysis immediately. To the detection of the degree of DNA damage the Comet assay[5] was used. Samples were centrifuged with LSM (Biotech, Austria) to obtain lymphocytes. PBS buffer was used to re-suspend the lymphocytes and adjust the concentration to 1 million cells/mL. 35 μL of the suspension was mixed with 85 μL of low melting point agarose and was spread onto pre-coated microscopy slides on 85 μL of solidified high melting point agarose. Cells were lysed for 1 hour at 4 °C in high salt and detergent solution to obtain the nuclear DNA on the agarose gel. The nuclear DNA was incubated for 45 minutes with the specific enzymes ENDO III for detection of oxidized pyrimidines and FPG to detect dam-

aged purines. Slides were then exposed to alkali for 40 minutes for DNA unwinding and cleavage of alkali-labile sites. Then the electrophoresis was applied for 30 minutes at 4 °C and DNA migrated to the anode and created comets. After neutralization and staining with ethidium bromide, the gels were analyzed by a specialized semiautomatic software Lucia (Laboratory Imaging, Czech Republic) by fluorescence microscopy. The software measured the ratio of DNA intensity in the tail relative to the head of the comet and provided the ratio of single-strand breaks (SSB) of DNA, pyrimidine damage (ENDO) and purine damage (FPG). For comparison of results paired the Wilcoxon test was used. The statistical significance was at $p = 0.05$. Data are presented as a median and interquartile range.

21 patients were analyzed for SSB and data about damaged purine and pyrimidine nucleic acids were obtained from 19 patients. There was not a significantly higher amount of SSB, ENDO, and FPG after the surgery as described in Table 1.

Our results declare that spinal anesthesia does not significantly damage nuclear DNA. Due to the lower load of genotoxic agents, whenever possible patients should undergo joint and hip surgery in spinal anesthesia instead of general anesthesia.

Table 1: Results of the DNA damage

	Baseline	After spinal anesthesia	p value
SSB	4.42 (1.77–9.35)	4.80 (2.12–8.96)	0.20
ENDO	4.73 (2.68–12.69)	6.50 (3.26–12.51)	0.47
FPG	6.34 (4.82–4.69)	8.25 (4.69–14.07)	0.24

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EVALUATION OF *IN VITRO* TOXICITY OF POTENTIALLY ANTIMICROBIAL COMPOUNDS FROM THE GROUP OF AMINOBENZOIC ACID DERIVATIVES

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Determination of *in vitro* cytotoxicity for newly developed substances is an essential part of their preclinical testing. The aim of the study was to find out whether six potential

antimicrobial compounds from the group of aminobenzoic acid derivatives (PABA-Et, PABA-Me-3, PABA-Et-3, DAB-3, MABA-3, MABA-5) are toxic to human liver cells. A standard human liver cell line (HepG2) was chosen as the experimental model. The cells were incubated with the tested compounds at different incubation concentrations for 24 h. A colorimetric method based on the reduction of tetrazolium dye MTS in living cells to formazan was employed.[1] The standard toxicological parameter IC_{50} was calculated by nonlinear regression from a semilogarithmic plot of incubation concentration versus percentage of absorbance relative to untreated controls using GraphPad Prism 7 software. The determined IC_{50} suggested significant differences in cytotoxicity among the tested compounds. According to the IC_{50}, the compounds can be ranked in the following order: PABA-Et >> PABA-Me-3 > MABA-5 > DAB-3 ≈ PABA-Et-3 ≈ MABA-3. In conclusion, PABA-Et and PABA-Me-3 exhibit the lowest cytotoxicity in HepG2 cells.

The study was supported by grants of Charles University Progress Q42, SVV 260 414 and by the Czech Science Foundation (17-27514Y).

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INTERACTION OF SELECTED ANTICANCER DRUGS OF THE CYCLIN-DEPENDENT KINASE INHIBITOR GROUP WITH THE ABC DRUG TRANSPORTERS

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ABCB1 (Pgp, P-glycoprotein) and ABCG2 (BCRP, breast cancer resistant protein) are members of ATP-binding cassette (ABC) efflux transporter family. Physiologically they are expressed in the cellular membrane and protect body tissues against potentially toxic xenobiotics including drugs. They represent also one of the tumor defence mechanisms when being able to efflux a wide variety of cytotoxic drugs out of the cancer cells leading to treatment failure.

BRAF protein plays an important regulatory and signal role in MAPK/ERK pathway affecting cell division, differentiation and secretion. Mutations of BRAF lead to over-activity in MAPK/ERK pathway in many cancer cells and can be therefore targeted by anticancer therapy. Cobimetinib and dabrafenib are relatively new anticancer therapeutics, which are used in treatment of melanoma carying the BRAF mutation.

The aims of this project were to investigate whether the kinase inhibitors cobimetinib and dabrafenib could inhibit the efflux transporters ABCB1 and ABCG2 and reverse drug resistance to ABCB1 and ABCG2 substrates *in vitro*. Using the Hoechst accumulation

assay we studied the inhibitory effect of these drugs to MDCKII cell lines overexpressing ABCB1 and ABCG2 transporters. The XTT assay was further used to study the antiproliferative effect of cobimetinib and dabrafenib and their impact on cytotoxicity of daunorubicin and mitoxantrone, the model anticancer substrates of ABCB1 and ABCG2, respectively.

We found that cyclin-dependent kinase inhibitors cobimetinib and dabrafenib are able to significantly inhibit ABCB1 and ABCG2 efflux transporters in MDCKII-ABCB1 and MDCKII-ABCG2 cell lines with cobimetinib showing higher inhibitory effect on ABCB1, compared to ABCG2. Contrary to cobimetinib, dabrafenib revealed preferential inhibition of ABCG2.

Both drugs, cobimetinib and dabrafenib can significantly reverse daunorubicin resistance in MDCKII-ABCB1 cells. Moreover, dabrafenib is able to reverse resistance of mitoxantrone in MDCKII-ABCG2 cells. We also showed that the presence of neither ABCB1, nor ABCG2 affected resistance of cobimetinib in MDCKII-ABCB1 and MDCKII-ABCG2 cells, indicating that these transporters do not play a role in the cellular resistance to these drugs. All the cell lines showed high resistance to dabrafenib demonstating no antiproliferative effect in up to 50 μM regardless of presence of ABCB1 or ABCG2. We demonstrate anti-melanoma agents cobimetinib and dabrafenib as inhibitors of ABCB1 and ABCG2 abile to reverse drug resistance to daunorubicin and mitoxantrone. These results may be taken into account when optimizing the cobimetinib and dabrafenib containing therapeutic regimens for the treatment of multidrug resistance melanoma patients.

The study was supported by Charles University (SVV 260 414).

STUDY OF IMPACT OF SELECTED TYROSINE KINASE INHIBITORS ON MULTIDRUG RESISTANCE MEDIATED BY ABC DRUG EFFLUX TRANSPORTERS

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Alectinib and brivanib are tyrosine kinase inhibitors (TKis) intended for the therapy of lung cancer and other solid tumors. While alectinib has already been marketed, brivanib is currently undergoing advanced stages of clinical evaluation. In this work, we investigated possible interactions of these novel drugs with ABC drug efflux transporters ABCB1, ABCG2 and ABCC1 that play a significant role in the phenomenon of multidrug resistance (MDR) of cancer cells. Using daunorubicin and mitoxantrone accumulation assays in MDCKII cells stably transduced with human ABC transporters, we observed significant inhibition of ABCB1 and ABCG2 but not of ABCC1 by alectinib at clinically relevant concentrations. Brivanib inhibited all three examined transporters in these assays. In the follow-up experiments, we investigated the potential of these interactions to combat MDR mediated by tested ABC transporters. Employing MTT proliferation method, we

demonstrated the ability of both drugs to increase sensitivity of MDCKII-ABCB1 and MDCKII-ABCG2 cells to cytostatic substrates daunorubicin and mitoxantrone, respectively. In contrast, no such result was recorded for the combination of daunorubicin and brivanib in MDCKII-ABCC1 cell line. In the final study, employing quantitative real-time reverse transcription PCR technique, we tracked the changes in *ABCB1*, *ABCG2* and *ABCC1* expression levels following exposure to the tested TKis in lung carcinoma A549 and colon adenocarcinoma LS174T cells. Consequently, no or only slight changes in the mRNA expression levels of tested transporters were detected in either cell line. In conclusion, our results show that both alectinib and brivanib exhibit potential to inhibit ABC drug efflux transporters and that these interactions could be favourably exploited for overcoming cytostatic MDR of cancer cells in oncological practice. On the other hand, these drugs do not seem to be able to substantially change MDR phenotype of cancer cells. Finally, our results can serve as a valuable foundation for follow-up *in vivo* studies that could support the rationality of our conclusions.

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BENEFICIAL EFFECTS OF 11β-HSD1 INHIBITION ON COGNITIVE PERFORMANCE IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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The increased life expectancy goes hand in hand with aging and aging-related cognitive impairments. Alzheimer's disease (AD) is the most common type of dementia being an irreversible and progressive brain disorder with loss of cognitive functions. Amyloid plaques and neurofibrillary tangles are the two main neuropathological hallmarks. Another features as loss of neurons, oxidative stress and inflammation contribute to the pathophysiology of AD. Recent studies suggest that excess of glucocorticoid action exerts deleterious effects on the hippocampus and causes impaired spatial memory, a key feature of age-related cognitive dysfunction. Furthermore, it has been demonstrated that aged mice with cognitive deficits show increased gene expression of 11β-hydroxysteroid dehydrogenase type 1 in the hippocampus and parietal cortex. The senescence accelarated mouse-prone 8 (SAMP8) strain is a spontaneous animal model of accelerated aging. Many studies indicate that SAMP8 harbor the behavioral and histopathological signatures of AD. In the present study, we evaluated the neuroprotective effects of 11β-HSD1 inhibition, influence of mild chronic stress and/or diet on congnitive performance in different groups of SAMP8 by conducting behavioral and cognitive tests such as Open Field, Novel Object Recognition, Morris Water Maze, among others.

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THE INTERACTION OF COPPER WITH REDUCED AND OXIDIZED GLUTATHIONE

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Glutathione (GSH) is an important endogenous direct antioxidant and less is known that it is also binding both cuprous and cupric ions. This study was aimed at the profound analysis of its chelation effect in four different (patho)physiological pH conditions (4.5, 5.5, 6.8, 7.5). Similar analysis was performed for comparison with its oxidized form (GSSG). Firstly, the complex formation with both Cu^{+} and Cu^{2+} was tested by comparing spectra of the pure substance, Cu salt and that of the mixture. In order to determine the stoichiometry of the complex, two non-competitive methods were used: Complementary approach and the Job's method. In the Job's method the concentration of both components was changing, while total concentration was constant. For the complementary approach the molar concentration of substance was changing while the concentration of metal was constant. Both reduced and oxidized gluthatione formed complexes of stoichiometry 1:1 with cupric ions, which were changing under some conditions into 3:1. GSSG made cuprous complexes of the stoichiometry 2:1 or 1:1, GSSG:Cu^{+} depending on conditions, while GSH 2:3 or 2:3 changing into 2:1 depending on conditions.

This study have shown that GSSG, considered to be the only oxidized and inactive form of GSH, is also able to form complexes with cuprous and cupric ions.

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INVOLVEMENT OF PDIA3 IN OXIDATIVE STRESS RESPONSE

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PDIA3 is a member of the protein disulfide isomerase family (PDI) and it is a stress-responsive protein. It is also involved in various cellular signaling pathways and has various

functions in the cell. The best known location is in the endoplasmic reticulum where it plays a major role mainly in the proper folding and quality control of glycoproteins, and participation in the assembly of the major histocompatibility complex class I. However, its existence has also been described in many other cell compartments, such as nucleus, mitochondria, cell surface or cytosol, where it interferes with various processes. While in some instances these roles need to be confirmed by further studies, a lot of observations confirmed its involvement in the signal transduction from the cell surface and the regulatory processes in the nucleus. The aim of our work was to find out what is its role in the cell stress exposure in the MDA-MB 468 and MCF-7 cell lines. These are breast tissue cells that have been chosen because of their resistence to environmental conditions and the small need for their proliferation. After calculating the optimal concentration, these cells were exposed to stress in the form of *tert*-butyl hydroperoxide; we observed expression of the PDIA3 protein after 3, 6 and 12 hours intervals along with the control sample. We also pretreated cells with 17β-estradiol before stress exposure because it is assumed that different levels of protein expression in both cell lines after exposure to stress are related to whether cells are 17β-estradiol positive or negative. Our study therefore extends the knowledge of PDIA3, illuminating the stress response processes in the MDA-MB 468 and MCF-7 cell lines. While the expression of the protein in the MCF-7 cell line is almost unchanged after treatment with *tert*-butyl hydroperoxide, the MDA-MB 468 changes significantly. The difference can also be seen when the cells were pretreated with 17β-estradiol.

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STUDYING THE IMPACT OF THE CIRCADIAN RHYTHM REGULATION ON THE HYPOTHALAMIC METABOLIC PATHWAYS

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Circadian rhythm orchestrates the "internal clock", that allows organisms to comply with daily changes in light and temperature caused by rotation of the Earth. This rhythm maintains body homeostasis, regulating the secretion of insulin, glucose homeostasis, thus impacting on the numerous physiological functions, namely feeding patterns. Circadian clocks are formed by transcription-translation feedback loop of specific set of genes, known as the clock genes, present in all cell types. The coordinator of all these clocks lies within the hypothalamus, namely in suprachiasmatic nucleus (SCN).[1]

Circadian rhythm disruption or deregulation increases the risk for obesity.[2] On the other hand, obesity is also correlated with circadian rhythm alterations.[3] Since these mechanisms

are still poorly understood, the aim of this work was to identify an underlying mechanism common to both metabolic dysfunction and circadian rhythm deregulation.

Hypothalamic cell line mHypoE-N42 was used to investigate the *in vitro* fluctuation in the mRNA and protein expression of clock genes within 24h day cycle. We also evaluated the circadian amplitude of serine/threonine kinases mTOR and RPS6K, as the relevant players in metabolic pathways. The results obtained using this cell line suggested a connection between the transcription factor BMAL1 and RPS6K on protein levels. To evaluate this finding in more detail, we performed an *in vivo* approach, inducing the alteration of the normal circadian rhythm, using wild-type (WT) mice, kept under constant dark or constant light condition, fed with chow-diet *ad libidum* for 72 hours and then sacrificed. Their hypothalamus was tested for the levels of clock genes, *mTOR* and *RPS6K*. Results showed unchanged mRNA levels of clock genes of the positive (daytime) side of the cycle (*Bmal1 and Clock*) and significant changes in the clock genes from the negative (nighttime) part (*Per, Cry*) at the 72h timepoint. Under constant dark condition, the mTOR pathway was over-activated, however, without the concomitant increase in RPS6K phosphorylation. Considering that evidences point to Bmal1 as a regulator of RP6SK, we believe that the maintenance of Bmal1 expression is preventing the subsequent activation of this kinase mediating the mTOR pathway. This could also explain the unchanged body weight and metabolic fitness in constant dark conditions, even with increased mTOR activation. Overall, we show evidences of metabolic pathways modifications upon alteration in the dark and light cycle. The central pacemaker in the hypothalamus proved to be very resilient to light and dark cycle modifications, so as the next step, peripheral tissues will be examined, to further strengthen the results.

The study was supported by Erasmus+ project and CNC – Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal.

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EFFECT OF MEBENDAZOLE ON THE ACTIVITY OF SELECTED ENZYMES IN TAPEWORM *HYMENOLEPIS DIMINUTA*

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The resistance of parasitic helminths to anthelmintic drugs is a growing worldwide phenomenon and a concerning issue. Xenobiotic metabolizing enzymes play an important role

in drug resistance development as they can lower the concentration of the anthelmintics in the parasite's body and therefore protect the parasite from the anthelmintic effect. Effect of drug metabolizing enzymes on drug resistance development was already described in the group of roundworms and flukes, but very limited information is available about this topic in tapeworms. In our study, we tested the possibility of anthelmintic mebendazole to affect the activity of these enzymes and possibly to influence the drug resistance development in rat tapeworm (*Hymenolepis diminuta*).

Our first goal was the infection of the definitive host (rat) using the cysticercoids from infected intermediate hosts mealworm beetles (*Tenebrio molitor*). After the successful infection and development, adult tapeworms were isolated from the rat's intestines and incubated with mebendazole (1 and 10 μM) for 24 hours. Following the incubation second goal was to prepare cytosol-like, microsomes-like and mitochondria-like subcellular fractions and to measure the activity of selected xenobiotic metabolizing enzymes.

The results of our study indicate that mebendazole can affect the activity of certain xenobiotic metabolizing enzymes of rat tapeworm. In the cytosol-like fraction we observed an increase in activity of catalase, peroxidase, superoxide dismutase, aldo-keto reductase 1A1, glutathione-S-transferase. In mitochondria-like fraction we observed an increase in activity of aldo-keto reductase 1C. Furthermore, we observed a decrease of activity in glutathione reductase in cytosol-like fraction, aldo-keto reductase 1A1 and carbonyl reductase in mitochondria-like fraction.

THE *IN VITRO* AND *EX VIVO* STUDY OF INTERACTIONS OF ANTIVIRALS WITH INTESTINAL EFFLUX TRANSPORTERS

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P-gp, MRP2 and BCRP are efflux transporters, members of the family of ATP binding cassette (ABC) transporters. These transporters are located on the apical membrane of the intestinal epithelium, where they may limit absorption of orally administered drugs. Study of drug interactions with/on intestinal efflux transporters is necessary to provide safe and effective treatment. The Caco-2 cell line is FDA recommended *in vitro* model of intestinal barrier and it is used for bidirectional testing of substrates and inhibitors of ABC transporters in preclinical research. However, this methodology has several shortcomings, so the need of introduction of new experimental models is increasing and the *ex vivo* method based on human or rat intestine is a promising option. Precision-cut intestinal slices (PCIS) represent a mini-model of the organ and contain all types of cells of the tissue. We used both *in vitro* model using Caco-2 cell monolayers for drug transport study and *ex vivo* method of PCIS for accumulation study and rhodamine123 (RHD123) as a model substrate of P-gp. We analyzed interactions of selected protease inhibitors (lopinavir, ritonavir,

saquinavir, atazanavir) and nucleoside reverse transcriptase inhibitors (abacavir, zidovudine, tenofovir disoproxil fumarate) on this efflux transporter. Of tested antiretrovirals, lopinavir, ritonavir, saquinavir, and atazanavir caused concentration dependent decrease in the efflux ratio and increase in *ex vivo* accumulation of RHD123 in *in vitro* and *ex vivo* experiments, respectively. In conclusion, we confirmed that lopinavir, ritonavir, saquinavir, and atazanavir might inhibit intestinal P-gp and thus increase the absorption of P-gp substrates. Importantly, we demonstrated that rat PCIS provide comparable results with those obtained using Caco-2 model. Therefore, rat PCIS may represent more physiological alternative to currently preferred *in vitro* method and the establishing of human PCIS would be an additional step closer to real clinical environment.

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QUANTIFICATION OF ATHEROSCLEROTIC LESIONS SIZE IN CHOLESTEROL FED AND DAUNORUBICIN TREATED RABBITS BY MEANS OF STEREOLOGICAL METHODS

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Daunorubicin (DAU) was the first anthracycline (ANT) antibiotic with impressive clinical activity in the treatment of acute pediatric leukemias. Later, it has been shown that anthracyclines have broad range of therapeutic activities against both hematological and solid tumors, including breast, ovarian and gastric carcinoma. On the other hand, ANTs are the most dangerous anticancer drugs with respect to cardiovascular toxicity characterized by mostly irreversible morphological changes and cell death. Indeed, ANTs are used also in patients with hypercholesterolemia so the effect on atherogenesis in blood vessels is of interest. Thus, we hypothesized that DAU treatment and cholesterol diet will aggravate atherosclerosis when compared to rabbits fed by cholesterol diet only. Seven adult male New Zealand White rabbits were used. Control group of rabbits (n = 5) received saline *i.v.* once weekly for 10 weeks since the beginning of the 4th week, the second DAU group (n = 2) was fed with cholesterol diet (0.2% cholesterol) and clinically relevant doses of daunorubicin (3 mg/kg, ≈ 50 mg/m^2, *i.v.*).

The systematic uniform random sampling was applied for the quantification of atherosclerotic lesions. The samples from aortic arch, right femoral artery, right carotid artery and mesenteric artery were taken for the evaluation. Serial sections of vessel (7 μm) were cut on a cryostat and hematoxylin-orcein staining was used for the histological and morphometric evaluation. The results of the morphometric analysis showed that the biggest atherosclerotic plaques were detected in aorta when compared to other vessels with statistical significance when compared to right carotid artery. On the other hand, atherosclerotic plaque size was not significantly different between rabbits treated with DAU and cholesterol diet compared to rabbits on cholesterol diet only in any studied blood vessel. The

results of this pilot study showed that combination of DAU treatment and cholesterol diet did not aggravate atherosclerosis in aortic arch, right femoral artery, right carotid artery and mesenteric artery when compared to rabbits fed with cholesterol diet.

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PHYLOGENETIC AND TAXONOMIC CHARACTERIZATION OF NEW HALOARCHAEA RELATED TO *NATRONOMONAS*

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This work continues with the characterization of new archaea related to the genus *Natronomonas,* isolated from hypersaline waters of solar salterns located near the city Huelva in South-West of Spain. These microorganisms need a high concentration of salts in the environment for their survival and growth. Ana Durán-Viseras started with their characterization and I was participating in the continuation of the project during my Erasmus+ stay at the University of Sevilla.

To complete the phylogenetic studies, as a part of MLSA (Multilocus Sequence Analysis), we amplified the *rpoB'* gene of potential new species of archaea, two isolated strains F17-44 and F12-1, and we created a phylogenetic tree. Based on these results we came to a conclusion that they represent new genus; this fact was later confirmed by the analysis of polar lipids profile by HPTLC (High Performance Thin Layer Chromatography). We determined the guanine and cytosine DNA content, which is part of the taxonomic characterization of new prokaryotic species. We started the phenotypic characterization.

The results of this polyphasic characterization indicate that the strains F17-44 and F12-1 may represent two different species of a new genus of haloarchaea, similar to the genus *Natronomonas*.

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EXERCISE AS MEDICINE: GROWTH HORMONE RESPONSE TO HIGH-INTENSITY INTERVAL TRAINING

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The present research focused on growth hormone response to exercise. It contributes the rationale of a wider project related to the beneficial effect of high-intensity interval training and therefore related to the topic exercise is medicine.[1] We hypothesized that, as shown by Booth *et al.*,[2] in several systems of our organism (such as cardiovascular system, skeletal system *etc*.) our genome is maladapted, because of reduced physical activity with respect to our ancestors.

Therefore, also the growth hormone response to exercise is depressed. Several mechanisms such as increased blood lactate concentration, increased hydrogen ion concentration in the blood, afferent signals from muscle metabolic receptors *etc*. have been proposed as stimuli for the GH response to exercise. The aim of the present research is the study of a possible metabolic stimulus of a relative muscular hypoxic, that is the ratio between energy demand and oxygen availability, as the main regulator of the growth hormone production.

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ESTABLISHMENT OF THE METHOD FOR *CANDIDA* SPP. *IN VITRO* BIOFILM FORMATION

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Candida albicans is a member of the healthy microbiota, asymptomatically colonizing the gastrointestinal tract, reproductive tract, oral cavity and skin of most humans. In healthy individuals is often harmless, kept in balance with other members of the local microbiota, but during host immunosuppresion or alteration in the microbiota can cause superficial mucosal infections and life-threatening infections, as well. Many strains of *Candida* spp. are capable to form biofilm, especially on abiotic surfaces, *e.g.* catheters, pacemakers, dentures, prosthetic joints. *C. albicans* yeasts in biofilm display dramati-

cally different phenotypes from those of their planktonic counterparts, *e.g*. increased resistance to antimicrobial agents, making biofilm-based infections a significant clinical challenge.

Our goal is to design a high-throughput biofilm susceptibility assay enabling to determine anti-biofilm activity of newly synthetized substances with previously proved antimycotic activity. For this purpose, we have optimized cultivation conditions for *in vitro Candida* spp. biofilm formation and methods for quantification, detection and visualization of these biofilms. Ten different *Candida* spp. strains have been used (reference strains, clinical isolates). The model with 96 microtitre well plate covered by lid with incorporated pegs was chosen and the impact of plastic surface with/without hydroxyapatite coating has been evaluated, as well. For quantification and visualization of adherent yeasts/biofilms different techniques, such as direct cell counting method, biofilm biomass quantification by crystal violet assay, optical density of homogenized biofilm measurement, measurement of metabolic activity of biofilm-forming yeasts, visualization of biofilm by fluorescent microscopy and evaluation of live/death cells forming biofilm by fluorescent microscopy, were used.

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EFFECT OF FENBENDAZOLE ON EXPRESSION OF SELECTED *ARABIDOPSIS THALIANA* ENZYMES

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Fenbendazole (FEN) is a broad-spectrum benzimidazole anthelmintic that is widely used to prevent and treat helminthoses in farm animals. The use of anthelmintics is associated with the risk of their transfer to the environment, where they can potentially affect non-target organisms, including plants. They come into contact with anthelmintics and their metabolites mainly due to the fertilization of farmland with manure and/or come directly into contact with the excrements of treated animals on pastures. There have not been many studies focused on the cell physiology and cell metabolism of plants affected by anthelmintics, which lead to our participation in a study which dealt, in part, with the influence of FEN on the transcriptome and gene expression in model plant *A. thaliana* – a dicotyledonous plant, which is a very common model organism of higher plants. Its genome was also published in 2000, allowing extensive research into gene expression.

The aim of this work was to monitor expression of selected *A. thaliana* genes in leaves and roots after exposure of 5 μl of FEN after 24 and 72 hours and to verify RNA-microarray

analysis by quantitative PCR. RNA isolation was performed from both plant tissues, which was transcribed into cDNA by reverse transcription. This was further modified according to the protocol and analysed using quantitative real-time PCR. Expression of the selected genes was compared to the control group that was not affected by FEN. It was found that only roots provide significant changes in the expression of selected genes. Therefore, RNA-microarray verification was only performed on root samples. A good match was found between the results of the two methods.

The study was supported by the Czech Science Foundation (GAČR, grant No. 15-05325s) and by Charles University in Prague (research project SVV 260 416).

IN VITRO ASSAYS FOR INVESTIGATING NUCLEIC ACID DELIVERY

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Gene therapy is emerging as a potential treatment owing to its ability to deliver genetic material inside the cell. Reporter gene based transfection process can be used to study gene expression. Delivery by nanocarriers can access of oligonucleotides to their pharmacological targets within cells.[1] Cationic polymers based on polyethylenimine form complexes with plasmid DNA referred to as polyplexes. In the present work, different types of polyethylenimine based polyplexes were employed to study gene expression and splice correction, as a measure of intracellular delivery of plasmid DNA and antisense oligos, respectively.

The first goal of this work was to modify the currently used protocol for this assay. Both protocols can be performed but in the modified version we can directly skip one additional step during preparation of BCA standard curve. The main reason was to avoid additional step in the multi-well plate format. The optimized protocol was then tested and used in transfection experiments. A further objective before transfection of B16 cell line with linear polyethylenimine and different types of plasmids was to confirm the healthy status of cells by observing the morphology.

Further, the goal was to determine the expression of Thy1.2 in cancer cells after transfection.

In the last part of this project, polyplexes based on three different polyethylenimines such as linear polyethlenimine (LPEI), branched polyethylenimine (BPEI) and disulfide crosslinked polyethyleimine (c-LPEI) together with antisense oligonucleotides were investigated for splice correction *in vitro*. The aim of this part was to compare polyethyleneimines and achieve splice correction by polyplex based transfections by measuring luciferase expression in HeLa pLuc 705 cell line.

The study was supported by ERASMUS+ project.

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THE EFFECT OF FLUBENDAZOLE ON THE EFFICACY OF PACLITAXEL IN BREAST CANCER CELLS

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Flubendazole (FLU), a benzimidazole-based compound, is common anthelmintic drug for veterinary use. Its molecular mechanism of action is based on specific binding to β-tubulin subunit of microtubules. FLU was identified as having previously unrecognized antileukemia or antimyeloma activity. FLU altered microtubule structure and inhibited tubulin polymerization by interacting with a site on tubulin similar to commonly used anticancer drugs – taxanes. The aim of this study was to evaluate the effect of FLU on antiproliferative efficacy of paclitaxel (PTX) treatment in breast cancer cells.

For this purpose, three breast cancer cell lines were used – non-metastatic line MCF-7 and two metastatic cell lines MDA-MB-231 and BT-474. The proliferation of the cells treated by FLU and PTX and their combinations was followed up using xCELLigence real-time analysis and end-point method WST-1. The protein level was determined using western blot analysis. The activity of caspases was assayed by Promega Caspase-Glo Assays. The PTX concentration was evaluated by LC-MS analysis (QQQ).

MCF-7 and MDA-MB-231 have been shown as the most sensitive to PTX and FLU treatment, so we selected them for further experiments. Unfortunately, combination of FLU and PTX had not higher antiproliferative effect than PTX alone. In addition, the levels of molecules participating in mechanism of action, such as vimentin and tubulin, were not significantly changed after combination treatment. However, in both cell lines higher levels of caspase 2 and caspase 3/7 were observed comparing to PTX alone, especially in MDA-MB-231 cells. Finally, PTX concentration was measured using LC/MS analysis and the results showed decreased concentration of PTX inside the cells after combination treatment of PTX and FLU, compared to PTX alone. We assume that both tested drugs are probably not able to enter the cells at the same time in sufficient quantity and therefore PTX cytostatic effect can’t be potentiated by FLU in breast cancer cells.

IN VITRO EVALUATION OF THE PHOTODYNAMIC ACTIVITY OF NOVEL AMPHIPHILIC (AZA)PHTHALOCYANINES

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Neoplastic diseases are nowadays one of the most common reasons of death in developed countries. That is the reason why a great attention is dedicated to the development of new methods for treatment those diseases. One of such modern methods is the photodynamic therapy. This is a selective, minimally invasive method with minimum side effects. The principle of this type of therapy is application of inactive drug, called photosensitizer (PS), followed by exposure to activating light with suitable wavelength in the presence of molecular oxygen. Therefore, the photodynamic therapy needs three basic components: the PS, light and oxygen. All of these components are non-toxic on their own. However, their combination leads to the inception of the photochemical reaction in which reactive oxygen species (ROS) are generated, especially singlet oxygen. These very reactive molecules damage target cells, which subsequently die via apoptosis or necrosis.[1,2]

As has been said, the main part of the photodynamic therapy is the PS, which is the substance that absorbs the light with specific wavelength and converts it to ROS production inducing oxidative stress. Nowadays, few of these compounds exist, which were introduced to the clinical practice.[3]

The objective of this study is assessing the effectiveness of the novel PSs from the group of azaphthalocyanines *in vitro*. All of the studied compounds were evaluated on malignant human cervical cell line (HeLa). Cytotoxicity after the exposure to activating light (phototoxicity) and intrinsic toxicity in the absence of light (dark toxicity) were determined. Further the subcellular localization PSs after acumulation in cells was assessed using confocal microscopy. Morphological changes after photoactivation of submitted substances, were evaluated as well. All of studied compounds proved to be efficient PSs with low dark toxicity rendering them suitable for further study.

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HPLC-HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF *IN VITRO* AND *IN VIVO* METABOLISM OF SCOPARONE

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Scoparone is an active ingredient of *Artemisia scoparia*, a medicinal plant used in traditional Chinese medicine. It has been studied for various pharmacological effects such as upregulation of conjugation enzymes included in excretion of bilirubin, reduction of proinflammatory cytokines, lowering of plasma lipids levels and inhibition of platelet aggregation.[1] In this study, metabolism of scoparone was studied by LC-MS method using Q-ToF device. Scoparone was incubated with liver microsomes obtained from 6 different mammal species including human to study *in vitro* oxidation. In total, six metabolites were detected in the incubation samples. Scopoletin and isoscopoletin were identified as major metabolites in every species, however, the rates of scoparone oxidation as well as a ratio of formed isoscopoletin and scopoletin varied. Furthermore, *in vivo* metabolites in human were studied in urine samples obtained from two healthy volunteers after oral administration of scoparone. Nine metabolites were detected in the urine samples in total, major metabolites being glucuronide and sulphate conjugates. The highest levels of metabolites were detected in the urine samples taken three hours after scoparone administration suggesting rapid elimination. Unfortunately, conjugation metabolites could not be fully identified in conditions of this study, however, it has been proved that this LC-MS method is suitable for further research of scoparone metabolism both *in vivo* and *in vitro*.

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EFFECT OF SESQUITERPENES ON RAT LIVER BIOTRANSFORMATION ENZYMES

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Sesquiterpenes are secondary metabolites produced by higher plants, bacteria and fungi. They ensure the survival and competitiveness of plants. Sesquiterpenes are composed of three isoprene units. They possess anti-inflammatory, antimalarial, antibacterial, antiviral, antioxidant, analgesic, antifungal and anticancer activity. Sesquiterpenes, the main components of plant essential oils, have been used in folk medicine and as spices for

years.[1] The aim of the present study was to find out the effect of three structurally related sesquiterpenes α-humulene (HUM), β-caryophyllene (CAR), and β-caryophyllene oxide (CAO) on the activities of the main drug-metabolizing enzymes. Precision-cut tissue slices from the rat liver (*Rattus norvegicus*) were chosen as model system. Thickness of the slices was 200–250 μm and diameter 8 mm. Liver slices were pre-incubated for 30 minutes and then incubated for 8 and 24 hours. Samples of liver slices and incubation medium were taken upon replacing the medium after pre-incubation (time 0 hours) and then after 8 and 24 hours. Concentration of sesquiterpenes was 10 μM. It was found that activity of aldo-keto reductase AKR1A, cytochrome P450 (CYP2B/3A) and NADPH-quinone oxidoreductase 1 (NQO1) was influenced in comparison to control. Activity of AKR1A1 and CYP2B/3A affected by CAR and HUM showed decrease after 8 hours of incubation. Activity of CYP2B/3A affected by CAO showed decrease after 24 hours incubation. Activity of NQO1 affected by HUM showed increase after 24 hours of incubation.

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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 *CADM1* gene (Cell Adhesion Molecule 1) in oropharyngeal squamous cell carcinoma (OPSCC) and compare methylation levels in HPV positive and HPV negative tissue samples.

CADM1 methylation in bisulfite converted DNA was detected using methylation specific high-resolution melting analysis (MS-HRM) on Bio-Rad CFX96 Touch™ within range of 70 samples (35 tumors and 35 metastases) and 38 control tissue samples (non-cancerous

palatine tonsils). Selection of primers was based on Fisser et al.[3] We considered sample to be methylated above cut-off limit 10%.

Methylation was detected in 31% of the samples (11/35). In all cases methylation status was the same in both samples of one patient (tumors and associated metastasis). All 38 control samples were unmethylated which also applies to all 6 HPV negative cancer samples. Methylation was detected in 38% of HPV positive cancer samples (11/29).

CADM1 methylation in OPSCC is increased by the presence of HPV infection, which is corresponding to methylation of the *CADM1* gene in cervix carcinoma.[4] *CADM1* gene methylation levels are 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.

The study was supported by MH CZ – DRO (University Hospital Hradec Králové, 00179906).

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THE EFFECT OF METHYLVIOLOGEN ON SECONDARY METABOLITES PRODUCTION IN *FAGOPYRUM ESCULENTUM* cv. BAMBI *IN VITRO* CULTURES

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The subject of this study was to evaluate the effect of abiotic elicitor on the rutin production in callus and suspension cultures of buckwheat. The cultivar of buckwheat used for this research was *Fagopyrum esculentum* Moench cv. Bambi. It was cultivated in Murashige and Skoog nutrient medium with the addition of a growth regulator 2,4-dichlorofenoxyacetic acid in concentration of 1 mg/l. The elicitor used in this study was a solution of methylviologen, added to the cultures in three different 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 affecting the cultures for 6, 12, 24, 48, 72 or 168 hours under the same conditions as the cultures were cultivated until then. After the defined period of time, cultures were collected, dried out and stored for further analysis of rutin content. To control samples (without elicitor treatment) 1 ml of 96% ethanol was added and they were collected after 6, 24, 72 or 168 hours. Release of rutin into the nutrient medium was also investigated. Rutin content in each sample of cultures and in each sample of nutrient medium was later determined by HPLC.

Any significant increase in the production of rutin was not observed in this study. The samples either showed 0.00 mg/g DW of rutin or some of the suspension cultures samples showed the minimal detectable amount of rutin, which was 0.1 mg/g DW, but this amount was often found in both examined samples and their corresponding control samples which led to no statistical output. Apart from five sub-zero outputs, there were three actual non-zero results found in suspension cultures – 0.1 mg/g DW of rutin after the addition of methylviologen in c_1 concentration when collected after 168 hours, and then cultures after 48 and 168 hours of cultivation with the elicitor in c_3 concentration. The release of rutin to the nutrient medium was not detected in any sample at all.

Therefore, the positive effect of methylviologen elicitation on *in vitro* cultures of buckwheat cultivar *Bambi* and their rutin production was not confirmed in this study.

The study was supported by PROGRESS Q42 and SVV 260 416.

CYTOTOXIC AND CHOLINESTERASE INHIBITORY ACTIVITY OF EXTRACTS FROM SELECTED SPECIES OF THE *CENTAUREA* L. GENUS

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In the screening of biologically active secondary plant metabolites carried out at the Department of Pharmaceutical Botany, selected species of the genus *Centaurea* (Asteraceae) were investigated. This study deals with a preliminary phytochemical research and biological activities of *Centaurea cyanus*, *C. benedicta*, *C. soltitialis*, *C. jacea*, *C. scabiosa*, *C. pseudophyrgia*, and *C. stoebe* seed extracts. The phytochemical screening of summary ethanolic and alkaloid extracts was performed by TLC and mass spectrometry analyses. Furthermore, the alkaloid extracts were investigated for their potential activity to inhibit human erythrocyte acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) along with elucidating their cytotoxicity against selected 9 tumor cell lines. *C. cyanus* alkaloid extract showed promising and selective anticholinesterase activity against BChE (IC_{50} BChE = 22.62 ± 3.62 μg/mL, IC_{50} AChE = 221.50 ± 44.56 μg/mL). The alkaloid extracts of other *Centaurea* species were considered inactive against cholinesterases (IC_{50} values were > 100 μg/mL). Cytotoxicity of alkaloid extracts was investigated at a screening concentration of 50 μg/mL against tumor cell lines Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7, and SAOS-2 along with MRC-5, a non-tumor cell line used as the control. Doxorubicin (1 μmol/L) was used as a positive standard. Cytotoxic activity of the extracts was determined after 48 hours incubation as cell viability and obtained results were expressed as percentages relative to the control (100%). The *C. cyanus* alkaloid extract demonstrated the best cytotoxic activity and the extract was effective against MOLT-4

(16 ± 7% of cell viability). However, cytotoxic activity of all *Centaurea* alkaloid extracts was low against the selected tumor lines in comparison with doxorubicin.

This study was supported by SVV 260 412.

BIOLOGICAL ACTIVITY OF SELECTED HAEMANTHAMINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY IN RELATION TO ALZHEIMER'S DISEASE AND ONCOLOGICAL DISEASES

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The plants of the Amaryllidaceae family are known to contain a specific type of compounds, namely the Amaryllidaceae alkaloids. One of them is haemanthamine (HA), an isoquinoline alkaloid, which exhibits a wide and important range of biological activities, including antitumor, antiviral, antioxidant, antimalarial and anticonvulsant. The recent studies showed that haemanthamine has also apoptopic effect on leukemia cells and strong cytotoxic potential against gastrointestinal cancer cells.[1] Acetylcholinesterase and butyrylcholinesterase-inhibitory activity of HA was also tested (IC_{50} HuAChE, HuBuChE > 1000 μM).

In the present work, selective modifications on the structure of HA were carried out to study relationships between structure and biological activity. Fifteen derivatives of HA were prepared and purified using analytic and preparative TLC. The chemical structures were elucidated by combination of MS, HRMS, 1D and 2D NMR spectroscopic techniques, and by comparison with literature data. Prepared compounds were tested on the their inhibitory potency of cholinesterases. The cytotoxic activity of prepared compounds was studied on panel of cancerous and noncancerous cells. The most promising biological activities were shown by aromatic esters labeled as LC-70 and LC-73.

The study was supported by SVV 260 412.

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ALKALOIDS OF *NARCISSUS PSEUDONARCISSUS* cv. DUTCH MASTER: ISOLATION, STRUCTURAL IDENTIFICATION, PREPARATION OF ANALOGUES, BIOLOGICAL ACTIVITY

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The purpose of this work was to isolate the substances from the fraction ND 15-9, which was obtained by column chromatography of the alkaloid extract of *Narcissus pseudonarcissus* cv. Dutch Master. The preparative TLC method was used to separate this fraction, and three compounds were isolated in the pure state NDS1, NDS2 and NDS3. NMR, GC/MS and optical rotation were used to determine their structure, and the data obtained were compared with literature data. In addition, studies of their biological activity was performed.

Isolated substances were identified as epimaritidine, crinine and tetrahydromasonine. Their inhibitory activities (IC_{50} AChE > 1000 μM, IC_{50} BuChE > 1000 μM) versus human erythrocyte AChE and plasma BuChE were negligible compared to galanthamine (IC_{50} AChE = 1.71 ± 0.1 μM, IC_{50} BuChE = 42.3 ± 0.1 μM), huperzin A (IC_{50} AChE = 0.033 ± 0.001 μM, IC_{50} BuChE > 1000 μM) and berberin (IC_{50} AChE = 0.71 ± 0.1 μM, IC_{50} BuChE = 30.72 ± 3.5 μM). On the basis of the obtained data, it can be concluded that isolated substances are unusable in AD therapy. The results of POP inhibitory activities are negligible in crinin, for epimaritidine the IC_{50} is 0.79 ± 0.4 mM and for tetrahydromasonine IC_{50} 0.75 ± 0.9 mM. Both alkaloids showed a low POP inhibition compared to berberin (0.14 ± 0.02 mM).

The study was supported by SVV 260 412.

SECTION OF CHEMICAL SCIENCES: SYNTHETIC PART

COMPOUNDS COMBINING PYRAZINAMIDE AND 4-AMINOBENZOIC ACID FRAGMENTS AS POTENTIAL ANTITUBERCULARS

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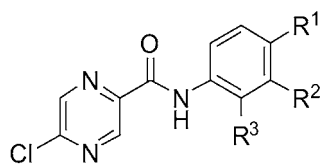
Previous experiences with pyrazinamide derivatives have shown that 4-(5-chloropyrazine-2-carboxamido)-2-hydroxybenzoic acid is a promising non-toxic antimycobacterial

agent (MIC for *Mycobacterium tuberculosis (M. tbc)* H37Rv was 3.13 μg/mL).[1] To prove the necessity of R^1 = COOH, R^2 = OH substituents on the phenyl ring (that is in the context of the fragment of *p*-aminosalicylic acid) for optimal antimycobacterial activity, a series of 13 derivatives were synthesized and tested for *in vitro* growth inhibition of *M. tbc* H37Rv, *M. kansasii*, *M. avium*, *M. smegmatis* and *M. aurum*. Active derivatives presented an inhibiting activity against *M. tbc* H37Rv and *M. smegmatis* with MIC values in the range of 6.25–50 μg/mL and 62.5–250 μg/mL, respectively. No activity has been detected against the other strains. 4-(5-Chloropyrazine-2-carboxamido)-2-hydroxybenzoic acid remained with the best MIC value (6.25 μg/mL for *M. tbc.* H37Rv). Changes in substituents operated on the phenyl ring maintained activity against *M. tbc.* H37Rv, worsened it or led to a complete lost. Esterification of the carboxylic moiety with *n*-propanol did not increase the activity in most compounds. Structure-activity relationship deduced from this experience shows that R^1 = COOH and R^2 = OH substituents (which is the *p*-aminosalicylic fragment) confer the best antimycobacterial activity and absence of hepatoxicity.



R^1 = COOH, COO-*n*-Pr
R^2 = H, OH
R^3 = H, Cl, CH_3, OCH_3

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SYNTHESES OF PRECURSORS FOR PREPARATION OF ASYMMETRIC AZAPHTHALOCYANINES ON A SOLID PHASE

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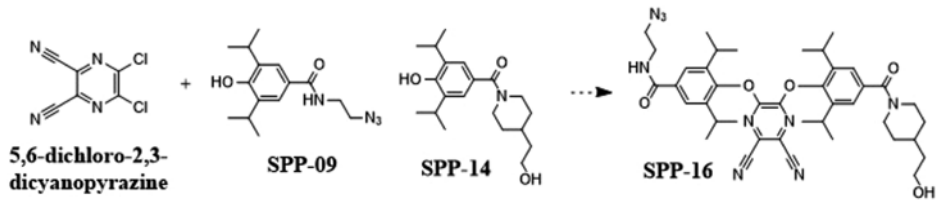
The aim of the project was the synthesis of precursors for cyclotetramerization of asymmetric fluorescent tetrapyrazinoporfyrarin (TPyzPy) bearing one moiety suitable for binding to solid phase and the other intended for binding of final TPyzPz to oligonucleotides. Procedure was started by preparation of the essential intermediate 4-hydroxy-3,5-diisopropylbenzoic acid,[1] which was functionalized to amides with amines bearing an azido group or a primary alcoholic moiety. Several amidation methods[2] were evaluated and based on their efficiency the most yielding one was selected. Two prepared intermediates *N*-(3-azidopropyl)-4-hydroxy-3,5-diisopropylbenzamide **(SPP-09)** and (4-hydroxy-3,5-

diisopropylphenyl)(4-(2-hydroxyethyl)piperidin-1-yl)methanone **(SPP-14)** were used for replacement of chlorines in 5,6-dichloro-2,3-dicyanopyrazine to obtain targeted *N*-(3-azidopropyl)-4-((5,6-dicyano-3-(4-(4-(2-hydroxyethyl)piperidine-1-carbonyl)-2,6-diisopropylphenoxy)pyrazin-2-yl)oxy)-3,5-diisopropylbenzamide **(SPP-16)**.



The financial support from the Charles University (SVV 260 401) is gratefully acknowledged.

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SYNTHESIS OF NOVEL AZAPHTHALOCYANINES WORKING AS DARK QUENCHERS

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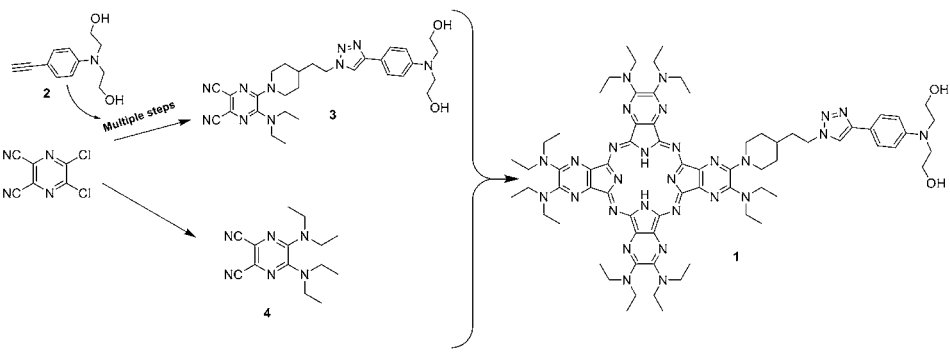
Azaphthalocyanines (AzaPcs) are analogues of well known phthalocyanines with pyrazines that replaced the benzene rings. They can be used in many applications due to their promising broad spectrum of properties. For example they can be used as photosensitisers in the photodynamic therapy or as pH sensitive dyes. The alkylamino substituted AzaPcs



can be used as dark quenchers of fluorescence in DNA hybridization probes. The AzaPcs for this application have typically three quaters same and the last quarter could be modified with wide range of functional groups.

The goal of this project was to synthesize unsymetrical AzaPc **1**, which will be substituted with "T-based molecule" – carrying two hydroxyles that could be incorporated into the oligonucleotide chain. The synthesis consisted of preparation of diol **2** using Sonogashira coupling and then it was attached to the azide-bearing substituted pyrazine-2,3-dicarbonitrile using the "click" method to make **3**. The starting material **3** was then cyclotetramerized with molecule **4** in 1:3 ratio to obtain AzaPc **1** that was isolated from the mixture of six congeners by column chromatography.

The study was supported by Grant Agency of Charles University (No. 1168217) and SVV 260 401.

PYRAZINAMIDE DERIVATES AS POTENTIAL ANTIMICROBIAL COMPOUNDS

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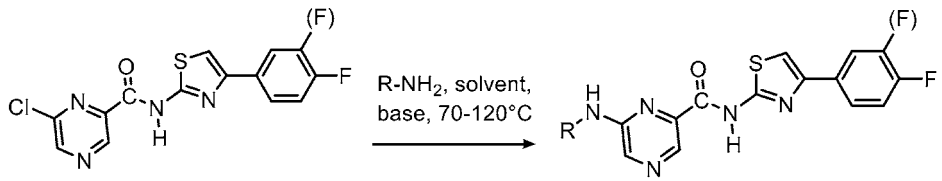
A new series of pyrazinamide (PZA) derivatives was designed, docked into β-ketoacyl-ACP synthase III (FabH) of *Mycobacterium tuberculosis* (*Mtb*), synthesized, characterized by analytical data and *in vitro* tested for antimycobacterial activity against *Mtb* H37Rv and several non-tuberculous mycobacteria in a Microplate Alamar Blue Assay. The structure of presented compounds combines the first-line antitubercular PZA, 4-arylthiazole-2-amine scaffold with formerly identified antimycobacterial activity[1] and alkylamino chains. 6-Chloro-*N*-(4-[4-fluorophenyl)thiazol-2-yl]pyrazine-2-carboxamide exerted high *in vitro* activity against *Mtb* with MIC = 0.78 μg mL[1] and low cytotoxicity[2] and we expected increase of antimycobacterial activity due to introduction of alkylamino substituent in position C6 of the PZA core as in the case of C6 and C5 alkylamino derivatives of PZA synthesized previously by Servusová *et al*.[3] Generally, although the molecular docking study predicted strong stabilizing interactions near the catalytic triad of FabH (pdb: 1U6S), the alkylamino chains in C6 position of the PZA core decreased antimycobacterial activity against *Mtb* in comparison with the starting compound.



The study was supported by the Czech Science Foundation project No. 17-27514Y and by SVV 260 401.

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TUNING OF pK_A OF AZAPHTHALOCYANINE pH SENSORS

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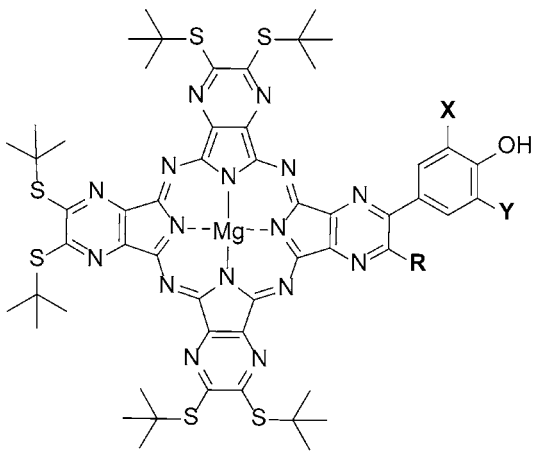
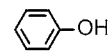
Azaphthalocyanines (AzaPcs) are macrocyclic complexes with characteristic properties. They have a large system of conjugated double bonds and are able to absorb light in far red region. One of the possibilities for molecule after the photon absorption to relax from singlet excited to the ground state is emission of a photon (fluorescence). Switching the fluorescence ON and OFF can be utilized in fluorescent sensors. Our research group recently developed the sensors based on AzaPc core containing a phenolic group as recognition moiety.[1] The fluorescent state of the molecule depends on the pH of the environment and the pK_A of the recognition moiety that can be modified by altering its substituents.

The synthesis of AzaPcs was initiated by the synthesis of their precursors. The starting material was in many cases 4-hydroxyacetophenon except for substance **5**, where the starting substance was anisaldehyde and substance **4**, where the reaction started up with 2,6-di(*tert*-butyl)phenol. Reactions with 4-hydroxyacetophenon were initiated by



	1	2	3	4	5
R	H	H	H	H	
X	H	Br	Br	tBu	H
Y	H	H	NO_2	tBu	H

the introduction of nitro group (**3**) and bromine (**2, 3**) to 4-hydroxyacetophenon by electrophilic substitutions. The products were then oxidized to corresponding ketoaldehyde using selenium dioxide and immediately condensed with diaminomaleonitrile to substituted pyrazine-2,3-dicarbonitrile. The synthesis was completed by the cyclotetramerization reaction of this dicarbonitrile with 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile using magnesium butanol as initiator. Six different congeners were obtained by this reaction that were separated by the column chromatography. Congener with one asymmetric part was isolated (see the structure below). The preparation of substance **5** was initiated by acyloin condensation of anisaldehyde in the presence of bis(benzimidazolium) catalyst. The product was then oxidized to diketone without any further isolation. After that, the hydroxy group was deprotected and the substance underwent a condensation reaction with diaminomaleonitrile similarly as in the previous reactions, resulting in pyrazine-2,3-dicarbonitrile, that later went through the cyclotetramerization. During the synthesis of substance **4**, the starting compound reacted with 5-chloropyrazine-2,3-dicarbonitrile and the product was cyclotetramerizated. In this case, the appropriate congener could not be isolated by column chromatography in the form of magnesium complex because of the similar retention factors. The product was converted to a metal-free derivative and after the purification the magnesium was chelated back again to the center of the macrocycle.

The study was supported by SVV 260 401 and by the Grant Agency of Charles University (1168217).

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PROPARGYLTACRINES – ACETYLCHOLINESTERASE INHIBITORS WITH ANTI-MAO ACTIVITY

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Alzheimer's disease is a serious neurodegenerative disease that affects aged humans, mainly. The causes of this disease are not clear, but the mechanism and risk factors are known. There is no drug for treatment. We can eliminate symptoms or slow the development of dementia. The treatment uses acetylcholinesterase inhibitors (AChE) and *N*-methyl-D-aspartate receptor antagonist. AChE inhibitors form balance of choline transmission and slow down the accumulation of β-amyloid as another mechanism of the disease. Tacrine is not used in therapy due to side effects (hepatotoxicity). But it is easily available and became the basis for formation of new compounds like hybrids and multitarget-directed ligands (MTDLs).[1]

The change of mood and depression are symptoms of the disease, too. It is believed, the products from the oxidation of amines are the causes of oxidative stress and can damage

neurons.[2] The monoamine oxidase (MAO) inhibition can relieve depression and oxidative damage of cells.

By using MTDLs approach in my work, I targeted at the synthesis of new molecules linking the tacrine moiety and propargylamine moiety of selegiline. Potentially, these compounds should inhibit the AChE and MAO enzymes and affect multiple target structures with one substance. It would ensure a sufficient concentration of neuromediators acetylcholine and catecholamines for right cholinergic transmission and less mood swings with depression.

The synthesis was based on a substituted tacrine (6-chloro, 7-methoxy, 7-phenoxy) with higher efficacy and lower toxicity.[3] We added a three-carbon linker chain between heterocycle and the propargyl to achieve better inhibition of AChE and MAO.[4] The one step reactions led to mono and bis products. We had to find right composition of the eluent for separating these two products. The next biological and clinical trials will show whether these compounds have some activity and benefit for the population.

The study was supported by Faculty of Military Health and Faculty of Pharmacy in Hradec Králové.

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COMPOUNDS COMBINING PYRAZINAMIDE AND 4-AMINOBENZOIC ACID AS POTENTIAL ANTITUBERCULARS

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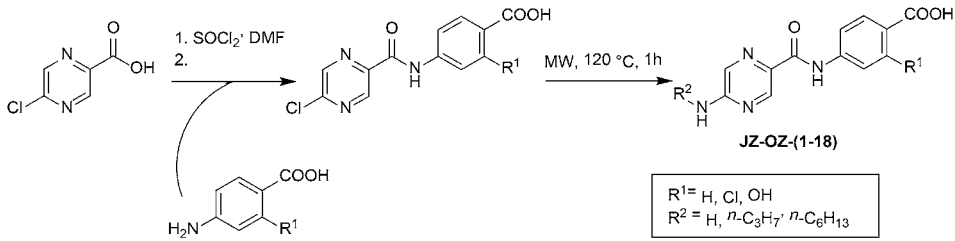
A series of new compounds combining pyrazinamide and 4-aminobenzoic acid was prepared and *in vitro* tested for antimycobacterial activity against *M. tuberculosis* H37Rv, *M. avium, M. kansasii, M. aurum* and *M. smegmatis*. Previously prepared 4-(5-chloropyrazine-2-carboxamido)-2-hydroxybenzoic acid (R^1 = OH) exerted micromolar activity against *M. tbc* H37Rv and low *in vitro* cytotoxicity in HepG2 cells.[1] *p*-Aminosalicylic acid (PAS) has significant antitubercular properties based on its resemblance to 4-aminobenzoic acid and interference with the folate pathway in mycobacteria.[2] To assess the role of the PAS fragment, we designed and prepared derivatives with modified substitution on the phenyl ring (R^1). Further modification was the exchange of 5-Cl on the pyrazine core with alkylamino substituent (**JZ-OJ-1 to 18)**, which was a successful modification in our previous series.[3]

Some of the 5-propylamino compounds (incomplete results) proved micromolar activity against *M. tbc* H37Rv. Structure-activity relationships will be discussed.



The study was supported by the Czech Science Foundation (project No. 17-27514Y) and by SVV 260 401.

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STABILITY OF MAGNESIUM PHTHALOCYANINES IN ACIDIC ENVIRONMENT

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Phtahlocyanines (Pcs) are classified as analogues of porphyrins condensed with four isoindoline units via a nitrogen atom and display a deep blue color due to their wide 18π electron conjugation. Along with subphthalocyanines, these compounds get potential attention as useful dyes that are applicable to organic solar cells, photodynamic therapy, organic electronic devices and other appliances.[1] Phthalocyanines are capable to form compounds with diverse types of metals. Pcs can be used as fluorescence probing due to their suitable absorption and emission in the red region of the visible spectrum. Red or near-infrared excitation and emission is important biological application since longer wavelength light penetrates deeper into tissues, it is less scattered and the autofluorescence of endogenous chromophores are limited. Physicochemical properties of Pcs and their analogues are highly depending on central metal and peripheral substitution. The presence of heavy metals increases molecular relaxation via intersystem crossing which can cause high quantum yield of singlet oxygen formation and low quantum yield of fluorescence. Heavy atom effect is defined as the increase of the spit-orbit coupling in compounds substituted or complexed with heavy atoms. Magnesium is the lightest cations that is stable in the center of these macrocycles, and it is also the metal of choice for porphyrinoid compounds used for fluorescence probing in the red region[2]. We have conducted numerous experiments to determine the stability of the magnesium phthalocyanine complexes in water, organic solvent, liposomes, microemulsions and nanoparticles. Seven buffers ranging from pH 1 to 7 were prepared and a certain amount of each buffer was added into sample to monitor

the changes of the absorption and fluorescence spectra for a certain period, usually for up to 24 hours. Due to low concentration of nanoparticles, I was able to collect only its fluorescence spectra. The transition from a metal complex to a metal-free complex can be observed from the absorption spectra when the curve is split in half which indicates the formation of a metal-free complex. Protonation followed by the formation of metal-free complexes occurred in the presence of water and organic solvents. Our experimental data shows liposomes can protect the compounds from the acidic environment of the system compared to microemulsion. On the other hand, in the case of nanoparticles a decrease of the emission spectra occurs, but it is independent of the pH value. The aim of the project was to determine the stability of magnesium phtahlocyanines in water, organic solvents, liposomes, microemulsions and nanoparticles.

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SYNTHESIS OF PYRAZINAMIDE COMPOUNDS WITH ACTIVITY AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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In the early phases of drug design and development, scientists must overcome many steps involved in identifying potential drug-like or lead-like compounds. This has led to the need of creating large sets of chemical data which will aid in improving the identification of pharmacophores and active compounds. Various scientific fields especially pharmacology, medicinal chemistry and biochemistry have begun to employ the use of computer sciences to aid in the screening of potential leads with more specificity with regards to bioactivity of substances or drug-like compounds. The emphasis of this project was to create a database containing a collection of chemical substances (pyrazinamide compounds) synthesized overtime in the Department of Pharmaceutical Chemistry with the aim of having anti-mycobacterium (and possible antifungal) activity, and further utilize this database to predict certain pharmacokinetic and bioavailability properties.[1] This project seeks to demonstrate how certain molecular descriptors can be used as reliable chemoinformation to determine the likeliness or possibility of developing a lead-like or drug-like compound by utilizing computer software.[2]

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SYNTHESIS AND ANTIINFECTIVE EVALUATION OF SUBSTITUTED *N*-(PYRAZIN-2-YL)BENZENESULFONAMIDES

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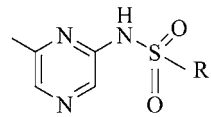
Tuberculosis (TB) is among the ten leading causes of death, especially in developing countries. Even though it is an old disease with established treatment regimen, there has been an increased resistance to anti-TB drugs.[1] The anti-tubercular pyrazinamide has caught the attention of researchers as the different theories for its mechanism of action have made it an interesting entity for further investigation. Here we will discuss *N*-(pyrazine-2-yl)benzenesulfonamides (Figure) as a new derivatization approach based on synergism methodology between pyrazinamide and sulfonamides. Sulfonamides exert their antimicrobial effect by competitive inhibition of folic acid synthesis and subsequent inhibition of bacterial growth and reproduction.[2] I have contributed to the synthesis and purification of 9 compounds in a series of total 20 pyrazinesulfonamides. The biological testing results will be discussed in the presentation.



R: Aryl Substituents

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (projects SVV 260 401 and SVV 260 416), as well as by the Grant Agency of Charles University (project C-C3/1572317) and the Czech Science Foundation (project no. 17-27514Y).

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TUMOR-TARGETING CHEMOTHERAPY: SYNTHESIS OF PRECURSORS FOR NEW ANTI-CANCER AGENTS

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Breast cancer is one of the most incident pathologies in women. Furthermore, the triple negative breast cancer (TN) is known as the most challenging kind of breast cancer to treat.

TN has negative histochemical confirmation for estrogen receptors (ER), progesterone receptor (RP) and human epidermal growth factor receptor 2 (HER2).[1] The lack of receptors, which are usually used for clinical stratification, is not allowing targeted therapies. Our group had previously developed small molecules,[2] derived from pyrrolo-thiazoles, with *in vitro* anti-cancer activity against three cell lines of breast adenocarcinoma: MCF7 (ER+ and PR+), HCC1806 (TN) and HCC1954 (HER2+).[3] Our recent work presents new synthetic pathways of thiazolidines, as percursors of pyrrolo[1,2-*c*]thiazoles, possessing more hydrophilic groups and additional modifications that are expected to provide more efficient anti-cancer agents.

The study was supported by Coimbra Chemistry Centre

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SALICYLANILIDE DERIVATIVES: A PROMISING SOLUTION TO ALZHEIMER'S DISEASE

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Based on previous knowledge about salicylanilide derivatives ((thio)phosphates, carbamates) proposed as inhibitors of acetyl- (AChE) and butyrylcholinesterase (BChE),[1,2,3] 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 or 3-phenyl-3-hydrobenzo[*e*][1,3,2]oxazaphosphinin-4-one 2-oxides. Their ability to inhibit both cholinesterases was evaluated using Ellman's method. AChE was inhibited with IC_{50} values within the range of 48–66 μM.

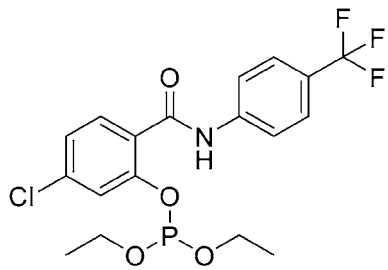

Fig. 1: The most active BChE inhibitor

5-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl diethyl phosphite (Fig. 1) exhibited superior inhibition of BChE (IC_{50} = 2.37 μM) and it was selected for advanced tests (type and kinetics of inhibition, cytotoxicity).

The study was supported by the Czech Science Foundation project no. 17-27514Y.

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SYNTHESIS OF CERAMIDES WITH DEUTERATED SPHINGOSINE CHAINS

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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, *stratum corneum*. In this layer, Cer along with cholesterol (Chol) and free fatty acids form multilayer lamellae of intercellular lipid matrix.

To evaluate the skin lipid arrangement, skin membrane models with labelled (deuterated) lipids have been used. Therefore, the goal of this work was to synthesize deuterated Cer, *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 by using biophysical studies.

Synthesis of deuterated Cer started from elimination of 1-pentadecanol-d_{31} to obtain a terminal deuterated alkene. Next, a vinylation of (*S*)-Garner's aldehyde led to obtain an intermediate, which was treated in Grubbs metathesis with terminal alkene. The product of Grubbs metathesis (a protected deuterated sphingosine) was then deprotected under acid conditions; free sphingoid base was acylated by protonated or deuterated lignoceric acid using water soluble carbodiimide. Synthesized *d*-CerNS and *dd*-CerNS were incorporated into *stratum corneum* membrane models (mixtures). 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 (5 wt%). Overall, four types of model membranes with different representation of deuterated methylene (CD_2) chains, were studied by infrared spectroscopy. A phase behaviour (*e.g.*, conformation, lateral arrangement and miscibility) of lipid model membranes was investigated. The results of this study could be helpful in explaining the (patho)physiological arrangement of skin lipids in *stratum corneum.*

The study was supported by the Czech Science Foundation (project no. 16-25687J) and by the Grant Agency of Charles University (project no. 88615).

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL UNCHARGED CHOLINESTERASE REACTIVATOR AGAINST NERVE AGENT INTOXICATION

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Nerve paralytic agents and pesticides are strongly toxic organophosphorus compounds with high affinity for acetylcholinesterase (AChE) enzyme, which is present in nervous and neuromuscular synapses. This intoxication leads into an acute cholinergic crisis, which can lead to death. The therapy must be quick and includes application of atropine, reactivators of AChE and diazepam. Among the commonly used reactivators molecules of mono- or bis-quarternary aldoxime type are found. These compounds are permanently charged, so their penetration through the hematoencephalic barrier is very limited (up to 10%, often only 1–3%).

Within the presented project, a non quarternary reactivator of AChE which could present a higher ability to penetrate through biological membranes has been prepared with different synthetic methods. *In vitro* tests of his capability to reactivate the AChE has been performed with Ellmans method. The AChE was previously exposed to nerve paralytic agents (dichlorvos, paraoxon, sarin, soman, tabun and VX). The efficiency of reactivation has been determined with two different concentrations – 10 and 100 μM. The activity of the new non quarternary reactivator was compared with standard oxime reactivators of AChE – obidoxim and pralidoxim.

The new non quarternary reactivator was synthesized with more step synthesis. Excelent results has been achieved within *in vitro* testing. In comparison with standard reactivators, it achieved better reactivation of AChE inhibited with dichlorvos (91%), sarine (65%) and the VX agent (35%).

This work was supported by the Ministry of Defence of the Czech Republic through a Long-term organization development plan 1011.

EMPLOYING NMR SPECTROSCOPY IN STRUCTURAL ELUCIDATION OF NATURAL COMPOUNDS

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Presented alkaloid was isolated from *Papaver rhoeas* (Papaveraceae) at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové. Papaveraceae family is rich in specific alkaloids, mainly in isoquinoline alkaloids.

The isolated substance was characterized employing basic ^{1}H and ^{13}C NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC, gCOSY and NOESY, supported by EI-MS spectra.

Isolated compound has been already described in the literature as rhoeagenine (Fig.1). The isolated alkaloid was subjected to the screening of its biological activities on acetylcholinesterase and butyrylcholinesterase. Other biological activities will be investigated.

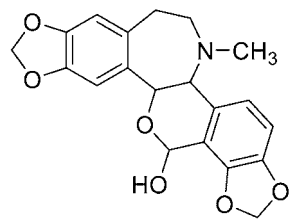

Fig. 1: Rhoeagenine

This work was supported by the Czech Science Foundation (project 18-17868S) and SVV 260 401.

OPTIMALIZATION OF THE SYNTHESIS OF 32-HYDROXYDOTRIACONTANOIC ACID

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The acylceramides belong among ultralong chain ceramides. They are essential components of the *stratum corneum* and play crucial role in proper function of skin barrier (they help preventing the excessive water loss and penetration of exogenous substances to the organism).

The 32-hydroxydotriacontanoic acid forms the backbone of all the acylceramides. The carboxyl group of this acid is bound to a primary amino group of the sphingoid bases and the ω-hydroxy group is predominantly esterified with linoleic acid. In the *stratum corneum*, this acid may remain in the form of acylceramides or it can be linked to the surface of corneocytes.[1]

The recent literature describes the synthesis of 32-hydroxydotriacontanoic acid with relatively low yields, around only 35%. The most problematic part of the synthesis is the connection of two shorter fragments leading to the ultralong chain.[2] The main aim of this research project was to optimalise the reaction conditions to increase the yield. One possi-

bility is to modify the reaction conditions in the previously described Wittig reaction. Since Wittig reaction belongs among olefination reactions, we wanted to examine additional olefination reactions. This project is mainly focused on Julia and Julia-Kocienski reactions and their modifications. We expected that if we change the reaction time or temperature of Witting reaction during the ylide formation, we might see an improvement. Unfortunately, we were not able to increase the yields. The situation changed when we used modified Julia-Kocienski reaction with 16-[(1-cyclohexyl-1*H*-tetrazol-5-yl)sulfonyl]hexadecanoic acid as a starting material. In this case, we were able to increase the yields even over 70% which greatly improves the described reaction pathway.

The study was supported by SVV 260 401 and by the Czech Science Foundation (project no. 16-25687J)

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TOTAL SYNTHESIS AND STUDY OF HUMAN 6-HYDROXYCERAMIDES IN MODEL LIPID MEMBRANES

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Ceramides (Cer) as members of sphingolipid family, play an important role in cell signalling. Besides, Cer also occur in the epidermis. In the uppermost skin layer (stratum corneum), Cer along with free fatty acids and cholesterol (in equimolar ratio) form intercellular multi-lamellar lipid matrix, where Cer play another role in the skin barrier function. 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 sphingoid base (*e.g.*, sphingosine; C18) and 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 the most unusual sphingolipids. In contrast to sphingosine-based Cer, 6-hydroxysphingosine-based Cer (**H-Cer**) are unique only for the epidermis. In addition, **H-Cer** are not typical for all mammals.[1] Moreover, the function and biosynthesis of **H-Cer** in the skin are still enigmatic. Several dermatological studies showed that lower concentrations of **H-Cer** in skin correlate with skin diseases, such as atopic dermatitis.[1]

The major limitation of understanding the importance and uniqueness of **H-Cer** is that these species are not commercially available. Therefore, the aim of this work was to explore 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 synthesis (totally 7 reaction steps)

of **H** involved an alkynylation of (*S*)-Garner's aldehyde (a protected L-serinal) with protected (±)-pentadec-1-yn-3-ol followed by selective two-step trans-reduction of triple bond. In this step, a mild and selective [Cp · $Ru(CH_3CN)_3$]PF_6-catalyzed Trost's hydrosilylation followed by protodesilylation was used. Subsequently, Cer NH (*N*-lignoceroyl 6-hydroxysphingosine) and Cer EOH (with ester-linked linolenic acid) have been prepared.

Additionally, the phase behaviour and biophysical properties of Cer NH have been studied using model lipid membranes. Model membranes were prepared as equimolar mixtures of Cer NH, (deuterated) lignoceric acid, and cholesterol with an addition of cholesteryl sulfate. Model mixtures were then investigated by **thermotropic IR spectroscopy**. From these experiments, we studied the chain order, phase transitions, lateral packing and miscibility of Cer NH and other skin lipids.

These results could help to **answer** our simple question, why **H-Cer** occur only in the epidermis. In the future, prepared **H** will be studied for its antimicrobial activity and will serve as a precursor for the synthesis of other physiological **H-Cer** subclasses, which have not been prepared yet (*Cer AH* and *Cer OH*).

This work was supported by the Czech Science Foundation (project 16-25687J) and SVV 260 401. We thank Mrs. Iva Vencovská for her technical assistance.

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NUCLEOPHILE ASSISTED GOLD(I) CATALYZED CYCLIZATIONS

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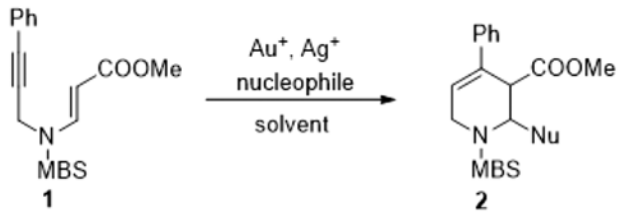
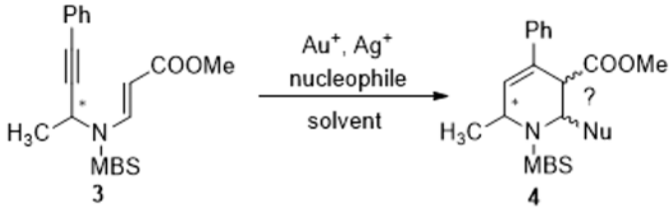
Gold(I)-catalyzed cyclizations of substituted enyne **1** in the presence of a nucleophile (*e.g.* methanol) was developed. Screening of various gold catalysts, silver co-catalysts



Scheme 1. Nucleophile-assisted cyclizations



Scheme 2. New stereogenic center

and solvents was performed, and the cyclization step optimized. Under optimized reaction conditions, we observed the formation of substituted tetrahydropyridine **2** with excellent diastereoselectivity. To extend our methodology, we are now interested in the preparation and further cyclization of enyne **3** with a chiral center that could govern the stereoselectivity of cyclization.

This work was supported by SVV 260 401 and the Grant Agency of Charles University (project no. 262416) and the Czech Science Foundation (project no. 18-17868S).

NMR SPECTROSCOPY: AN ADVANCED APPROACH TO EVERYDAY ANALYSIS

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Nowadays, nuclear magnetic resonance spectroscopy is not only an irreplaceable analytical tool used for both systematic elucidation of completely unknown chemical structures and determination of system condition and kinetics, but also a method used in everyday analysis of samples with either predicted or unidentified content. Employing the behaviour of nuclei with non-zero spin under appropriate conditions in a strong magnetic field with radio frequency ranged pulses applied, various useful data about the individual nuclei and their surroundings can be obtained.

A modern chemist should be familiar with the standard NMR methods used to analyse every sample, but when the contents do not meet the predictions or cannot be identified in a standard manner, careful advanced analysis comes to play. The goal is to employ more or less complicated experiments requiring the least amount of time while yielding rigorous results, thus having the maximal possible effectivity. I have prepared three seemingly simple cases representing the daily struggle of synthetic and analytical chemists, which required an advanced methodical approach to solve.

This project was supported by SVV 260 401.

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SYNTHESIS AND EVALUATION OF NOVEL ANTIMYCOBACTERIAL ISONIAZID ANALOGUES

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Isoniazid (INH) is a first-line drug against tuberculosis with a selectivity for *Mycobacterium tuberculosis* (*Mtb*.). The mechanism of action consists primarily in the inhibition of InhA and thus cell wall biosynthesis. However, the development of resistance has limited its therapeutic potential and many structural modifications have been synthesized.[1]

This work is focused on synthesis and evaluation of novel INH analogues, predominantly the hydrazones of INH with various oxocarboxylic acids that are further modified on free carboxyl group by various amines or phenols to yield amides and esters. The hydrazone bond was also reduced in some compounds. Additionally, a series of 2-benzoylhydrazine-1-carboxamides was prepared and they were cyclized[1] to 5-phenyl-1,3,4-oxadiazole-2-amines subsequently.

The prepared derivatives were characterized and their *in vitro* antimycobacterial activity (*Mtb.* H37Rv, *M. avium*, two strains of *M. kansasii*) was evaluated. The best activity against *Mtb.* showed the anilides of INH-based hydrazones substituted by an electron-withdrawing group, an additional aromatic ring or a long alkyl (MIC values of ≤ 0.25 μM). Derivatives of 4-iodo- and 4-CF_3(O)-anilines exhibited a comparatively high effectivity against atypical mycobacteria (MIC ≥ 2 μM). Importantly, the presented compounds are selective, non-toxic for mammalian cells (HepG2) and almost all of them are comparable or significantly superior to parent INH. Their activity against drug-resistant mycobacterial strains is currently under investigation.

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

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NOVEL AMINO-DECORATED POLYAMIDOAMINE DENDRIMERS WITH ETHYLENEDIAMINE CORE: SYNTHESIS AND POTENTIAL APPLICATION IN (TRANS)DERMAL DRUG DELIVERY

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Dendrimers are characterized as synthetic, spherical macromolecules with tree-like branched structures. Their well-controlled size (3–10 nm), ease of functionalization, high water solubility, well-defined chemical structure, and biocompatibility make these nanomaterials attractive for a wide spectrum of promising biomedical applications.[1] Peptide dendrimers and polyamidoamine (PAMAM) dendrimers have been used to date as effective transdermal or topical drug delivery systems, with the latest in a much greater extent.[2] The structural characteristics of the aforementioned molecules guided us to develop lower generations of novel dendritic structures containing amide groups and amino-branching points in their interior. The new poly(amidoamine) dendrimers were fully characterized and will be evaluated for their effect on skin permeability.

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AMARYLLIDACEAE ALKALOIDS DERIVATIVES OF AMBELLINE AND THEIR BIOLOGICAL ACTIVITY

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Plants of the family Amaryllidaceae belong to the widely-expanded species. They contain a large amount of Amaryllidaceae alkaloids (AA), which are known for their biological activity. The most important activities are antiviral, antimalarial, anticancer and anticholinesteratic.

One of the most abundant AA in genus *Nerine* is ambelline. The biological activity of this compound has been studied only little. So far, this compound showed moderate antiplasmodial effect against the chloroquine-resistant *P. falciparum* Dd2. This compound is structurally similar to haemanthamine. Some synthetic derivatives of haemanthamine

prepared in our laboratory showed important biological activities connected to potential treatment of Alzheimer's disease and oncological diseases. This fact has led us to prepare ambelline analogues.

Ambelline has been previously isolated from fresh bulbs of *Nerine bovdenii*. A series of aliphatic and aromatic ambelline derivatives has been synthesized and evaluated for anti-cholinesterases activity. Moreover, the cytotoxic activity has been studied on a panel of selected cancerous and noncancerous cell lines. The most interesting biological activities have been demonstrated by aromatic derivatives of ambelline labeled as LC-92 (inhibition of butyrylcholinesterase) LC-104, LC-106 and LC-108 (cytotoxic activity).

This project was supported by SVV 260 292.

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AROMATIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AND THEIR BIOLOGICAL ACTIVITIES

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Modern research has shown that Amaryllidaceae alkaloids represent a rich reservoir of potential small molecules exhibiting several medicinal properties through various mechanisms. Among the many Amaryllidaceae alkaloids, galanthamine has been given a great amount of attention due the fact that it possesses potent acetylcholinesterase inhibition activity, and is distributed worlwide for the treatment of Alzheimer's disease. Some of Amaryllidaceae alkaloids 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 Amaryllidaceae alkaloids, which displays significant *in vitro* cytotoxic activity against several different types of cancer cell lines (*e.g.* MOLT-4, HepG2, HeLa, MCF-7, SK-BR-3, A549, Caco-2, HT-29).[1,2]

HA has been isolated from wide range of Amaryllidaceae plants, and is one of the most abundant Amaryllidaceae alkaloids. We isolated HA from the bulbs of *Narcissus* cv. Dutch Master in large amounts as a start material for the preparation of its derivatives. Some of the previous prepared derivatives have shown promissing 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 an 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 SVV UK 260 292.

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PREPARATION OF LYCORINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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The plants of the Amaryllidaceae family are one of the most important sources of biologically active alkaloids. Lycorine, a phenanthridine alkaloid, isolated from various species of the Amaryllidaceae plant family, has attracted considerable attention due to its promising biological activities. Specifically, its anticancer properties have been evaluated *in vitro* and *in vivo* in various preclinical models of human cancers.[1] Further biological effects manifested by lycorine are: antiviral, antibacterial, antifungal, antiplasmodial, anti-oxidant, anti-inflammatory and insect antifeedant effects, as well as ascorbic acid biosynthesis and RNA inhibitory activity. So far, lycorine was used for preparation of many derivatives by modification of different functional groups in its molecule, and screened for a various biological activities such as anticancer activity, inhibition of cholinesterases, antiplasmodial, antitrypanosomal, antiviral and anti-*Trichomonas vaginalis* activity.

The present work deals with the preparation of lycorine derivatives and their biological activity connected to the treatment of Alzheimer's disease and anticancer activity. Twelve lycorine derivatives were prepared and purified using common chromatographic methods. The chemical structures were elucidated by MS and NMR experiments. All prepared compounds were screened for their cholinesterases inhibitory activity. Moreover, the cytotoxic activity has been studied on a panel of selected cancerous and noncancerous cell lines. Promissing butyrylcholinesterase inhibition activity has been demonstrated by 1,2-di-*O*,*O*′-benzoyllycorine (IC_{50} = 29.65 ± 6.81 μM).

The study was supported by SVV UK 260 412.

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ENZYME KINETIC EVALUATION OF SEVERAL POTENTIAL INHIBITORS OF CERTAIN HUMAN CYSTEINE AND SERINE PROTEASES

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Cysteine and serine proteases are enzymes involved in many physiological processes. The imbalance between them and their endogenous inhibitors is associated with various diseases such as cancer and osteoporosis. Synthetic inactivators could be useful in the treatment of these enzyme-mediated pathological conditions. Therefore, there are ongoing attempts to develop low-molecular weight inactivators for therapeutically relevant cysteine and serine proteases. As potential inhibitors of selected human proteases compounds synthesized in Prof. Gütschow's group were investigated. They belong to imidazole compounds derived from *N*-protected cyclohexylalanine, 2-phenyl-7,8-dihydroimidazo[1,2-*a*] pyrazin-6(5*H*)-one derivatives, α,β-unsaturated peptidomimetic compounds, carbamates, an *N,N*-dibenzylcrotonamide derivative and peptoides.

This project has been focused on the evaluation of new potential inhibitors against human cathepsins B, L, S and K, which belong to cysteine proteases, as well as representatives of serine proteases, human leukocyte elastase and human thrombin. Furthermore, the structure-activity relationships of a small series of low molecular weight compounds were investigated.

Enzyme inhibition assays and their corresponding kinetic evaluation were introduced. In the course of this project, a human leukocyte elastase inhibition assay was modified and the determination of the Michaelis-Menten constant (K_M) was performed. Based on the obtained data, the inhibitory potency of the tested inhibitors was characterized by methods of linear and non-linear regression analysis to calculate different inhibition parameters.

Most of the imidazole derivatives have shown significant inhibition against human leukocyte elastase. The best inhibitor from this group has been compound 3161 ($IC_{50}/(1 + [S]/K_M) = 0.60 \pm 0.03$ μM) containing a methyl- and phenyl-substituted imidazole moiety. Carbamate 3167 ($k_{inac}/K_i = 12.44$ μM^{-1} s^{-1}) was considered as an irreversible cathepsin B inhibitor. The *N,N*-dibenzylcrotonamide derivative, compound 3110 ($k_{inac}/K_i = 2292.8 \pm 240.74$ μM^{-1} s^{-1}) containing an α,β-unsaturated Michael acceptor substructure, was considered as an irreversible inhibitor of cathepsin K. Any other investigated compounds did not show any inhibitory potency.

In summary, some potent inhibitors of some tested enzymes have been found. The imidazole derivatives proved reversible inhibitory potential against human leukocyte elastase. Results with compound 3167 confirmed that the introduction of a carbamate structure is a possible way for the development of the new cathepsin B inhibitors. The

N,N-dibenzylcrotonamide derivative (compound 3110) demonstrated the potential of Michael acceptors as irreversible cathepsin K inhibitors. Further analogous compounds need to be investigated in the future.

DEFINITION OF THE CARBOHYDRATE BINDING CAPACITIES OF THE NOVEL ENTEROTOXIN LT-IIC

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Diarrhoea as a disease is still the leading cause of malnutrition and a major cause of deaths in children under 5 years of age in the low-income countries. Additionally, it is the most common health problem associated with travelling to the developing countries. In all the mentioned cases, enterotoxigenic *E. coli* (ETEC) is one of the most frequent causes.

ETEC is defined as a pathogenic strain of *E. coli* producing enterotoxins. So far, two types of enterotoxins have been identified: heat-stable (ST) and heat-labile (LT). LTs are further divided into two categories based on their relatedness with cholera toxin to type I (LT-I) and type II (LT-II). All these enterotoxins have been found to bind to carbohydrate structures on glycosphingolipids by their respective B subunits, however, their binding patterns differ. While LT-I, LT-IIa and LT-IIb have been previously studied in terms of binding specificities, the newest LT-IIc was tested only on few commercially available ganglio-series gangliosides.

In this study, the binding capabilities of this novel enterotoxin were re-examined by series of binding assays using more ganglio-series and some neolacto-series gangliosides as well as other glycolipids and glycoproteins, to establish the basics of the recognition pattern and to characterize the optimal binding sequence. At the end, inhibition studies using pure carbohydrates were carried out.

As previously described, ganglio-series gangliosides with Siaα3Galβ3GalNAc carbohydrate chain sequence were bound by the B subunits of LT-IIc (LT-IIc-B) and the strongest binding was noted for Neu5AcGD1a. Similarly strong binding was noted for neolacto-core gangliosides Neu5Acα3nLc$_4$Cer and Neu5Gcα3nLc$_6$Cer with the similar terminal sequence Siaα3Galβ4GlcNAc. No binding to gangliosides carrying a disialo motif (Siaα8Siaα3-) or an α6-linked Neu5Ac occurred. Furthermore, no binding was noted for asialo glycolipids or glycoproteins, underlining the importance of the sialic acid in LT-IIc-B carbohydrate interactions.

THE MONITORING OF AGENT BZ AFTER INTRAMUSCULAR ADMINISTRATION

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Agent BZ (3-quinuclidinyl benzilate) is a potential military incapacitant with anticholinergic activity causing delirium, cognitive dysfunction, hallucinations, and inability to perform tasks.[1] BZ is being currently used as a pharmacological tool (*e.g.* to induce experimental cognitive impairment) and moreover, the misuse for military purposes could not be excluded.[2] Previously, LC-MS/MS method for the BZ determination in rat plasma has been developed in the Department of Toxicology and Military Pharmacy (Faculty of Military Health Sciences). Since the ability of BZ to cross the blood-brain barrier is largely unknown, the aim of the present study was to optimize the sample preparation procedure for brain tissue.

Both plasma and brain tissue samples were taken from animals administered intramuscularly with BZ at dose of 2 mg kg^{-1} and 10 mg kg^{-1} Samples were collected 1, 3, 5, 10, 20, 30, 60, 120, 240, and 360 minutes after its administration. BZ was isolated from plasma using solid phase extraction. Brain tissue samples were prepared using mechanic blender, sonicated by ultrasonic homogenizer and followed by protein precipitation by acetonitrile. Atropine was utilized as an internal standard in LC-MS/MS. The maximum concentration of BZ in plasma and brain tissue was found 3 and 5 minutes after its administration, respectively. These observations will help to optimize dosing regimens in *in vivo* models of cognitive deficit.

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HPLC SEPARATION OF BIOCONJUGATES OF AZAPHTALOCYANINES

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Bioconjugates of azaphthalocyanines are studied as dark quenchers in molecular probes in real-time PCR. The detection is based on energy transfer between a fluorophore and a

quencher labelled to the oligonucleotide chain. The fluorophore emits a radiation and the dark quencher switches it off. The efficiency of fluorescence quenching depends on the distance between fluorophore and quencher. Probes can be mono-labelled, in which two oligonucleotides are used, one attached to a fluorophore and the other one to a quencher. So called double-labelled probes contain both (fluorophore and quencher) on the same oligonucleotide chain. In the case of random coil, an unbound probe does not emit any fluorescence because fluorophore and quencher are close to each other. After hybridisation with a complementary chain the quencher cannot absorb the fluorescence any more.

The aim of the work was to study a chromatographic behaviour of model double-labelled probes in order to find suitable conditions for separation of mono-labelled oligonucleotides as the major impurity. The double-labelled probes containing commercially available BBQ1 as well as azaphthalocyanine as dark quenchers were used. The fluorophores were fluorescein (FAM) and a cyanine dye (Cy5).

Different conditions for the separation of model mixtures containing mono- and double-labelled probes on two stationary phases were tested. The better results were obtained on a polymer based column in comparison with the C18 stationary phase. The polymeric based column is not available in dimensions suitable for semipreparative purposes and if this type of separation is required, the C18 phase could be employed as the alternative column.

HPLC ANALYSIS OF QUETIAPINE AND ITS TWO BIOLOGICALLY ACTIVE METABOLITES ON ZIRCONIA-BASED REVERSED STATIONARY PHASE

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The purpose of the present work was a bioanalytical evaluation of quetiapine and its two biologically active metabolites – 7-hydroxyquetiapine and norquetiapine using High Performance Liquid Chromatography (HPLC).

Quetiapine has demonstrated efficacy in schizophrenia, bipolar disorder and in the treatment of specific symptom clusters such as agitation and sleep problems in mood disorders.[1]

Separation was performed on zirconia reversed-phased column ZirChrom-PBD. Zirconia-PBD column has enhanced chemical and thermal stability.[2] The separation was performed by gradient elution. The final mobile phase (MF) consisted of two components:

- MF A – acetate buffer, 6 mM, pH 4.0 : ACN, in the ratio 90:10 (V/V)
- MF B –10 mM trifluoroacetic acid, pH 1.9 : ACN in the ratio 40:60 (V/V)

The flow rate was 1 ml/min, the temperature was set at 30 °C. The detection was carried out at 254 nm. The analytes were isolated from plasma using LLE. LLE was validated according to FDA and ICH. Specificity, accuracy, precision, recovery, linearity, robustness, limit of detection and quantification, and stability were monitored.

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POTENTIAL USE OF ZIRCONIUM DIOXIDE IN SPE

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Sample preparation is an important step before the analysis itself. It enables removal of the ballast components, which could interfere with an analyte or even disable the analysis. Solid phase extraction (SPE) belongs among the most popular sample preparation techniques. Silica is the most often used sorbent for SPE, however new, more durable sorbents with different selectivity have appeared. This work focuses on employment of zirconium dioxide and its ability to retain analytes having Lewis base character via ligand exchange. This work took up the previous study, which dealt with the extraction of ibuprofen from two different pharmaceutical preparations by zirconium dioxide.[1] We tested to use zirconium dioxide in extraction of indomethacin as a Lewis base from suppositories. The influence of conditioning solvents with different polarity and extractions solvents was investigated. 10% acetonitrile in methylene chloride was found to be the best solvent for conditioning of zirconia. Methanolic ammonia solution was used as eluent. After some experiments the concentration and the volume of the eluent was set at 0.25M NH_3 in MeOH and 3 mL. Diclofenac was used as the internal standard. The recovery was found to be nearly 83%.

This study was supported by project SVV 260 401.

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CHIRAL HPLC DETERMINATION OF NABUMETONE AND ITS METABOLITES IN HUMAN LIVER CYTOSOLIC AND MICROSOMAL FRACTIONS

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A non-steroidal anti-inflammatory prodrug nabumetone is transformed in the liver to active metabolite (6-methoxy-2-naphthyl)acetic acid. The differences of *in vitro* biotrans-

formation of nabumetone (rat vs human liver cytosolic and microsomal fractions) were studied in previous communication.[1] Study of stereospecificity of enzymes and the calculation of enantiomeric ratios of the biotransformed chiral metabolites is an equally important characteristic of enzymes such as the Michaelis constant. Calculation of enantiomeric ratios of chiral metabolites biotransformed from prochiral drugs *in vitro* is used to identify new enzymes responsible for the biotransformation of a given prochiral drug.[2]

This work deals with the optimization of separation and detection conditions for chiral LLE-HPLC-PDA analysis of nabumetone and its six metabolites in extracts from human liver microsomal and cytosolic fractions. Differences in biotransformation of nabumetone and racemic mixture of 3-OH-nabumetone between men and women were studied *in vitro* in extracts from liver cytosolic and microsomal fractions, using chiral LLE-HPLC-PDA analysis. Enantiomeric ratios of all chiral metabolites of nabumetone were calculated.

The results of the biotransformation of nabumetone and 3-OH-nabumetone were compared in both sexes (males, females) *in vitro* in human liver cytosolic and microsomal fractions. At the same time, the enantiomeric ratios of chiral metabolites of nabumetone in males and females were compared. Female cytosolic carbonyl-reducing enzymes were 1.74-fold more stereospecific for (+)-reduced nabumetone than in men. Three new potential metabolites of 3-OH-nabumetone were detected by incubation of 3-OH-nabumetone *in vitro* with human liver cytosolic and microsomal fractions.

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DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS FOR CHIRAL SEPARATION OF PROMISING POTENTIAL DRUG AGAINST ALZHEIMER'S DISEASE

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Our work was focused on the development of a HPLC-UV method for the determination of K 1277 enantiomers of systematic name *N*-(2-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl) amino)hexyl-2-amino-3-(1*H*-indole-3-yl)propylamide dihydrochloride. These tacrine-tryptophan hybrids could be considered as promising candidates of potential drugs against Alzheimer's disease. The aim was to find the optimal chromatographic conditions for separation of K 1277 enantiomers synthesized from tacrine and tryptophan fragments. Two chiral stationary phases (CSPs) were studied to develop an efficient analytical method applicable in various pharmacological studies. Teicoplanin and *tris*(3,5-dimethylfenyl) carbamate cellulose as chiral selectors were chosen based on previous experiences.[1] The Dionex UltiMate 3000 chromatograph was used for separation optimization using

a mixture of acetonitrile, water and sodium perchlorate as a mobile phase. Applying the optimized chromatographic method, the *R*-enantiomer was eluted from the *tris*(3,5-dimethylfenyl)carbamate cellulose based CSP in 4.6 minute being followed by the *S*-enantiomer in 6.5 minute with resolution 4.3. The repeatability in terms of relative standard deviation and the limit of detection of the analytical method were determined to be lower than 2% and 0.543 μg/ml, respectively. Finally the analytical method was applied for separation of K 1277 enantiomers in human plasma.

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

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The aim of this study was to develop ultra-high performance supercritical fluid chromatography method (UHPSFC) with UV detection for determination of agomelatine and its six potential impurities. UHPSFC system Acquity UPC[2] with PDA detector was used with Torus Diol column (3.0 × 100 mm, 1.7 μm). Column temperature was set at 40 °C and BPR (back pressure regulator) pressure at 2000 psi. Flow rate of mobile phase was 1.5 mL/min. The detection wavelenght was set at 225 nm. CO_2 modified with the mixture of methanol/acetonitrile (1:1) with addition of 0.1% ammonium hydroxide was selected as a mobile phase. Gradient started at 10% methanol/acetonitrile with additive and was increased up to 30% in the three minute.

Method was validated according to the International Conference on Harmonisation (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 except of impurities (7-methoxynapht-1-yl)ethylamine hydrochloride (0.5–100 μg/mL), (7-methoxynapht-1-yl)acetic acid (0.2–100 μg/mL) and (7-methoxynapht-1-yl)acetonitrile (0.07–70 μg/mL). Method was validated for API (active pharmaceutical ingredient) and subsequently for agomelatine tablets. The accuracy and precision of method was determined at three different concentration levels for API and three different concentration levels for impurities. Accuracy and precision have to be in the range of 95%–105% and RSD ≤ 5, respectively. The impurity concentrations represent 0.05% of API as required by ICH quality guideline Q3A for drug substance (reporting treshold 0.05%) and Q3B for drug product (reporting treshold 0.1%). The results for accuracy and precision were 98.5%–104.9%, RSD ≤ 1.9 for impurities and 99.6%–104.0%, RSD ≤ 1.3 for API. The

evaluation of interday precision was carried out on three different days. Each day new samples at three concentration levels were prepared. The interday accuracy and precision was confirmed by 97.6%–104.1%, RSD ≤ 4.7 for impurities and 99.6%–105.6%, RSD ≤ 1.8 for API.

Each tablet was dissolved in 20 mL of ACN and measured by UHPSFC-PDA method. The results for accuracy and precision in tablets samples were 95.9%–105.5%, RSD ≤ 3.1 for impurities and 99.3%–101.3%, RSD ≤ 0.6 for API. The interday accuracy and precision was confirmed by 95.6%–104.1%, RSD ≤ 4.8 for all impurities with one exception – *N*,*N*-bis[2-(7-methoxynapht-1-yl)ethyl]amine (RSD ≤ 6.1), results for API were 100.1%–101.1%, RSD ≤ 1.6.

In conclusion, this method was found to be suitable for agomelatine impurities determination in pharmaceutical quality control.

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DETERMINATION OF MYCOTOXIN CITRININ USING ON-LINE SPE HPLC ON MOLECULARLY IMPRINTED POLYMERS

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The aim of this work was to develop a fast method for mycotoxin citrinin (CIT) determination using high performance liquid chromatography (HPLC) and column switching technique in combination with on-line solid phase extraction (SPE) on citrinin-selective molecularly imprinted polymer (MIP). The most suitable sorbent for selective extraction was chosen from six newly synthetized MIP after evaluation of their binding capacity and selectivity. Selectivity was tested by comparing molecularly imprinted and non imprinted polymers and the ability of the MIP polymer to selectively separate citrinin from the test sample. The best MIP was filled into pre-column (20 × 3 mm) and connected to HPLC column-switching system. Three different matrix types were injected to this chromatographic system. The 50 µl of the sample was injected onto MIP extraction column and washed out of interferences with methanol/0.5% water solution of acetic acid in a ratio of 25:75 (v/v) at a flow rate of 1 ml min^{-1} for 1 minute. After the valve was switched, the analyte was eluted from the MIP column to a Kinetex® Biphenyl (100 × 4.6 mm, 5 µm particle) chromatographic column eluted by mobile phase consisting of acetonitrile/0.5% acetic acid, which flowed through the column at a rate of 1 ml min^{-1} for separation by gradient elution. Fluorimetric detection was set at wavelengths Ex 335 nm, Em 500 nm. Total sample analysis time including on-line extraction was 9.5 minutes. The limit of quantification for this method was 5 µg kg^{-1} for cereal matrix and 25 µg kg^{-1} for red yeast rice and food supplements based on red yeast rice. CIT was analyzed in 9 samples of which 6 samples were food supplements available on the Czech market. The measured amounts

of CIT in food supplements were low. In most cases even below the detection or quantification limit and therefore all samples met the maximum limits of CIT for food supplements (2000 μg kg^{-1}).[1]

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UHPLC-MS/MS METHOD FOR THE DETERMINATION OF MARAVIROC IN PLACENTAL PERFUSATE

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Maraviroc is an antiretroviral drug acting as an entry inhibitor blocking the chemokine co-receptor 5 (CCR5). It is used as the second choice therapy in HIV 1 positive patients; the lack of knowledge on its safety in pregnancy and transplacental transfer, however, has limited its administration to pregnant women so far. The goal of this work was to develop fast and selective method for the analysis of maraviroc in perfusates of human placental cotyledon performed in order to clarify its transfer from mother to fetus. The ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was the method of choice. BEH C18 column and gradient elution with the mobile phase A (water with 0.1% formic acid) and B (acetonitrile) at 0.35 mL/min flow rate and 40 °C temperature were used for the separation. The mass spectrometry conditions were set up as follows: electrospray ionization in positive mode, capillary voltage 1.0 kV, RF lens 0.1 V, extractor 3.0 V, ion source temperature 130 ºC, cone voltage 35 V, desolvation gas flow 1000 L/h, and temperature 450 ºC. Selected reaction monitoring (SRM) mode was used for quantitation with collision energy 20 eV (SRM 1, quantifier transition) and 30 eV (SRM 2, qualifier transition). Liquid-liquid extraction (LLE) was chosen for sample preparation due to the high lipophilicity of maraviroc. The LLE optimization included optimization of solvent type, solvent to sample ratio, extraction temperature, shaking intensity, and extraction time. The best results were obtained when dichloromethane was used as the extraction agent (recovery > 90%). The optimized method was fully validated in the calibration range 1–1000 ng/mL at five concentration levels (1 ng/mL, 2.5 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL) with the lower limit of quantification (LLOQ) 1 ng/mL and limit of detection (LOD) 0.33 ng/mL. Precision (RSD %) and accuracy (% bias) was determined for each concentration level: 1 ng/mL (RSD = 8.1%, bias = +17.5%), 2.5 ng/mL (RSD = 13.0%, bias = +13.5%), 50 ng/mL (RSD = 2.6%, bias = +3.3%), 500 ng/mL (RSD = 2.7%, bias = +0.4%), 1000 ng/mL (RSD = 1.7%, bias = −0,6%). Further parameters of validation were

linearity ($R^2 = 0.9994$) and matrix effects (99.1%–109.2%) for four concentration levels: 1 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL. The internal standard of maraviroc, maraviroc-d_6, was used for quantification in all experiments. The method finally enabled sensitive and selective determination of maraviroc in placental perfusion samples. We believe these data could help to gain better knowledge on transplacental transport of maraviroc and its safety in pharmacotherapy of HIV-1 positive pregnant women.

The study was supported by the Grant Agency of Charles University (projects 616216/C/2016 and SVV 260 412), and the project STARSS reg. no.: CZ.02.1.01/0.0/0.0/15_003 /0000465 co-funded by ERDF.

CHROMATOGRAPHIC DETERMINATION OF OXIDATIVE STRESS BIOMARKERS USING *IN VITRO* MODELS

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Even though reactive oxygen/nitrogen species (ROS/RNS) are physiologically generated in biological systems, their excessive production may cause severe damage of cellular components. Excessive production of ROS/RNS can occur in response to various stressors such as xenobiotics, radiation or pathological processes.[1] Oxidative stress has also been reported to cause adverse effects of some therapeutic drugs including acetylcholinesterase (AChE) oxime reactivators which are used in therapy of organophosphate poisoning.[2]

In this study, we determined the effect of obidoxime, methoxime, asoxime, pralidoxime and trimedoxime on redox homeostasis in cultured human hepatoma (HepG2) cells. The cells were incubated with oximes at concentration corresponding to their IC_{50} for 1, 4 and 24 hours. Intracellular ROS levels were determined using two fluorescent probes (2′,7′-dichlorodihydrofluorescein diacetate and dihydroethidium). Malondialdehyde and 3-nitrotyrosine were measured using LC-MS/MS. Additionally, non-protein thiols and non-protein disulfides were evaluated to reflect antioxidant capacity. Individual reactivators displayed distinct quantitative and/or qualitative changes in redox homeostasis reflecting different role of oxidative stress in their intrinsic toxicity. Future perspectives are to test new AChE reactivators synthetized at Department of Toxicology and Military Pharmacy in order to minimalize their unwanted side effect related to oxidative stress.

This work was supported by the Ministry of Defence of the Czech Republic through a Long-term organization development plan 1011.

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THE SEPARATION OF MOLECULAR PROBES CONTAINING AZAPHTALOCYANINES AS DARK QUENCHERS

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This work dealt with the development of a suitable HPLC purification method for AzaPc-labelled oligonucleotides, which are tested in real-time PCR. The attention was focused on the chromatographic behaviour of the studied molecular probes on different phenyl stationary phases. The influence of various mobile phases components (*e.g.* methanol, acetonitrile, triethylaminoacetate, tris(hydroxymethyl)aminomethane) were tested for the separation of double-labelled probes, which contain azaphthalocyanine as a dark quencher and different fluorophores. Impurities present in the sample include mainly unmodified oligonucleotide chain and some minor unspecified impurities and also mono-labelled oligonucleotide chains (containing only a fluorophore or a quencher). The sufficient separation of double-labelled probes from mono-labelled ones was unsuccessful using a gradient elution. The isocratic method seemed to be more appropriate for this purpose. Thus, we focused on the optimization of the isocratic conditions.

SECTION OF TECHNOLOGICAL SCIENCES

STUDY OF FLOW BEHAVIOUR OF PARTICLE SIZE FRACTIONS OF LACTOSE EXCIPIENTS

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This work studies the flow behaviour of the particle size fractions in a range of 0.080–0.400 mm of two lactose powders. Anhydrous lactose (AL) is well suited for formulations containing moisture sensitive drugs due to the absence of crystal water and can be utilized for tabletting, granulation, lyofilization or in dry powder inhalers. The lactose monohydrate (Tablettose 80, LM) is used in capsule and sachet filling, effervescent tablets and orally disintegrating tablets. The influence of particle size on the bulk density, the tapped density, the Hausner ratio (HR) and the angle of repose (AOR) was evaluated. The results showed that the values of bulk and tapped density, HR and the AOR were lower for LM showing its better flowability. Further, the mass flow rate through a circular orifice in a range of 0.6–1.5 cm of the stainless-steel conical hopper was studied and modeled with

the Beverloo and/or Jones & Pilpel power regression equation, respectively. The precission of the flow rate prediction using the actual equation parameters was the main criterion. The flow rate increased with the particle size reaching the maximum between 0.245–0.346 mm fraction for AL and 0.245 mm fraction for LM; then it decreased again. At both mathematical models, the precission of the flow rate prediction for the powder size fractions was approximately 3%. In summary, Tablettose 80 produced by granulation showed better flow behaviour than anhydrous lactose owing to better particle properties which confirmed its suitability in direct compression.

This study was supported by the Grant Agency of Charles University project no. 322315/2015.

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FROM SUGAR TO FILM COATED TABLETS – A MODERNIZATION OF PRODUCTION TECHNOLOGY

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Tablet coating represents an important procedure in pharmaceutical technology. The coating can protect an active pharmaceutical ingredient from negative environmental conditions as well as ensure the drug release at a specific time and a specific site in the organism. Generally, the main types of tablet coating are sugar and film coating. As the sugar coating has a number of disadvantages such as a time-consuming production process, the difficult process of standardization, the impossibility to produce a modified release mechanism, it is nowadays rarely being used in pharmaceutical companies. This work deals with the replacement of sugar coating with film coating technology for the multivitamin product Spofavit®**.** During the pilot batch, the commercial coating mixture Nutraficient® Food Supplement Coating, Opadry AMB was applied on two types of tablet cores: the original composition tablet cores and the modified ones with added lactose. The weight variation, hardness, friability and disintegration properties were tested. After processing the results of the pilot batch, a scale-up from the pilot to the production scale for the original composition tablet cores was performed with the utilisation of a Glatt Coater 1500 coating machine. The resulting film coated tablets showed good stability and disintegration properties. Once the manufacturer fulfils the necessary legal requirements, Spofavit® film coated tablets will be ready for sale on the Czech market during 2018 and the routine production.

The study was supported by Zentiva, k.s.

CHOLESTEROL IN HUMAN SKIN BARRIER: PERMEABILITY AND BIOPHYSICS

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Intercellular lipid membranes in the stratum corneum (SC), composed of equimolar mixture of ceramides, free fatty acids and cholesterol (Cer, FFA, Chol) are crucial for barrier function of mammalian skin.[1] Chol is required for proper lipid organization of SC; however, it stays un-clear, why is it present in an amount so high that it separates from other lipids.[2] Experiments using synthetic model membranes with decreased Chol content suggested that molar ratio of Cer:Chol:FFA 1:0.4:1 is sufficient for lipid barrier formation and its complex functionality.

The aim of this work was to manipulate Chol content directly in human SC and study the effects of decreased Chol on the SC permeability and microstructure.

Ex vivo SC obtained from healthy Caucasian donors was extracted by methyl-β-cyclodextrin (CD) to reduce natural Chol content. The extracted SC did not show significant changes in Cer or FFA whilst the amount of Chol was lowered to 78%. SC barrier properties were evaluated by measurements of transepidermal water loss (TEWL), electrical impedance and permeabilities for theophylline (TH) and indomethacin (IND). Significant difference between TEWL of CD-extracted and control sample was not detected. Decreased electrical impedance and permeability to TH, and a slight increase in permeability to IND were found. That corresponds to synthetic membranes with similar Chol content, suggesting that barrier function of SC with lower than natural amount of Chol is not significantly impaired. Molecular organization, investigated using ATR-FTIR spectroscopy, did not reveal significant changes. Furthermore, powder X-ray diffraction suggested that the separated Chol in SC is relatively stable and that CD treatment rather decreased the intensity of a mixed lipid phase.

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OPTIMIZATION OF POLYMERIC NANOPARTICLES SEPARATION AND PURIFICATION PROTOCOL

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Poly(lactic-co-glycolic) acid (PLGA) is one of the most successful polymeric molecule invented for biomedical use. PLGA's biggest advantage lies in its biodegradability and nontoxicity.[1] It has been approved by EMA for human use. Because of rapidly increasing number of protein or nucleic acid based drugs the need for sophisticated drug-delivery systems grows. PLGA nanoparticles (NPs) present exactly such drug-delivery system capable of encapsulating large variety of compounds.[2] Drug release profile and size of NPs can also be modified. It is considerable advantage when compared to conventional application forms.

Within this study we have researched optimization of separation and purification of drug loaded NPs. They were prepared by nanoprecipitation of PLGA in aqueous stabilizer solution.[1] Separation and purification of NPs was done using multiple cycles of centrifugation. We have evaluated purification of particles prepared from five different PLGA polymers. Different centrifugation times have been applied to find the most effective way. Water and two types of stabilizers each one in two concentrations have been used as purification media. Incorporated fluorescent dye Rhodamine B was used as a model drug because of its simple quantification by spectrophotometry. Separated NPs have been characterized by recovery yield (RY) of PLGA and encapsulation efficacy (EE).

NPs prepared in 1% Kolliphor® P188 and separated in water show the highest EE. However, NPs produced and separated in Pluronic® F-127 have generally higher RY. As the most suitable polymers for Rhodamine B encapsulation proved linear PLGA 7-3 (LA-GA) and tripentaerythritol-branched PLGA.

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THE USE OF A HIGH-SPEED MIXER IN THE MIXING OF POWDERS

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The high-speed mixer uses a dual mixing mechanism based on eccentric centrifugal movement and is particularly intended for mixing of liquids or semi-solids. This work is aimed at using high-speed mixer[1] in mixing of powders. To assess the significant factors affecting homogeneity of the acetylsalicylic acid (ASA) and microcrystalline cellulose (MCC) mixture, the reduced experimental plan 3 according to Box-Behnken design[2] was used. In 13 experimental runs, three factors (weight of the mixture, speed rotation, mixing time) at three levels were studied. In order to evaluate the resulting homogeneity of the mixture by near infrared spectroscopy (NIR), standard sample deviations (SD) were used. The experimental plan allowed to obtain the *surface response graphs* for the combinations of the observed factors. The results showed that a mixture weight of 150 g, rotational speed of 900 rpm and a mixing time of 30 sec was the best combination out of the factor levels used. The result was confirmed with the complete mixing experiment. Although the good homogeneity of mixture was detected using the high-speed mixer it cannot be recommended for powder mixing in pharmaceutical technology in general. Heating of the mixture and changes in its flowability were observed, particularly, with the high speed and longer time of mixing.

The study was supported by SVV 260 401.

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COMPARATIVE EVALUATION OF USING DRY BINDERS IN A PHYSICAL MIXTURE OR AS A COPROCESSED DRY BINDER IN MATRIX TABLETS WITH EXTENDED DRUG RELEASE

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This work evaluates and compares the properties of directly compressible tabletting materials and matrix tablets containing the combination of α-lactose monohydrate and microcrystalline cellulose in the ratio of 3:1 in a physical mixture and in a coprocessed dry binder. Polyvinyl alcohol is used as the retarding agent at the concentrations of 30, 40 and 50%. Tested parameters are compressibility, compactibility and the rate of drug release from tablets. Compressibility is evaluated by means of the energy profile of the compression process. Compactibility is evaluated by means of the tensile strength of the tablets. Dissolution testing is performed using rotating basket method.

The values of total energy of compression, plasticity and tensile strength of the tablets were higher in the tabletting materials with the coprocessed dry binder. Increasing additions of polyvinyl alcohol decreased the values of total energy of compression, plasticity, tensile strength of tablets and drug release rate. The dissolution behavior of tablets, which

contained physical mixture or coprocessed dry binder and the same amount of polyvinyl alcohol was comparable.

EVALUATION OF DISSOLUTION PROFILE OF SUPPOSITORIES CONTAINING DICLOFENAC SODIUM SALT

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Suppositories are a promising dosage form, which is advantageously used in cases where it is not possible to use other dosage form (*e.g.* in children, non-cooperating patients or patients with swallow inability).[1] Dissolution tests serve as qualitative tests of medicinal products and provide valuable information during formulation of new ones.[2]

The aim of this work was to study the dissolution process of diclofenac sodium from suppositories. The tested suppositories were prepared by three methods (manually, by Unguator technology and industry made ones). All suppositories contained 100 mg of the active ingredient suspended in the solid fat base. Dissolution tests for lipophilic solid dosage forms by flow-through apparatus containing suppository cell according to the Czech Pharmacopoeia were performed at three media flow rates.

It was found that the maximum release of diclofenac in all suppositories occurred within three hours. The time required for maximal active substance release shortened with the increasing flow rate of the medium and the most significant changes in concentration occurred in first ten minutes of dissolution test. At the lowest flow rate, the initial increase in concentration was slow and the total amount of released diclofenac was statistically lower. This was probably caused by insufficient flushing of diclofenac from molten suppository mass in the chamber of flow-through cell. The standard deviations of the results were greatest in handmade suppositories and much smaller in industrial and Unguator-made ones. According to these findings, it can be assumed that the variance of the results and the rate of diclofenac release depend on the dissolution medium flow rate and also on the suppository preparation method.

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EVALUATION OF CO-PROCESSED EXCIPIENTS INTENDED FOR ORODISPERSIBLE TABLETS FORMULATION

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Co-processed excipients (CPE) can be defined as a combination of two or more substances, which are physically modified by an appropriate process (*e.g.* spray-drying). These excipients are currently increasingly used for production of tablets by direct compression. Due to the availability of a wide range of initial materials for their production, it is possible to obtain a large number of combinations with required characteristics and better properties than simple physical mixtures. Although the composition of these CPE may be similar, the small changes in the component's characteristics can make them to behave differently after tableting.[1]

The aim of this work was a comparison of compressibility (using force-displacement record) of CPE (Starlac®, Combilac®, Cellactose 80®, Disintequik ODT® containing lactose and Ludiflash®, SmartEx QD 50®, SmartEx QD 100® containing mannitol) and properties of obtained tablets (tensile strength, friability, disintegration and water absorption ratio) prepared using compression pressures of 78, 130, 182 MPa. CPE containing lactose have lower values of plasticity compared with CPE containing mannitol but higher values of released elastic energy. This observation is also reflected in values of tensile strength where the highest values were measured for Ludiflash® and lowest for Starlac®. CPE containing mannitol imply higher ejection force. All samples fulfil the requirements of Eur. Ph. for ODT tablets disintegration (3 min).[2] The highest water absorption ratio was measured for Cellactose® while the lowest for Starlac®. Generally, it is impossible to select the best CPE, as their different properties fit different needs of manufacturers and final products.

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STUDY OF CONSOLIDATION BEHAVIOUR OF PARTICLE SIZE FRACTIONS OF LACTOSE EXCIPIENTS

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Study of consolidation behaviour is an important part of the characterization of powder materials. In this work, the bulk flow properties (the bulk density of free arranged powder and the density of solids, *i.e.* the true density determined with a pycnometer) as

well as the consolidation properties (the tapped density and the Hausner ratio) of particle size fractions of two lactoses were evaluated. While Tablettose 80 (TB 80) is prepared by the granulation process, Lactopress Anhydrous (LA) is produced by the rapid drying of the lactose solution at higher temperature. To investigate the consolidatïon behaviour, the consolidation dynamic is recommended instead of Hausner ratio value. Two methods were used to assess the consolidation dynamics and to evaluate the changes in the powder layer. Since the relative change of volume of the powder layer in dependence on the number of taps did not fit the experimental data optimally, the dependence of the porosity factor on the number of taps was used.[1] From the linear regression, the angle of the internal friction AIF (°) was estimated. Although Tabletose 80 and Lactopress Anhydrous have generally a quite regular shape, the differences in AIF for particle fractions resulted from their different bulk properties. The AIF = 46° observed for the largest fraction of TB 80 was the result of the rough structure of granules while AIF = 46° detected for the smallest fraction of LA resulted from the agglomerates of fine particles.

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SECTION OF SOCIAL AND CLINICAL PHARMACY

ANALYSIS OF ANTIBIOTIC ADMINISTRATION IN PROPHYLAXIS

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Antibiotic prophylaxis (AP) plays an important role in reduction of surgical site infection. The aim of this work was to analyze antibiotic administration in prophylaxis in surgical procedures in the health facility (HF) in the context of the internal standard of HF and results of the research on available AP work. This cross-sectional observational study ran from 5 February 2018 to 9 February 2018 in surgical departments of HF. Patients aged ≥ 18 who underwent surgery in defined period of time were included in this study. The process of AP was recorded in the prepared form during the operation: patient identification, date of operation, type and length of operation, sending and receiving department, choice of antibiotic (ATB), dosage, route of administration and time of administration of ATB. The medical documentation was used to complete data of AP and patient characteristics.

Data from the study were compared with both the review of available guidelines and internal standard of HF. The data were processed using descriptive statistics. 197 patients (103 men and 94 women) with average age of 56.5 ± 15.72 years attended the study. Patients were hospitalized on average for 7 ± 5.21 days, 21.8% of patients underwent urological procedure, 16.2% general and abdominal surgery procedure and 14.2% neurosurgery procedure. 125 (63.5%) patients received AP, 11 (5.6%) patients without prophylaxis should have received AP and, in contrast, for 14 (7.1%) patients AP was indicated excessively. Cefazolin was administered in 52% of operations and co-amoxicillin in 25.6% of operations. The choice of ATB did not correspond in 20.7% to the standard of HF and in 25.2% to the review of available guidelines. The dosage of ATB did not correlate in 18.0% with the standard of HF and in 66.7% with the the review of available guidelines. The results indicate the need to carry out further investigations, the findings should serve as a groundwork for optimizing AP in the HF.

The study was supported SVV 260 417.

ANALYSIS OF ANTICOAGULANT UTILIZATION IN THE CZECH REPUBLIC IN THE PERIOD FROM 2007 TO 2016

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Anticoagulants are drugs that prevent blood coagulation. They are most commonly used in the prevention and treatment of thromboembolic diseases, but their indications and usage are becoming more widespread, which can also manifest itself in their consumption.

The objective of this work was to assess the consumption of oral and parenteral anticoagulants in the Czech Republic from 2007 to 2016, based on the data from the State Institute of Drug Control (SIDC).

The research consisted of a retrospective analysis of the SIDC database. This database contains reports of drug supplies from distributors to pharmacies and other health care facilities. All oral and parental anticoagulants approved in the Czech Republic were included in the study. Drug utilization was calculated as number of defined daily doses per thousands of inhabitants per day (DID). The data was processed in the Microsoft Excel 2007 programme. The data on the number of residents was acquired from the Czech Statistical Office.

Oral anticoagulants hold a dominant position. Especially warfarin continuously holds a dominant position among anticoagulants. Its consumption remains almost constant, from 2007 to 2016 raised from 10.03 DID to 11.61 DID. The consumption of NOAC grew rapidly from 2012. Total consumption of NOAC increased from 2007 to 2016 from 0.002 DID to 5.27 DID. The consumption of LMWH increased in regular intervals. Total consumption of LMWH increased from 2007 to 2016 from 3.46 DID to 9.38 DID.

In conclusion, the results showed increasing utilization of anticoagulants, especially in the group of new oral anticoagulants. Warfarin, despite its food and drug interactions, the need for constant INR testing, and the development of new oral anticoagulants is still widely used with nearly stabile consumption.

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ANALYSIS OF CARDIOTOXICITY OF HIGH DOSE GLUCOCORTICOID THERAPY

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Glucocorticoids (GC) are life-saving drugs used in the treatment of some inflammatory diseases, but they have adverse effects on many organ systems. Serious adverse cardiovascular (CV) toxicities, including sudden death, have been reported in occasional patients who have been given pulse infusions of GC (1 g/day *i.v.*).[1] The aim of this retrospective study was to determine the influence of high dose GC treatment on electrocardiographic (ECG) parameters, in particular prolongation QTc. Second endpoint was to analyze the influence of CV comorbidities, and co-medication on ECG in treated patients. We included 311 patients with connective tissue disease and systemic vasculitis, 66.9% females and 33.1% males. We used data from medical records, analyzed the ECG parameters incl. QT/QTc interval before and after the aplication of pulse GC. A 2005 European protocol proposed the use of a QTc value greater than 440 ms in males and 460 ms in females as a definition of a prolonged QTc.[2] QTc was prolonged in 30 (9.6%) subjects, in 27 (90%) of them after pulse therapy. In comparison, patients with normal *vs.* abnormal QTc parameter, higher frequency of CV diseases was found in patients with QTc prolongation: arterial hypertension 19 (63.3%), valve regurgitation 4 (13.3%), atrial fibrilation 4 (13.3%), ischemic heart disease 2 (6.7%), myocardial involvement 2 (6.7%) and 13 (43.3%) of them used drugs that prolong QTc. In conclusion, patients with underlying CV diseases and those using drugs that prolong QTc were in higher risk of prolongation QTc induced by GC, but ventricular arrhythmia was not observed.

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ANALYSIS OF DRUG-RELATED PROBLEMS IN GENERAL PRACTITIONERS OFFICE

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A drug-related problem is according to Pharmaceutical Care Network Europe an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes. There are six primary domains for problems with 21 subdomains. With code P1 it is adverse reaction, which means side effects or toxic effects like allergic reaction after use of ampicilin or cancerogenity after long term use of some drugs. Second group with code P2 contains drug choice problem when patient gets inappropriate drug or drug form or there is some duplication in therapy. P3, dosing problem, is about too high or too low dose or duration of treatment, that can be too short or too long. Drug use problem (P4) usually means wrong drug administration or when patient does not take it at all. Interactions (P5), potential and manifested. The last group with code P6 includes any other problems like when patient isn't satisfied with therapy despite proper use.[1]

The aim of the work was to describe and analyze the most frequent drug-related problems occurring in general practitioners office. To identify problems from documentation and suggest a solution to doctors. The data collection was held at the GPs office from Februrary 20 to March 29 in 2018. We checked fifty patients who were selected by doctors. Selection by GP was based on an amount of used drugs and frequency of medical examinations. After last audit, April 15 2018, all drug-related problems were sorted by PCNE Classification for Drug related problems V5.01 and data were evaluated. After consultation with doctors we wrote down their acceptance of the intervention.

The study was supported SVV 260 417.

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ANALYSIS OF ADVERSE EVENTS IN PATIENTS WITH HIGH DOSE GLUCOCORTICOID PULSE THERAPY

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Intravenous pulse glucocorticoid (GC) therapy seems to be very beneficial in inflammatory conditions and is considered to have a low risk of adverse events.[1] However, the pulse regimen possess additive non-genomic effects to the genomic adverse effects,[2] which were not completely observed. The aim of this work is to analyze occurrence of adverse drug events, analyze its risk factors and find selected, more susceptible populations with underlying risk factors.

In our retrospective study, patients were administered 1000 mg methylprednisolone in 3 to 5 doses during 5 to 10 days. Analysis includes 330 rheumatic patients and data were collected from their medical records. With average age 51 years and 222 women (74%), the most dominant diagnosis were connective tissue diseases (n = 195, 59%) and systemic vasculitis (n = 119, 36%). In 40 patients (12%) some adverse event occurred. In 23 subjects (7%) cardiovascular etiology was present, 11 subjects (3%) developed steroid diabetes mellitus or decompensated their preexisting diabetes mellitus and occurrence of other adverse events was uncommon (< 1%). However, not all adverse events lead to termination of pulse therapy. The reason to terminate the therapy was present in 28 patients (8.5%).

As a retrospective analysis has its limitations, we got to observe only what is being monitored in GC pulse regimen by default. Occurrence of serious adverse events was common (1–10%), but when assessing right risk management having all risk factors of serious events in mind, our study presents GC pulse therapy as relatively safe.

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EVALUATION OF REGISTRATION RATES OF POTENTIALLY INAPPROPRIATE MEDICATIONS IN CENTRAL AND EASTERN EUROPE

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During the last decades, proportion of geriatric patients in the world population increases. This phenonemon is caused by advances in medicine, social care and working conditions. A problem, however, lies in a very frequent polypharmacotherapy and polymorbidity in older adults. With the aim to reduce the frequent adverse drug events in older patients, the explicite criteria of PIMs (potentially inappropriate medications) have been created. The aim of this work was to determine the registration rates of PIMs in Eastern and Central Europe, available in all 22 existing explicite criteria in the scientific literature.[1]

A comprehensive set of 345 PIMs published in all explicite criteria in peer-reviewed or impact factor journals by the year 2015 have been created. Every drug was included in the analyzed list only once, disregarding the other conditions of inappropriateness (*e.g.* drug-disease interactions, dosing, etc.). The exception were PIMs available in non-sustained and sustained-release forms, because each of this drug form can be identified under a specific ATC code. Using data of the EU COST Action IS 1402 initiative (2015–2018), registration rates of PIMs available in 22 different explicite criteria were evaluated in total sample and in samples of individual Eastern and Central European countries, particularly the Czech Republic, Slovakia, Croatia, Estonia and Poland.

From the overall list of 345 PIMs, 145 (42.03%) were registered in the Czech Republic, 151 (43.77%) in Slovakia, 135 (39.13%) in Estonia, 126 (36.52%) in Croatia and 176 (51.01%) in Poland. The most specific criteria for Eastern and Central EU region was the EU-(7)-PIM list. Because PIMs enclosed in this list present maximally half of all available PIMs in analyzed countries, the application of solely these criteria is not adequate. According to our results, it is important to utilize PIM lists available in all until now published criteria in European countries.

In conclusion, the most specific criteria for Eastern and Central EU region were the latest published EU(7)-PIM criteria. In order to maintain higher level of objectivity and accuracy in future research in this region, it is important to use methodology that merge different EU tools together.[2,3]

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ANALYSIS OF DRUG-RELATED PROBLEMS POTENTIALLY LEADING TO HOSPITALIZATION

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Hospital admissions due to drug-related problems (DRPs) represent a relevant clinical issue with significant economic consequences.

The aim of this study was to determine the prevalence of DRPs potentially leading to hospitalization, to characterize these problems and to identify the most commonly implicated medication classes.

Over the period of six months we have evaluated 200 unplanned admissions to geriatric ward of the 3rd Department of Internal Medicine – Metabolic Care and Gerontology of University Hospital Hradec Králové in order to determine whether the hospitalization was drug-related. The DRPs were consequently classified according to PCNE classification.

The overall prevalence of hospital admissions related to DRP was 11.5%. The majority of DRPs (83%) were classified as an adverse drug event. Antithrombotics and diuretics were the most commonly implicated medication classes, followed by drugs acting on renin-angiotensin system. The associated outcomes were bleeding and electrolyte disturbances. Likely causes of the DRPs were insufficient monitoring, inappropriate combination of drugs, dose selection and patient-related causes.

The limitation worth mentioning is that certain aspects like adherence, self-medication, dietary and hydration habits were not assessed. Furthermore, relatively small sample size might have altered the results. Despite the limitations, this study reveals high-risk medication classes commonly associated with hospital admissions among geriatric patients.

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DRUG INFORMATION CENTRE SERVICE ANALYSIS I.

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This study deals with the analysis of enquiries of the Drug Information Centre (DIC) of Faculty of Pharmacy in Hradec Králové, Charles University, and University Hospital Hradec Králové. The DIC was established in 1994 and the activities are focused especially on responding to the drug-related enquiries from healthcare professionals. Enquiries from the period of 1994–2016 were included in this analysis. The data were collected from all recorded enquiries in the Excel database within 1994–2016. Data analysis firstly focused on the characteristics of the enquiries (quantity, healthcare professionals' characteristics such as region, position, enquiry type and urgency of the enquiries). The enquiry type included properties and content of the medicines, compatibilities, stability, extemporaneous preparation, pharmacokinetics, dosage, interaction, mechanism of action, adverse effects, indication/contraindication, administration, choice of alternative medication and availability on the market. ATC groups of the drugs in question were also assessed as defined by the WHO as well as the drug with the most frequent ATC code to the third level, which indicates pharmaceutical subgroup. Thereafter from this set, the most frequently occurring medicines defined by ATC code to the fifth level were selected, which represents the drug molecule. Descriptive statistics was employed in the analysis.

Altogether 2221 enquiries were gathered for this analysis between the years 1994 and 2016. The highest number per year was recorded in 2003 (201). The highest number of urgent enquiries appeared in 2001 (74), that represented 46% of all enquiries of that year. The most common overall type of enquiry answered were the adverse effects with 19.9% of all enquiries focused on this issue, followed by indication/contraindication (19.5%) and enquiries related to the properties of the medicinal substances (15.9%). Antithrombotic agents were the most common drug enquiries according to the ATC code specification. Overall, 111 enquiries were focused on this group. The most frequent molecule (the fifth level ATC analysis) represented warfarin (code B01AA03) which was enquired 41 times. Throughout the years, the number of enquiries answered by DIC decreased and the enquiry type has changed, the same trend was shown in ATC groups of medicines enquired.

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DRUG INFORMATION CENTRE SERVICE ANALYSIS II.

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Drug Information Centre (DIC) of the Faculty of Pharmacy in Hradec Králové (established in 1994) is the only university-based DIC in the Czech Republic. DIC is providing expert information about drugs, such as answering medicines-related enquiries received from healthcare professionals. The objective of this study was to gather and analyze information about activities of the DIC in 1994–2016. Detailed analysis of DIC activities was performed, in order to compile suitable data. All enquiries were summarized into a database using Microsoft Excel software. General characteristics of the enquiries were analyzed using the gathered information, such as an average number of enquiries per year or time needed for resolving one enquiry. Information about enquirers was considered as well, *e.g.* their profession and region, from which the enquiries were sent. Furthermore, the analysis was focused on professional drug information resources as well as their utilization for interaction-related enquiries and enquiries with a specific patient population. Data was analyzed using descriptive statistics. Analysis of DIC activity included 2221 enquires within years 1994–2016. The average number was 97 enquiries per year. The most enquiries were sent in year 2003 (201; 9.0%) and the least was in 2014 (22; 1.0%). The average time of processing 1 enquiry was 173 minutes. More than half (1117; 50.3%) enquiries were sent from pharmacists. Further, hospital physicians (364; 16.4%), scientists (182; 8.2%) outpatient physicians (99; 4.5%) and general practitioners (62; 2.8%) sent enquiries to the DIC. Enquiries were received most often from Královéhradecký region (904; 40.7%) and Prague (412; 18.5%). Enquiries without specific patient population occurred in 1663 (74.8%) cases. Enquiries focused on pregnancy and lactation occurred in 196 (8.8%), geriatrics in 140 (6.3%) and pediatrics in 134 (6.0%) cases. The most used information resources were Micromedex, that was used 1257 times (56.6%), AISLP used 1240 times

(55.8%), Medline used 954 times (43.0%) and Martindale used 652 times (29.4%). Information service of the DIC was used mostly by community pharmacists. In the analyzed period of time, wide range of discussed enquiries were resolved using multiple professional drug information resources, *e.g.* facto-graphic databases about the medicines.

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SELECTED MEDICINAL PRODUCTS

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The category of selected medicinal products (SMP) has been established by the Act No. 79/1997 Coll., on Pharmaceuticals. Medicines containing paracetamol, ibuprofen, moxastine teoclas, nicotine, activated charcoal or specific surface disinfectant, vitamins and herbal teas can be registered as SMP. SMP can be sold outside pharmacies by vendors of SMP, who obtained specialized qualification required by law. The number of vendors was constant in period between 1998–2011, but it started to grow exponentially in 2012 (from 150 vendors in 2011 to 2386 vendors in 2017). The financial volume of SMP market in 2017 was approximately 15.3 million CZK without markup and value added tax, while 75.9% share made SMP from ATC groups M01AE01 – ibuprofen, N02BE01 – paracetamol and N02BE51 – paracetamol, combinations excl. psycholeptics. The research was focused on time and local availability and financial affordability of SMP and provided information about usage of SMP recommended by vendor to treat acute tooth pain in 24-year-old male. The research was performed between 12 March 2018 and 16 March 2018 in regions: Chrudim, Hradec Králové, Pardubice and Rychnov nad Kněžnou. 50 vendors of SMP were selected from State Institute for Drug Control pharmacies database regarding proportional representation on the market in the Czech Republic. 14.0% of vendors did not sell SMP. The median of the distance between vendor of SMP and the nearest pharmacy was 526 meters. 97.6% of vendors had extended opening hours on Saturdays and Sundays (compared to 30.9% of pharmacies). No information or wrong information about usage of medicine was given by 42.5% of vendors, 32.5% of them made a reference to package leaflet. There were significant differences in SMP prices (*e.g.* from 33.00 CZK up to 69.90 CZK for Ibalgin 200, 12 tbl) depending on type of shop, location *etc*. The results show that the existence of this category shortens time and improves local availability, but prices of SMP are higher than those in pharmacies. Further research should be done including safety profiles of SMP concerning adverse effects and statistic of misuse in population.

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KNOWLEDGE AND ATTITUDES REGARDING CERVICAL CANCER AND HUMAN PAPILLOMAVIRUS VACCINATION IN CZECH SECONDARY SCHOOL STUDENTS

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Cervical cancer is the second most common type of cancer in women. The majority of cervical cancer cases are caused by human papillomavirus (HPV). Three vaccines have been approved to prevent HPV infection and related diseases. The study aimed to analyse the knowledge and attitudes regarding HPV, cervical cancer and HPV vaccination in secondary school students in the Czech Republic.

The study was carried out in May 2017 at four secondary schools. A questionnaire used for data collection comprised 15 items including questions on demographic characteristics, knowledge about cervical cancer and HPV, vaccination coverage, HPV perceived susceptibility and seriousness, and beliefs on HVP vaccination.

A total of 667 students participated (mean age: 16.8 y ± 1.18; 63% female); 20.5% were smokers, 24.7% God believers. Most of the students (97.0%) heard about cervical cancer and penile cancer, significant proportion (68.3%) knew HPV was the causative factor. Half of the respondents (49.9%) have heard about vaccination against HPV. Among cervical cancer risk factors HPV infection was reported in 58.1% cases followed by promiscuity (14.5%), irregular gynaecological screening (6.8%), immunity disturbances (4.2%), having sexual intercourse at an early age (1.7%), 12.6% did not know. Only 37.8% had been vaccinated against HPV (female: 56.5%; male: 5.7%). The main reported reasons for not being vaccinated were concerns about vaccine safety, HPV vaccine cost, doubts on vaccine effectiveness, distrust of vaccines, the vaccine has not been offered. Women compared to men had more concerns about HPV infection and probably for this reason, women were more convinced of HPV vaccination importance.

Knowledge about HPV, cervical cancer/penile cancer and HPV vaccine may influence attitudes to HPV vaccination and are important predictors in HPV vaccination uptake. Making information widely available would help to make informed decisions and improve HPV vaccine acceptance.

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THE IMPACT OF ACUPUNCTURE ON HEART RATE VARIABILITY IN PATIENTS WITH MIGRAINE

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Migraine is one of the most common disease which affect human beings. The popularity of an alternative therapeutic method is growing, in our case especially acupuncture. I've decided to evaluate its effect on heart rate variability in patients with migraine diagnosed. Goal of this work is to evaluate if acupuncture has an effect on HRV in patients with migraine. Secondary goal is to find out which factors may influence HRV parametres changes. HRV parameters were assembled for 33 patients participating in this study. Measurement was performed before acupuncture and after 12 weeks of acupuncture therapy. At first, results were analysed in KUBIOS software which is specially designed to study heart rate variability and all needed parametres. And then statistical analysis was done by paired t-test in SPSS software. No significance diference between HRV value after and before acupuncture was found. The most significant factor suggested connection with higher parasympathetic activity. Even if results was not so significant, maybe because of small group of patients which we had at disposal, we found a slight improvement. There is a chance of using HRV measurement to detect an effect of acupuncture on patients.

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THE IMPACT OF STRESS ON HEART RATE VARIABILITY IN PHARMACY STUDENTS I

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Academic stress is the predominant stress in pharmacy students. Psychological and emotional stress is a risk factor for a variety of chronic diseases. The aim of the study was to assess the co-relation between perceived stress and heart-rate variability (HRV) parameters. A secondary aim was to assess the association between the stress level and HRV in both genders and healthy vs.chronically ill students.

This was a cross-sectional study using a validated survey (May, 2016), conducted at the Faculty of Pharmacy in Hradec Králové. 205 second year pharmacy students enrolled in the Health Care Psychology class participated volunteerly in the study.

Subjective stress was measured using the Perceived Stress Scale (PSS-14) questionnaire. Heart rate variability was recorded by heart rate variability biofeedback device two weeks before the examination period. Five and one-minute HRV measurements were

obtained at rest. The study was approved by the Ethical Committee of the Faculty of Pharmacy in Hradec Králové.

The mean score for the PSS-14 was computed for all of the items. Artefacts of HRV measurements were corrected using the Kubios software and the values were subsequently log-transformed. The correlation between psychological stress and HRV parameters was evaluated by Spearman's rank correlation coefficient. An independent T-test or Man-Whitney-U test was used for continuous variables depending on normal distribution. P-values < 0.05 were considered significant. All analyses were performed using the Statistical Package SPSS, v. 12.0 (SPSS®, SPSS Inc., Chicago, IL, 2006).

Of 84.4% sophomore students, 173 students took part in the survey (a response rate of 84.4%). Perceived stress scale (PSS-14) negatively correlated with lnLF (rho = −0.2, p = 0.01) and Coherence 1 (rho = −0.2, p = 0.039). The total average PSS-14 score was 19 (SD = 6.30). Students with poor self-reported health status experienced higher level of perceived stress (PSS-3) during last month (3.00, SD = 0.866) than did their healthy peers (2.72, SD = 0.852; p = 0.041). Students with poor self-reported health status also reported lower values of lnLF (6.6, SD = 1.174) and lnHF (6.7, SD = 1.255) than did their healthy peers (lnLF: 7.0, SD = 1.170; lnHF: 7.1, SD = 1.139) (p = 0.025 and p = 0.029), respectively. There was no difference in the total average PSS-14 score by gender. Yet, Coherence 1 was higher in women (79.9, SD = 13.5) compared to men (73.7, SD = 4) (p = 0.039).

The study showed a weak correlation between perceived stress and heart rate variability variables. Nevertheless, students with poor self-reported health felt higher level of stress than their healthy peers with a measureable negative impact on their physical health. A repeated measurement of HRV and the level of perceived stress of sophomore students after two year of their study at the Faculty of Pharmacy would be desirable to see how students have been managing coping with stress overtime.

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ARTICLES AND PROCEEDINGS

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MALÝ J., LÁDOVÁ, K. (eds.): XVIII. sympozium klinické farmacie René Macha (XVIII. Symposium on Clinical Pharmacy René Mach). Hradec Králové, Farmaceutická fakulta, 2016, 78 pp. ISBN 978-80-906644-0-1

BERÁNEK, M.: Molekulární genetika pro bioanalytiky (Molecular genetics for bioanalysts). Praha, Karolinum, 2016, 194 pp. ISBN 978-80-246-3224-7.

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PATENTS

DOLEŽAL, M., ZÍTKO, J., JANĎOUREK, O., SERVUSOVÁ-VAŇÁSKOVÁ, B.: Substituovaný 2-(2-fenylhydrazinyl)pyrazin, způsob jeho přípravy, jeho použití a farmaceutický přípravek ho obsahující (Substituted 2-(2-phenylhydrazinyl)pyrazine, process of its preparation, its use and pharmaceutical composition containing thereof). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 306322 B6.

ĎOUBAL, S., KLEMERA, P., KUCHAŘOVÁ, M., REJCHRT, P.: Způsob a zařízení pro měření viskoelastických parametrů viskoelastických těles (Method of and apparatus for measuring viscoelastic parameters of viscoelastic bodies). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 306176 B6.

KARABANOVICH, G., ROH, J., HRABÁLEK, A., KLIMEŠOVÁ, V., PÁVEK, P.: Dinitrofenyloxadiazol nebo -triazol, jeho použití a farmaceutický přípravek ho obsahující (Dinitrophenyl oxadiazole or triazole, its use and a pharmaceutical preparation containing it). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 306408 B6.

NĚMEČEK, J., ROH, J., HRABÁLEK, A., KLIMEŠOVÁ, V., KARABANOVICH, G., PÁVEK, P.: Substituovaný dinitrofenyltetrazol, jeho použití a farmaceutický přípravek ho obsahující (Substituted dinitrophenyl tetrazoles, their use and pharmaceutical composition containing thereof). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 306321 B6.

ROH, J., NĚMEČEK, J., HRABÁLEK, A., KLIMEŠOVÁ, V., KARABANOVICH, G., PÁVEK, P., SYCHRA, P.: Substituovaný fenyltetrazol, jeho použití a farmaceutický přípravek ho obsahující (Substituted phenyltetrazole, its use and pharmaceutical composition containing thereof). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 306245 B6.

VINŠOVÁ, J., KRÁTKÝ, M., PARASKEVOPOULOS, G.: Substituovaný derivát kyslíkatých kyselin fosforu, jeho použití a farmaceutický přípravek ho obsahující (Substituted derivative of phosphorus oxyacids, use thereof and pharmaceutical composition containing it). Praha, Úřad průmyslového vlastnictví, 2016. Patent No. CZ 305738 B6.

DEGREES

Lectures for the Professorship Appointments, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2016

Doc. Ing. BARBORA SZOTÁKOVÁ, Ph.D.: Associate Professor, Department of Biochemical Sciences, Faculty of Pharmacy, Hradec Králové.
Discipline: Biochemistry, MŠMT 24 295/2007-30/1
Inauguration: 24. 11. 2014
Continuation: 9. 12. 2014
Title of Lecture: Metabolismus anthelmintik (Metabolism of anthelmintic agents) 9. 6. 2015
Appointment: 17. 5. 2016

Doc. PharmDr. KATEŘINA VÁVROVÁ, Ph.D.: Associate Professor, Organic and Bioorganic Chemistry, Faculty of Pharmacy, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 22. 9. 2016
Continuation: 11. 10. 2016
Title of Lecture: Role ceramidů ve zdravé i nemocné kožní bariéře (Role of ceramides in healthy and diseased skin barrier) 13. 12. 2016
Appointment: 19. 6. 2017

Doc. PharmDr. TOMÁŠ ŠIMŮNEK, Ph.D.: Associate Professor, Department of Biochemical Sciences, Dean of the Faculty, Faculty of Pharmacy, Hradec Králové.
Discipline: Biochemistry, MŠMT 24 295/2007-30/1
Inauguration: 21. 11. 2016
Continuation: 13. 12. 2016
Title of Lecture: Perspektivy výzkumu toxických a protektivních účinků léčiv na kardiovaskulární systém (Perspectives of the research of toxic and protective effects of drugs on cardiovascular system) 14. 3. 2017
Appointment: 13. 12. 2017

Habilitation Theses and Lectures (Associated Professor), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2016

RNDr. LENKA KUJOVSKÁ KRČMOVÁ, Ph.D.: Senior Lecturer, Department of Analytical Chemistry, Faculty of Pharmacy, Hradec Králové.
Discipline: Analytical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 6. 9.2016
Continuation: 11. 10. 2016
Habilitation Thesis: Vývoj chromatografických metod pro klinický výzkum (Development of chromatographic methods for clinical research), defended 13. 12. 2016
Title of Lecture: Moderní trendy ve zpracování biologického materiálu v klinickém výzkumu (Modern trends in processing of biological material in clinical research) 13. 12. 2016
Appointment: 1. 2. 2017

PharmDr. JAROSLAV ROH, Ph.D.: Senior Lecturer, Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Hradec Králové.
Discipline: Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
Inauguration: 7. 9. 2017
Continuation: 10. 10. 2017
Habilitation Thesis: Příprava a studium antituberkuloticky účinných látek ze skupiny dusíkatých heterocyklů (Preparation and study of antituberculous agents from the class of nitrogen heterocycles), defended 12. 12. 2017
Title of Lecture: Nové strukturní typy antituberkulotik v preklinické a klinické fázi vývoje (Novel structural types of antituberculous drugs in preclinical and clinical phases of development) 12.12. 2017
Appointment: 1. 3. 2018

PharmDr. MARTINA ČEČKOVÁ, Ph.D.: Senior Lecturer, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Hradec Králové.
Discipline: Human and Veterinary Pharmacology, MŠMT 24 295/2007-30/1
Inauguration: 18. 5. 2017
Continuation: 13. 6. 2017
Habilitation Thesis: Role membránových transportérů ve farmakokinetice a mnohočetné lékové rezistenci (Role of membrane transporters in multi-drug resistence), defended 10. 10. 2017
Title of Lecture: Farmakokinetické lékové interakce zprostředkované transportéry (Pharmacokinetic drug interactions mediated by transporters) 10. 10. 2017
Appointment: 1. 12. 2017

Mgr. JARMILA ZBYTOVSKÁ, Dr. rer. nat.: Senior Lecturer, Department of Pharmaceutical Technology, Faculty of Pharmacy, Hradec Králové.
Discipline: Pharmaceutical Technology, MŠMT 29 593/2011-M3
Inauguration: 20. 2. 2017
Continuation: 14. 3. 2017
Habilitation Thesis: Kožní bariéra a možnosti jejího ovlivnění z farmaceutického pohledu (Skin barrier and possibilities of influencing it from the pharmaceutical point of view), defended 13. 6. 2017
Title of Lecture: Nanonosiče pro dermální a transdermální podání léčiv (Nanocarriers for dermal and transdermal administration of drugs) 13. 6. 2017
Appointment: 1. 10. 2017

Doctoral Dissertation Theses (Ph.D.), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2016

PharmDr. MARCELA ŠAFRATOVÁ, Ph.D.: Studium inhibičního (toxického) vlivu alkaloidů vybraných druhů rostlin z čeledi Amaryllidaceae na některé lidské enzymové systémy (*in vitro* studie) III (Study of inhibition (toxicity) activity of alkaloids from selected plant species of Amaryllidaceae family on human enzyme systems (in vitro study) III). 20. 12. 2016.
RNDr. JIŘÍ PLÍŠEK, Ph.D.: Využití separačních metod v klinickém výzkumu II (Using of separation methods in clinical research II). 10. 3. 2016.
RNDr. BARBORA HONEGROVÁ, Ph.D.: Využití moderních separačních metod pro klinické účely (Using of modern separation methods for clinical practice). 10. 3. 2016.
PharmDr. MARIE VOLKOVÁ, Ph.D.: Mechanismy membránového transportu radioaktivně značených receptorově specifických peptidů v ledvinách (Mechanisms of membrane transport of radiolabeled receptor-specific peptides in the kidney). 22. 1. 2016.
PharmDr. PETRA SVAČINOVÁ, Ph.D.: Vliv kluzných látek na viskoelastické parametry lisovacího procesu (Influence of lubricants on viscoelastic parameters of compaction process). 3. 2. 2016.
PharmDr. PAVEL ONDREJČEK, Ph.D.: Studium vlivu typu plniva a typu a koncentrace kluzných látek na parametry rovnice lisování (Evaluation of the influence of filler sort and lubricant type and concentration on the parameters of the compaction equation). 3. 2. 2016.
PharmDr. JIŘÍ MIKUŠEK, Ph.D.: Syntéza a využití vybraných dusíkatých heterocyklů (Synthesis and utilization of selected nitrogen heterocycles). 27. 9. 2016.
PharmDr. LUKÁŠ OPÁLKA, Ph.D.: Syntéza lidských ω-*O*-acylceramidů a hodnocení jejich vlivu na bariérové vlastnosti kožních lipidových membrán (Synthesis of human ω-*O*-acylceramides and evaluation of their effects on barrier properties of skin lipid membranes). 27. 9. 2016.
PharmDr. RUDOLF ANDRÝS, Ph.D.: Vývoj a aplikace afinitního nosiče pro izolaci lidských karbonyl-redukujících enzymů (Development and applications of affinity carrier for isolation of human carbonyl-reducing enzymes). 23. 3. 2016.
PharmDr. HANA JANSOVÁ, Ph.D.: Studium možností farmakologické ochrany srdečních buněk před oxidačním stresem a antracyklinovými cytostatiky (Study of potential pharmacological protection of cardiac cells against oxidative stress and antracycline anticancer drugs). 29. 6. 2016.
PharmDr. DANIELA ČÍHALOVÁ, Ph.D.: Interakce inhibitorů cyklin-dependentních kináz s ABC efluxními transportéry *in vitro*: vliv na mnohočetnou lékovou rezistenci v protinádorové terapii (Interactions of cyclin-dependent kinase inhibitors with ABC efflux transporters *in vitro*: Impact on multidrug resistance in cancer therapy). 12. 2. 2016.
PharmDr. BARBORA SERVUSOVÁ VAŇÁSKOVÁ, Ph.D.: Deriváty pyrazinkarboxylové kyseliny jako potenciální antituberkulotika. (příprava a studium biologických vlastností) (Derivatives of pyrazinecarboxylic acid as potential antituberculotics (synthesis and biological evaluation). 24. 11. 2016.
PharmDr. ZUZANA NEUMANOVÁ PTÁČKOVÁ, Ph.D.: Interakce antiretrovirotik s lékovými efluxními transportéry a jejich vliv na transplacentární farmakokinetiku (Interactions of antiretrovirals with drug efflux transporters and their role in the transplacental pharmacokinetics). 12. 2. 2016 .
PharmDr. ONDŘEJ JANĎOUREK, Ph.D.: Deriváty pyrazinu jako potenciální antituberkulotika (příprava a studium biologických vlastností) (Derivatives of pyrazine as potential antituberculars (preparation and study of biological properties). 24. 11. 2016.

Mgr. KATEŘINA JEŽKOVÁ BLAŽÍČKOVÁ, Ph.D.: Vztah tkáňového a solubilního endoglinu k endotelové dysfunkci a možnosti jejich ovlivnění. (Tissue and soluble endoglin relation to the endothelial dysfunction and possible treatment). 2. 12. 2016.

Mgr. MILOSLAV MACHÁČEK, Ph.D.: Studium nových fotosensitizérů ze skupiny ftalocyaninů a azaftalocyaninů pro fotodynamickou léčbu nádorových onemocnění (Study of novel phthalocyanine and azaphthalocyanine photosensitizers for the photodynamic therapy of cancer). 22. 11. 2016.

PharmDr. JAN KUBEŠ, Ph.D.: Transportní mechanismy sekundárních metabolitů přes membrány rostlinných buněk (Transport mechanisms of secondary metabolites across membranes of plant cells). 20. 12. 2016.

Rigorous Theses (PharmDr.) Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2016

Mgr. BABUŇKOVÁ, EVA PharmDr.: Imunofenotypizace malignit ze zralých B-buněk (Immunophenotyping of mature B-cell neoplasms). 18. 3. 2016.

Mgr. BINDER, JIŘÍ, Ph.D., PharmDr.: *In silico* studium interakcí cholinesteras s jejich modulátory a návrh nových látek tohoto typu (*In silico* studies of cholinesterases interactions with their modulators and design of new compounds of this type). 11. 3. 2016.

Mgr. BOUKALOVÁ, PETRA PharmDr.: Markery zánětu a proliferace v srdeční stěně transgenního modelu myší s vysokými hladinami solubilního endoglinu (Markers of inflammation and proliferation in the heart of a transgenic mouse model expressing high levels of soluble endoglin). 19. 2. 2016.

Mgr. COUFALOVÁ, IVA PharmDr: Stanovení lipofility potenciálních léčiv (Determination of lipophilicity of potential drugs). 16. 9. 2016.

Mgr. ČERMÁK, PAVEL PharmDr.: Příprava a fotofyzikální hodnocení tetra-3,4-pyridoporfyrazinů vhodných pro fotodynamickou terapii (Preparation and photophysical evaluation of tetra-3,4-pyridoporphyrazines suitable for the photodynamic therapy). 29. 11. 2016

Mgr. ČÍHALOVÁ, DANIELA, Ph.D., PharmDr.: Interakce inhibitorů cyklin-dependentních kináz s ABC efluxními transportéry *in vitro*: Vliv na mnohočetnou lékovou rezistenci v protinádorové terapii (Interactions of cyclin-dependent kinase inhibitors with ABC efflux transporters *in vitro*: Impact on multidrug resistance in cancer therapy). 16. 9. 2016.

Mgr. DĚDKOVÁ, TEREZA PharmDr. Navození rezistence hlístic na albendazol (Development of nematodes resistance to albendazole). 17. 6. 2016

Mgr. DRASTÍKOVÁ, MONIKA Ph.D., PharmDr.: Molekulárně biologická vyšetření somatostatinových receptorů v diagnostice hypofyzárních nádorů (Molecular biology investigation of somatostatin receptors in diagnostics of pituitary tumors). 18. 3. 2016.

Mgr. DUBECKÁ, MICHAELA PharmDr.: Studium interakcí přírodních látek polyfenolické povahy s vybranými nukleárními receptory (Study of interactions of polyfenolic compounds on nuclear receptors). 19. 2. 2016.

Mgr. FOJTÍKOVÁ, VERONIKA PharmDr. Nanovlákenné membrány jako nosiče léčiv 12 (Nanofibre membranes as drug carriers 12). 18. 10. 2016.

Mgr. HAVLOVÁ, IVANA PharmDr.: Studium vlivu subchronického podávání antiretrovirotika emtricitabinu na expresi efluxních lékových transportérů v orgánech matky a plodu (Study of effects of prolonged administration of emtricitabine on expression of ABC efflux transporters in maternal and fetal organs). 16. 9. 2016.

Mgr. HONEGROVÁ, BARBORA, Ph.D., PharmDr.:Využití moderních separačních metod pro klinické účely (Using of modern separation methods for clinical practice). 7. 4. 2016.

Mgr. HORÁKOVÁ, JANA PharmDr.: Nanofibrous vascular grafts (Nanofibrous vascular grafts). 3. 11. 2016.

Mgr. HORDĚJČUKOVÁ, ANEŽKA PharmDr.: Využití NMR spektroskopie při strukturní analýze látek izolovaných z *Berberis vulgaris* L. a *Narcissus poeticus* cv. Pink Parasol (Use of the NMR spectroscopy for stuctural analysis of the substances isolated from *Berberis vulgaris* L. and *Narcissus poeticus* cv. Pink Parasol). 25. 5. 2016.

Mgr. HORŇASOVÁ, VERONIKA PharmDr.: Měď-chelatační vlastnosti isoflavonoidů (Copper-chelating properties of isoflavonoids). 19. 2. 2016.

Mgr. HOŠŤÁLKOVÁ, ANNA, Ph.D., PharmDr.: Studium obsahových látek vybraných taxonů z řádu Laurales a Ranunculales s potenciálně neuroprotektivní aktivitou (Study of chemical constituents of taxons from order Laurales and Ranunculales with potential neuroprotective activity). 13. 1. 2016.

Mgr. HOVORKOVÁ, JANA PharmDr.: Přírodní látky a jejich biologická aktivita VII. Screening vybraných alkaloidních druhů rostlin na cholinesterasovou aktivitu (Natural compounds and their biological activity VII. Screening of selected alkaloidal plant species for cholinesterase activity). 20. 6. 2016.

Mgr. HRUBÁ, LENKA PharmDr.: Optimalizace metod pro stanovení kvantového výtěžku produkce singletového kyslíku a kvantového výtěžku fluorescence u derivátů azaftalocyaninů (Optimalization of methods for determination of singlet oxygen production and fluorescence emission of azaphthalocyanine derivatives). 29. 11. 2016.

Mgr. JANOŠCOVÁ, PETRA PharmDr.: Příprava derivátů fenylkarbamoylpyrazin-2-karboxylové kyseliny jako potenciálních antituberkulotik (Preparation of phenylcarbamoylpyrazine-2-carboxylic acid derivatives as potential antitubercular drugs). 20. 5. 2016

Mgr. JANOUTOVÁ, ALENA PharmDr.: Deriváty pyrazinu jako potenciální léčiva IV (Pyrazine derivatives as potential drugs IV). 11. 3. 2016.

Mgr. JANURA, MICHAL PharmDr.: Gene expression analysis of selected UDP-glucosyltransferases from *Haemonchus contortus* (Gene expression analysis of selected UDP-glucosyltransferases from *Haemonchus contortus*). 18. 3. 2016.

Mgr. KARASOVÁ, JANA PharmDr.: Studium ledvinných transportních mechanismů radioaktivně značených protilátek (Study of renal transport mechanisms of radiolabeled antibodies). 16. 9. 2016.

Mgr. KARAŠČÁK, ROMAN PharmDr.: Management osteoporózy na úrovni praktických lékařů (Osteoporosis management at general practitioners level). 16. 12. 2016.

Mgr. KASSEMOVÁ, DOMINIKA PharmDr.: Biologicky aktivní metabolity rostlin. 9. Alkaloidy *Fumaria officinalis* L. a jejich biologická aktivita (Biologically active metabolites of plants. 9. Alkaloids of *Fumaria officinalis* L. and their biological activity). 7. 4. 2016.

Mgr. KONEČNÁ, KLÁRA Ph.D., PharmDr.: Proteomová analýza secernovaných proteinů *Francisella tularensis* (Proteome analysis of secerned proteins of *Francisella tularensis*). 17. 6. 2016.

Mgr. KUBÍKOVÁ, KLÁRA Ph.D., PharmDr.: Vliv výživy na metabolismus kostí (The effect of nutrition on bone metabolism). 17. 6. 2016.

Mgr. KVAPILOVÁ, RADKA PharmDr.: Hodnocení biologické aktivity pomocí *Artemia salina* (Evaluation of biological activity using *Artemia salina*). 20. 6. 2016.

Mgr. LEBLOCHOVÁ, HANA PharmDr.: Příprava a testování biskvartérních pyridiniových reaktivátorů acetylcholinesterasy (Preparation and testing of bisquarternary pyridinium reactivators of acetylcholinesterase). 20. 5. 2016.

Mgr. LIPTÁKOVÁ, LUCIE PharmDr.: *In vitro* štúdium novo syntetizovaných potenciálne kardioprotektívnych liečiv (*In vitro* study of newly synthesized potential cardioprotective drugs). 17. 6. 2016.

Mgr. LUDVOVÁ, LUCIE PharmDr.: Vliv substituentů na bazicitu azomethinových dusíků ftalocyaninů (The effect of substitution on the basicity of azomethine nitrogens of phthalocyanines). 11. 3. 2016.

Mgr. MADĚRYČOVÁ, ZUZANA PharmDr.: Hodnocení aktivit krevních cholinesteráz ve středoevropské populaci (Rating of blood cholinesterase activities in the population of Central Europe). 16. 9. 2016.

Mgr. ANNA MÁLKOVÁ PharmDr.: Syntéza arylsulfanyl ftalocyaninů a jejich aza-analogů (Synthesis of arylsufanyl phthalocyanines and their aza-analogues). 29. 11. 2016.

Mgr. MALÝ, LUKÁŠ PharmDr.: Studium biologické aktivity alkaloidů izolovaných z *Fumaria officinalis* L. (Fumariaceae) II (Study of biological activity of alkaloids isolated from *Fumaria officinalis* L. (Fumariaceae) II). 7. 4. 2016.

Mgr. MÜLLNEROVÁ, VERONIKA PharmDr.: Příspěvek ke studiu degradace tenkých vrstev z alifatických polyesterů (Contribution to a study of thin layers degradation made from aliphatic polyesters). 15. 3. 2016.

Mgr. NEUWIRTHOVÁ, TEREZA PharmDr.: Polymerní systémy s acyklovirem k aplikaci na sliznice (Polymeric systems with aciclovir for mucosal application). 15. 3. 2016.

Mgr. NOVÁ, MARCELA PharmDr.: Výsledky průběžného sledování a kontroly parazitóz v chovu kozy domácí (Results of continuous monitoring and control infections of breeding goat). 16. 9. 2016.

Mgr. NOVÁK, ZDENĚK Ph.D., PharmDr.: Analýza struktury alkaloidů metodami multidimensionální NMR spektroskopie (Analysis of the structure of alkaloids using multidimensional NMR spectroscopy). 25. 5. 2016.

Mgr. NOVÁKOVÁ, KATEŘINA PharmDr.: Analýza lékových problémů v lůžkovém zdravotnickém zařízení I (Analysis of drug-related problems in ward health care facility I). 28. 4. 2016.

Mgr. ONDREJČEK, PAVEL Ph.D., PharmDr.: Studium vlivu typu plniva a typu a koncentrace kluzných látek na parametry rovnice lisování (Evaluation of the influence of filler sort and lubricant type and concentration on the parameters of the compaction equation). 11. 2. 2016.

Mgr. ONDŘEJÍČEK, ALEŠ PharmDr.: Syntéza a *in vitro* testování takrin-troloxových derivátů jako potenciálních inhibitorů acetylcholinesterasy (Synthesis and *in vitro* testing of tacrine-troloxic derivatives as potential inhibitors of acetylcholinesterase). 11. 3. 2016.

Mgr. PLÍŠEK, JIŘÍ Ph.D., PharmDr.: Využití separačních metod v klinickém výzkumu (Using of separation methods in clinical research). 7. 4. 2016.

Mgr. PTÁČKOVÁ, ZUZANA Ph.D., PharmDr.: Interakce antiretrovirotik s lékovými efluxními transportéry a jejich vliv na transplacentární farmakokinetiku (Interactions of antiretrovirals with drug efflux transporters and their role in the transplacental pharmacokinetics). 16. 9. 2016.

Mgr. RAMBOUSKOVÁ, LUCIA PharmDr: Ovlivnění kontrakce močového měchýře reaktivátory acetylcholinesterasy (The effect of acetylcholinesterase reactivators on urinary bladder contraction). 16. 9. 2016.

Mgr. REJŠKOVÁ, LENKA PharmDr.: Biotransformační enzymy a metabolismus anthelmintik u *Fascioloides magna* (Biotransformation enzymes and metabolism of anthelmintics in *Fascioloides magna*). 17. 6. 2016.

Mgr. RICHTEROVÁ, LENKA PharmDr.: Chalkony a jejich analogy jako potenciální léčiva X (Chalcones and their analogues as potential drugs X). 11. 3. 2016.

Mgr. ŘÍHA, MICHAL Ph.D., PharmDr.: Screening nových látek chelatujících železo/měď – *in vivo* a *in vitro* studie (Screening of new iron- and copper-chelating substances – *in vivo* and *in vitro* studies). 19. 2. 2016.

Mgr. SIEGLOVÁ, KATEŘINA PharmDr.: Úloha lidských papilomavirů v etiopatogenezi zhoubných nádorů sinonazální krajiny (The role of human papilloma viruses in ethiopathogenesis of malignant tumors in sinonasal area). 18. 3. 2016.

Mgr. SOKOLOVÁ, SIMONA PharmDr.: Charakterizácia ľudskej warfarin reduktázy (Characterization of human warfarin reductase). 17. 6. 2016.

Mgr. SRBOVÁ, ALENA PharmDr.: Studium nového směsného suchého pojiva obsahujícího laktosu, mikrokrystalickou celulosu a kukuřičný škrob (A study of a novel dry binder composed of α-lactose monohydrate, microcrystalline cellulose and corn starch). 18. 10. 2016.

Mgr. STUCHLÍKOVÁ, LUCIE Ph.D., PharmDr.: Metabolismus a účinky nových anthelmintik u helmintů a jejich hostitelů (Metabolism and effects of new anthelmintic drugs in helmints and its hosts). 18. 3. 2016.

Mgr. SVAČINOVÁ, PETRA Ph.D., PharmDr.: Vliv kluzných látek na viskoelastické parametry lisovacího procesu (Influence of lubricants on viscoelastic parameters of compaction proces). 11. 2. 2016.

Mgr. SWIERKOSZOVÁ, MARTINA PharmDr.: Nanovlákenné membrány jako nosiče léčiv 11 (Nanofibre membranes as drug carriers 11). 18.10. 2016.

Mgr. ŠTOLCOVÁ, TEREZA PharmDr.: Vliv monepantelu na expresi biotransformačních enzymů ovce (Effect of monepantel on expression of biotransformation enzymes in sheep). 18. 3. 2016.

Mgr. TROJANOVÁ, ELIŠKA PharmDr.: Interakce vybraných přírodních látek s farnesoidním X receptorem (Interaction of chosen natural compounds with farnesoid X receptor). 16. 9. 2016.

Mgr. TYSOŇOVÁ, KRISTÝNA PharmDr.: Exprese proteinů TGF-β signalizace v aortě transgenních myší s vysokými hladinami solubilního endoglinu (Expression of TGF-β signaling proteins in aorta of transgenic mice expressing high levels of soluble endoglin). 16. 9. 2016.

Mgr. VALENTOVÁ, GABRIELA PharmDr.: Utilizace nutričních substrátů u kriticky nemocných pacientů na ventilátoru (Utilisation of nutrinional substrates in mechanically ventilated critically ill patients). 19. 2. 2016.

Mgr. VANĚČEK, VÁCLAV Ph.D., PharmDr.: Nanotechnologie a biomateriály pro využití v buněčné terapii míšního poranění (Nanotechnology and biomaterials for application in cell therapy of spinal cord injury). 19. 2. 2016.

Mgr. VÁŠKOVÁ, LUCIE PharmDr: Změny genové a proteinové exprese vybraných enzymů u myší vlivem brusinkových antioxidantů a obezity (Changes of gene and protein expression of selected enzymes in mice due to cranberry antioxidants and obesity). 18. 3. 2016.

Mgr. VAŠKŮ, TEREZA PharmDr.: Comparison of radiolabelled fatty acid (18F-FTHA) and 18F-FDG in imaging of brown adipose tissue (Comparison of radiolabelled fatty acid (18F-FTHA) and 18F-FDG in imaging of brown adipose tissue). 16. 9. 2016.

Mgr. VIRTOVÁ, BLANKA PharmDr.: Průběh a výsledky kultivace modelového parazita motolice jaterní (*Fasciola hepatica*) v ovci domácí (*Ovis aries*) pro potřebu biotransformačních studií (The progression and results of culturing the model parasite liver fluke (*Fasciola hepatica*) in sheep (*Ovis aries*) for the use of biotransformation studies). 16. 9. 2016.

Mgr. VRÁTNÁ, SANDRA PharmDr.: Porovnání účinku látek (VN 004 a VN 009) ze skupiny derivátů vasicinu na modelu izolované průdušnice (Comparison of the effects of substances (VN 004 and VN 009) from derivatives of vasicin on model of isolated trachea). 16. 9. 2016.

Mgr. VRBATA, PETR Ph.D., PharmDr.: Nanovlákenné membrány jako nosiče léčiv (Nanofibrous membranes as drug delivery systems). 21. 10. 2016.

Mgr. ZÁVODSKÁ, GABRIELA PharmDr.: Procedura referralu jako nástroj k ovlinění stavu registrace léčivého přípravku v EU (The referral procedure as a tool for influence the regulatory status of medicinal product in EU). 26. 2. 2016.

SOCIAL HAPPENINGS

IN MEMORY OF Prof. ZDENĚK FENDRICH, M.D., Ph.D.

On 6th January 2018, the Star of Bethlehem took with her to the eternal calm Prof. Zdeněk Fendrich, M.D., Ph.D., a long-term colleague and head of the Department of Pharmacology and Toxicology of the Faculty of Pharmacy of Charles University in Hradec Králové.

Zdeněk, as all his friends addressed him and thanks to his helpful and friendly nature, there were many, was born on September 16, 1942 in the foothills of the Orlické hory in Rychnov nad Kněžnou. In the foothills of these mountains, however in Letohrad (formerly Kyšperk), he and his seven siblings spent his childhood and youth. He graduated from the Grammar School in nearby Žamberk and then successfully graduated from the Medical Faculty of Charles University in Hradec Králové in 1965. After a one-year military service he joined the Institute of Pharmacology of the aforementioned faculty, led by an excellent teacher and scientist Prof. Vojtěch Grossmann, M.D. He dealt with the transport of drugs both *in situ* and *in vitro* from the gastrointestinal tract. With the establishment of the Faculty of Pharmacy of Charles University in Hradec Králové in 1969, under the leadership of its first dean Prof. RNDr. Jaroslav Květina, DrSc., dr. h. c., Zdeněk Fendrich joined the Department of Pharmacology and Toxicology, where he defended his dissertation in 1974. Thanks to his excellent language skills, he stayed for a long time abroad: 1977–78 at the Department of Pharmacology of the University of Tampere (Finland), 1983–87 at the Department of Pharmacology and Clinical Pharmacy of Ahmad Bello University in Zaria (Nigeria) and finally in 1989 at the Department of Obstetrics and Gynecology in Bern (Switzerland). In 1995, he received a professorship in Human and Veterinary Pharmacology at Charles University. From 1992 to 2007, he was the head of the Department of Pharmacology and Toxicology at the Faculty of Pharmacy in Hradec Králové. He had never abandoned the field of pharmacokinetics, namely the transport systems. He developed techniques to study transport across the placental barrier *in situ* in rat and human cotyledon perfusion *in vitro*. In this area, a part of his department

is still working today. He always supported and encouraged his colleagues, not only in the field of research mentioned above. His cheerful character and unusual narrative skills, even when he presented seemingly trivial events, made him a very popular companion and a leading personality in the working team as well as in professional or social events.

Prof. Zdeněk Fendrich, M.D., Ph.D. is the author or co-author of a number of monographs, textbooks, original and review articles. He actively participated in more than 60 international conferences and was a member of a number of national and international societies, such as the International Federation of Placenta Association.

Dear Zdeněk, rest in peace.

On behalf of colleagues of the Department of Pharmacology and Toxicology
Radomír Hrdina

IN REMEMBERANCE OF
Assoc. Prof. RNDr. PhMr. LIBUŠE KOPÁČOVÁ, CSc.

Assoc. Prof. RNDr. PhMr. Libuše Kopáčová, CSc., a longtime employee of the Faculty of Pharmacy of Charles University in Hradec Králové, passed away on 15th March 2018.

She was born on 17th January 1931 in Prague. She graduated from the Faculty of Pharmacy of Masaryk University in Brno in 1953. At the same faculty, she defended her dissertation in 1960 and began teaching in the Department of Biology and later in the Department of Pharmacology. After the merge of the Faculties of Pharmacy in Brno and Bratislava (1960), Libuše Kopáčová started teaching and working at the Department of Pharmacodynamics and Toxicology of the Faculty of Pharmacy of Comenius University in Bratislava. When the Faculty of Pharmacy of Charles University was established in Hradec Králové in 1969, she became an Assistant Professor at the Department of Pharmacological Propedeutics, renamed to the Department of Biological and Medical Sciences in 1990. She stayed at this department until her retirement. In 1983, she was appointed Associate Professor in Pharmacology. From 1971 to 1976 and then from 1990 to 1994, she was the head of department. She devoted her entire life to science and teaching in the field of human pharmacology and physiology, specifically focused on the cardiovascular and nervous systems. In the last period of her scientific work, she investigated the heart muscle contractility, especially the effects of cardiac glycosides on isolated guinea pig hearts. As the head of the Human Physiology Workgroup she always sought to develop the discipline. Assoc. Prof. PharmDr. Miloslav Hronek, Ph.D., who is now the head of the research workgroup Clinical Physiology of Nutrition and Metabolism and concurrently the deputy head of the Department of Biological and Medical Sciences, arose also from her working group. Even after retirement of Libuše Kopáčová, the studies of the cardiovascular system continue, albeit in a different form – the research of atherosclerosis. These studies are led by Prof. PharmDr. Petr Nachtigal, Ph.D., the current head of the department and a vice-dean of the Faculty of Pharmacy of Charles University. Assoc. Prof. Kopáčová lectured not only in the subject Human Morphology and Physiology, but also in the subject of Pathology. She was the tutor of a many diploma and postgraduate students. For many years, she was a member of professional societies with the annual participation in scientific symposia with many scientific contributions. She published about 30 scientific papers and is the author of the textbooks Pharmacology for Pharmacist I., Pharmacology for Pharmacist II. and Nervous System:

Textbook for Students of Pharmacy. She was awarded the Medal of PhMr. V. J. Žuffa of the Slovak Pharmaceutical Society.

Throughout her life, she stood beside her husband, a veterinarian, until he died of a serious illness. Together they raised a son, a medical doctor. Libuše Kopáčová enjoyed many happy moments with her two granddaughters, but also in her garden at the family house in New Hradec Králové neighbourhood.

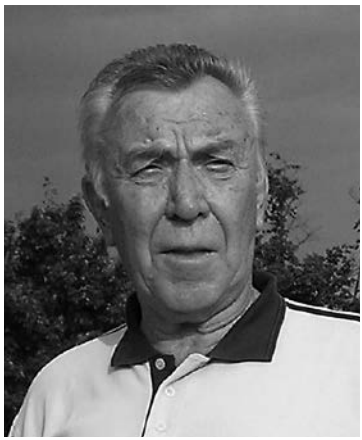
Libuše Kopáčová was a kind and wise woman who worked hard throughout her life in all the areas she was focused on. Even after her retirement in 1996, she worked part-time until 2002. She helped to fulfil the pedagogical and scientific activities of the department. After leaving the department, she kept in touch with the current members of the department, was interested in progress at the department and new matters coming with the development of the entire faculty. She was an enthusiastic admirer of the new University Campus building and was looking forward to the completion of other buildings.

With her passing away, a precious woman left us, leaving behind indelible traces, not only among her colleagues, but especially in hundreds of students whom she was a knowledgeable expert and kind guide.

Honour to her memory.

Z. Kudláčková and M. Kučerová

Assoc. Prof. RNDr. JIŘÍ HARTL, CSc. IS EIGHTY



Jiří Hartl was born on 26th March 1938 in Luleč. In the period 1953–57, he was a student of the Technical School of Chemistry in Brno, and after the leaving examination he started his pharmaceutical studies at the Faculty of Pharmacy in Brno. In 1960, the pharmaceutical education was re-organized in the then Czechoslovakia, the faculty in Brno was closed, and Jiří Hartl had to complete his studies at the Faculty of Pharmacy of Comenius University in Bratislava, Slovakia. He graduated in 1962 and became a junior lecturer in the Department of Pharmaceutical Chemistry of the faculty. He passed the examinations for the degree of Doctor of Natural Sciences (RNDr.) in 1966. Five years later, he moved to the newly established Faculty of Pharmacy of Charles University in Hradec Králové and started working in the Department of Pharmaceutical Chemistry. Within the course of Pharmaceutical Chemistry he was first specialized in drug synthesis, later he started to teach general and systematic medicinal chemistry. In 1973, he defended his dissertation entitled "Studies of 4,4-diaryl-2,3-dihalogenoisocrotonic acids with antineoplastic activity" and the scientific degree CSc. (equivalent to PhD.), was conferred on him. He was appointed an Associate Professor in Pharmaceutical Chemistry in 1985 and was the Head of the Department of Pharmaceutical Chemistry and Drug Control from 1990 to 2001. He was a member of the Scientific Board of the Faculty of Pharmacy in Hradec Králové, a member of the Board for Postgraduate Studies in Pharmaceutical Chemistry. For many years he was a Vice-Chairman of the Czech Pharmaceutical Society, the Chairman of its Synthetic Drugs Section and one of organizers of the annual conference Synthesis and Analysis of Drugs. He was also an active member of the Pharmacopoeia Committee of Ministry of Health. After his retirement Jiří Hartl continued his educational activities as a part-time staff member for many years.

The alumni and students know him as a lecturer and a fair examiner of the subjects Drug Synthesis (later named Technology of Synthetic Drugs) and Pharmaceutical Chemistry. His colleagues respect him as a friendly, responsible and reliable gentleman.

Dear Assoc. Prof. Hartl, allow us, on behalf of us and all our colleagues from the Department as well as all members of the Faculty to wish you on the occasion of your birthday good health, joy of life and contentment in the circle of your family.

M. Doležal and V. Opletalová

IN MEMORIAM OF PhDr. BĚLA ZAHRADNÍČKOVÁ

PhDr. Běla Zahradníčková, the retired long-time lecturer and later Head of the Department of Foreign Languages of the Charles University, Faculty of Pharmacy in Hradec Králové, passed away peacefully on 31 July, 2018. She was born as Běluše Jahodová on 1 November, 1929, in Ponětovice near the City of Brno, but she spent most of her childhood and early school years in the town of Vsetín, situated in the heart of scenic Moravian mountains. Her father worked as a design engineer in machine-building plants and her mother took care of him and their two daughters. During her school years the family moved to Brno, where she completed her grammar school attendance in 1949. Then she enrolled at the Masaryk University, Faculty of Arts in Brno, specializing in Czech and Russian Philology, graduating in 1953. Already as an undergraduate, she taught Russian at the Military Academy at Vyškov and after graduation she became a lecturer at the Departments of Languages at the Faculty of Arts and the Faculty of Medicine, Masaryk University. Beginning already in 1956, language teaching to pharmaceutical undergraduate and post-graduate students and writing textbooks for them was her main professional field. In Brno she married RNDr. Milan Zahradníček (1922–2009), later Associate Professor of Pharmaceutical Chemistry. When the Faculty of Pharmacy was established within Comenius University in Bratislava, Slovakia, in 1960, both of them moved there. She lectured at the Department of Foreign Languages, finally as Head of it. In 1962, she as a part-time student graduated in German Studies and started teaching also German. In 1971 she and her husband moved to the town of Hradec Králové, where the second Faculty of Pharmacy in the country was established within Charles University, Prague, in 1969. Here she became Head of the Department of Foreign Languages and taught German and Russian until her retirement in 2005.

Her publications primarily focused on two fields – writing textbooks and dictionaries and translating into German or Russian pharmaceutical texts of the researchers of the Faculty of Pharmacy, *Lingua ancilla pharmaciae* being her favourite saying. She has published several versions of textbooks and dictionaries for pharmaceutical undergraduates and other students, some of them in cooperation with colleagues. Her translations were published in Czech and Slovak as well as foreign journals, *e.g.* Československá farmacie, Farmaceutický obzor, Folia Pharmaceutica Universitatis Carolinae, Die Pharmazie, *etc*. She also translated other texts for the Kruh publishing house and other publishers.

Her experience and research in this field resulted in the dissertation *Adjectivized Active Participles in Russian Pharmaceutical Terminology*, which earned her the Philosophiae Doctor (PhDr.) degree at Masaryk University in Brno in 1977. The list of her publications accompanies the below-listed article published on the occasion of her sixtieth birthday.[1]

The main sphere of her activities, however, was teaching, in which she made full use of her linguistic and didactic abilities and skills. She also proved to be a good manager in establishing and managing the language departments in Bratislava and Hradec Králové. Her sense of exactitude and precision were exemplary. The members of the departments as well as students also appreciated her tactful leadership and friendly approach. She was always ready to help and give advice.[2]

Her teaching, scholarship and management have been awarded with several diplomas of honour and medals of the Faculty of Pharmacy and Charles University, but what is more important, she will remain in the memories of all who knew her.

Prof. PhDr. Bohuslav Mánek, CSc.

1. Mánek, Bohuslav: K životnímu jubileu PhDr. Běluše Zahradníčkové [On the Life Anniversary of PhDr. Běluše Zahradníčková]. Folia Pharm. Univ. Carol. XIV, 1989, 141–147 (accompanied with a bibliography of her publications).
2. Kunešová, Květa: Habent sua fata homines … (PhDr. Běla Zahradníčková celebrates her 80th birthday). Folia Pharm. Univ. Carol. XXXVIII, 2010, 105–106.

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17. Zahradníčková Běla: Textová učebnice jazyka německého pro farmaceuty [German for Pharmacists]. Praha, Karolinum, 1992 and 1996.
18. Holštejnová, Olga, Zahradníčková Běla: Textová učebnice ruštiny pro farmaceuty. Část 1. Odborné texty. [Russian for Pharmacists. Part I. Pharmaceutical Texts.]. Praha, Karolinum, 1993.
19. Zahradníčková Běla: Textová učebnice němčiny pro studenty Farmaceutické fakulty. 1. část. Odborné texty. [German for Pharmacy Undergraduates. Part I. Pharmaceutical Texts]. Praha, Karolinum, 2002 and 2005.
20. Zahradníčková Běla: Textová učebnice němčiny pro studenty Farmaceutické fakulty. 2. část. Slovník. [German for Pharmacy Undergraduates. Part II. Dictionary]. Praha, Karolinum, 2003 and 2005.
21. Numerous translations of pharmaceutical texts into German and Russian for various monographs and journals.

Prof. RNDr. PhMr. JAN SOLICH, CSc., Dr.h.c. DECEASED

Almost half a year after the celebration of his 90 years, prof. RNDr. PhMr. Jan Solich, CSc., Dr.h.c., one of founders of social pharmacy as a topic for research and teaching program for pharmacists at Charles University and promoter of clinical pharmacy in health care society, passed away on 2nd September 2018.

Jan Solich was born on 11th February 1928 in Nový Bohumín. After completing his studies at the classical grammar school and tirocinal (apprentice) practice in a pharmacy in Ostrava, he studied pharmacy at Masaryk University in Brno (1949–1951). As there was no faculty of pharmacy till 1951, future pharmacists were educated at the Faculty of Natural Sciences and were awarded PhMr. (pharmaciae magister) degree upon graduation.

In 1952, the Faculty of Pharmacy at Masaryk University Brno was established, and prof. Solich decided to expand and consolidate his knowledge in the newly opened pharmaceutical course of study. At the same time, he started his professional career as a lecturer at the Department of Galenic Pharmacy of this faculty. In the period 1955–1960, he worked as a senior lecturer at the Department of Pharmaceutical Practice. Simultaneously, he was enrolled into the science education in the field of Organization and Management of Healthcare at the Faculty of Medicine of Masaryk University and was awarded the degree CSc. (candidatus scientiarum) in this scientific discipline in 1960. In the same year he had to move from Brno to Bratislava (Slovakia) since the Faculty of Pharmacy of Masaryk University was abolished.

In Bratislava, he continued his work at the Department of Pharmaceutical Service of the Faculty of Pharmacy of Comenius University till 1971. For nine years (1960–1969) he was also head of its detached workplace – the faculty dispensing pharmacy in Brno which served for practical training of last year students. During his employment in Bratislava he habilitated (1963) and was appointed an associate professor (1964). Above that, he

obtained RNDr. (rerum naturalium doctor) degree as an extension of master's degree according to new higher education act in 1966.

In 1969, a new Faculty of Pharmacy belonging to the Charles University was established in Hradec Králové. Jan Solich transferred to this faculty in 1971 and remained faithful to it till his retirement in 1993. He served as the head of the Department of Organization and Management of Pharmacy and a vice-dean of the Faculty of Pharmacy as well. He was a chairman of the committee for the defense of dissertations in the field of Science of Healthcare since 1976 and was appointed a professor in the field in 1979. Besides, he also chaired commissions for final state exams and rigorous examinations. In the period 1976–1993, he was a Chairman of the Editorial Committee of the faculty. After his retirement he continued his work as an external teacher and was appointed Professor Emeritus in 2008.

During his academic career, prof. Solich tutored approximately 250 diploma theses, 210 rigorous theses and 25 doctoral dissertations. He lectured and led seminars in several subjects, such as Dispensing Pharmacy, Organization of Healthcare, Social Pharmacy, Social and Health Policy and Health Education. For many years (1965–1998) he lectured also in postgraduate education of pharmacist and pharmaceutical technicians. Besides his professional lectures at conferences and congresses, numerous lectures for general public must be mentioned. He published many articles in daily newspapers and various magazines as well. As a visiting professor he had lectures at pharmaceutical faculties in Switzerland, Germany, Denmark, Hungary and Sweden. In 1988, he was appointed *doctor honoris causa* at the Semmelweis University in Budapest.

First scientific activities of prof. Solich concerned galenic pharmacy. He was engaged in studies of preparation of tinctures, infusions and extracts by means of whirling extraction. He performed some stability studies as well, and his results were used in the 2nd and 3rd Czechoslovak pharmacopoeias. Later, within the Organization and Management of Healthcare, he solved problems concerning the need for pharmacist and their professional status in healthcare. He also devoted himself to the system of pre- and post-gradual education of pharmacist and pharmaceutical technicians. His extensive study on the requirements of various health care sectors for pharmaceutical services was a basis for development of standards for assuring the health care by pharmaceutical care. He promoted a new status of hospital pharmacy, published studies concerning the importance of psychology in the work of the pharmacist, mutual relationships in a team of pharmacists, co-operation between the physician and the pharmacist and between the pharmacist and the patient. Moreover, he dealt with the preparation of medicaments in pharmacies, incompatibilities, stability of drug preparations, mistakes in prescription *etc*. A lot of studies on the need and consumption of medicines have emerged under his supervision. Studies of prescriptions of outpatient physicians and hospital doctors served for the development of rational therapy. In order to reduce drug abuse and non-compliance, prof. Solich elaborated methodology of health education as a part of pharmaceutical activities and promoted it in both professional circles and in public. All his life he emphasized that the pharmacy will have such a position in society, that pharmacists build with their expertise and irreplaceability. His bibliography was published on occasion of his 85th anniversary.[1]

Prof. Solich was always active beyond his academic duties, too. He was a member of the Czech Medical Association of J. E. Purkyně, the Czech Pharmaceutical Society and

the Society of Social Medicine and Healthcare Administration. In some periods he served as a committee member of these institutions. For the propagation of the Czechoslovak and Czech pharmacy he was named honorary member of some foreign scientific and professional societies (Slovakia, Poland, Hungary, Bulgaria and Germany). He was also a member of the Scientific Council and Pharmacopoeial Committee of Ministry of Health.

Besides, he was a member of editorial boards of journals Československá farmacie (Czechoslovak Pharmacy), Journal of Social Pharmacy (Stockholm) and Folia Pharmaceutica Universitatis Carolinae. The latter journal has been published since 1977 and prof. Solich was the editor of initial sixteen numbers. In the publishing house Avicenum, he was the chairman of the Committee for Pharmacy (1982–1990).

After his retiring, Prof. Solich was active as consulting and revising pharmacist of the Military Health Insurance Company in Hradec Králové (1995–2000). Later he was engaged in the Association of Pensioners of Czech Republic (member of central board and from 2002 to 2008 chairman of the association), Senior Council of Czech Republic (vice-chairman in the period 2005–2010), the Czech Healthcare Forum (member of the administrative council since 2009) and the Government Council for Older Persons and Population Ageing (member from 2006 to 2010). His regional and local activities are important as well. Due to his efforts the advisory service for pensioners has been available in Hradec Králové since 1992 (as a part of the Association of Pensioners of Czech Republic, municipal organization in Hradec Králové).

In 2006 he became the head of the newly established Counseling center for seniors in the Hradec Králové region and led it for more than 10 years. He also founded geronto-pharmaceutical advisory service with nationwide competence.

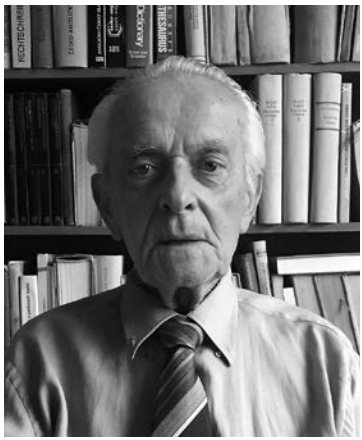
By passing away of prof. Solich, Czech pharmacy lost one of its outstanding personalities. Tribute to his memory.

On behalf of colleagues from the Department of Social and Clinical Pharmacy and other members of Faculty of Pharmacy in Hradec Králové

Jiří Vlček and Veronika Opletalová

1. SOLUTIO. Praha, Medon, 2013. Available from: http://www.medon-solutio.cz/online2013/index.php?linkID=txt28&lang=1.

JUBILEE OF Assoc. Prof. JAROSLAV SOVA



In October 2018, Assoc. Prof. RNDr. PhMr. Jaroslav Sova, CSc. celebrated his significant jubilee – 90th birthday. Assoc. Prof. Sova devoted his entire active life to Czech pharmaceutical university education. After graduating from Faculty of Pharmacy of Masaryk University in Brno, he took up a position at the Research Institute for Organic Synthesis in Pardubice-Rybitví, where he stayed only for one year. Then, he returned to the Faculty of Pharmacy in Brno as a postgraduate student and after the successful defense of his dissertation thesis and having obtained the degree CSc., he started his long-term academic career at the same faculty. In 1960, when the Faculty of Pharmacy in Brno merged with the Faculty of Pharmacy of Comenius University in Bratislava, he moved to Bratislava. The Faculty of Pharmacy of Charles University in Hradec Králové he joined in 1971, only one year after its establishment. He stood at the birth of the new pharmaceutical faculty and participated in building its laboratories and study programs. He worked for a total of 26 years at the Faculty of Pharmacy in Hradec Králové and during this time, he educated a number of pharmacists who can remember him as a hardworking and honest teacher. He gained a number of friends and supporters with his approach and attitude to his colleagues and students. His whole active life is connected to the Department of Inorganic and Organic Chemistry (today's Department of Organic and Bioorganic Chemistry), where he was the Head of the Subdepartment of General and Inorganic Chemistry for nearly 20 years. He taught courses in Organic Chemistry, Laboratory Techniques, and, above all, General and Inorganic Chemistry.

Assoc. Prof. Sova was an influential university teacher. He is a co-author of a few textbooks. General and Inorganic Chemistry, which was published in the co-operation with teachers from the Faculty of Pharmacy in Bratislava, the textbook of Organic Chemistry and the monograph Chemical Drugs, which became the basis of the concept of teaching pharmaceutical chemistry. He also contributed to the publication of the textbook Seminars

on General and Inorganic Chemistry. Assoc. Prof. Sova is the author of a number of scientific publications and articles in Czech and international journals.

Educational and scientific qualities of Assoc. Prof. Sova were officially recognized only after 1989. In the following year, he was awarded the academic title of Associate Professor. After November 1989, he actively participated in the executive of the faculty, where he held the post of the Vice-dean for Study Affairs for two three-year periods.

On behalf of all our colleagues at the Department, we wish Associate Professor Sova good health in the coming years, much joy of life and contentment in the close circle of his family.

Věra Klimešová and Karel Palát

A FEW WORDS IN MEMORY OF Assoc. Prof. MILAN ČELADNÍK, CSc.

At the beginning of October, we were surprised to hear that Associate Professor Milan Čeladník, one of the founding academics of the Faculty of Pharmacy in Hradec Králové, a long time Head of the Department of Inorganic and Organic Chemistry and, of course, an outstanding and respected teacher of the school, passed away.

I spent many years at the school with Professor Čeladník. First, I met him as a strict teacher, whom I and my fellow students held in high respect. He was my seminar teacher, and I should say that we used to prepare for his seminars most thoroughly. Yet he usually rebuked us that we could have been prepared better.

I have many more recollections of his style of teaching. Passing the exam when Mario, as his nickname at the school was, was the examiner, used to be considered a significant achievement. The rumour had it that a student who had passed the exam in Organic Chemistry could start considering himself or herself a graduate.

However, following the defence of my Diploma Thesis at his Department, I gradually learnt that Professor Čeladník was very strict and almost uncompromising on one hand, but also an extremely kind and nice person on the other. He was willing to do very much for his Department and mainly for his discipline, Organic Chemistry.

Departmental meetings were, without exception, held in a very friendly atmosphere, and we always heard something new, sometimes even juicy stories, from Professor Čeladník's youthful days at the Faculty of Pharmacy in Brno and then in Bratislava. From his stories we learnt that academic ground has always been not only a place where new knowledge originated, but also an environment in which lots of fun and interesting moments could be experienced. Needless to say that he tried to create and maintain this kind of atmosphere at our Department.

Associate Professor Milan Čeladník is the author of many educational texts, a textbook of Organic Chemistry for Pharmacists, and also one of the co-authors of the Bible of pharmaceutical chemists – Chemical Drugs by B. Melichar *et al.* He also started to explore pyridine-based antituberculotic agents and published many papers on this topic in scientific literature.

Tribute to his memory.

Alexandr Hrabálek

IMPORTANT JUBILEE OF Prof. RNDr. LUDĚK JAHODÁŘ, CSc.

Prof. RNDr. Luděk Jahodář, CSc. was born on the 17th of December 1948 in Budyně nad Ohří. He graduated from Secondary Medical School in Prague, in the field Pharmacy Technician. Since the secondary school he has been intensively interested in pharmacognosy, the science of substances of natural origin affecting human health. From this reason, he moved to Bratislava for seven years to study pharmacy at the Comenius University (1967–1972). In Bratislava was then the only Faculty of Pharmacy in the former Czechoslovakia. Hence, he had to study in Slovakia. He has also finished the doctorate in the natural sciences (RNDr.) there.

In Bratislava, he met his life partner, a classmate of the studies, and followed her back to eastern Bohemia. At that time, the Faculty of Pharmacy of Charles University in Hradec Králové was in building process, and in this institution Luděk Jahodář gradually realized his fifty years-career of university teacher starting from a Scientific Assistant, through the Associate Professor (1988, Bratislava) to the degree of a Professor of Pharmacognosy, that he obtained at the Charles University in Prague in 1995. From 1997 till 2014, he worked as a Head of Department of Pharmaceutical Botany and Ecology. He was the Dean of the Faculty of Pharmacy of the Charles University in the years 1994–1997. In this period, he played a major role in the development and the construction of a teaching building with greenhouses in the Garden of Medicinal Plants.

A significant part of his life was devoted to professional societies. For many years, he headed the Section of Natural Drugs of the Czech Pharmaceutical Society. For a long time, he worked in the committee of the society and in the time period 2003–2014, he presided the Czech Pharmaceutical Society. For many years, he has also been a member of the Czech Pharmacopoeia Commission and the Chairman of the Advisory Council for Medicinal Plants of the Ministry of Health of the Czech Republic.

Dealing with medicinal plants and their metabolites for more than 35 years, he studied chemical structure of natural compounds and their biological activity. His research subject included several dozen plant species, but the dominating position was occupied

by the taxa from the family of Ericaceae – *Arctostaphylos uva-ursi*, to which he devoted almost 20 years of his research. He lectured Pharmaceutical Botany, Phytotoxicology, Forensic Botany and selected chapters in Phytotherapy. He is the main author of a number of award-winning publications, such as Medicinal Plants in Current Medicine (2010), Pharmacobotany: Seed Plants (2006), Natural Toxins and Poisons (2004), Macroscopic and Microscopic Atlas of Drugs (1999) and Plants Causing Poisoning (2018). In the books Plants Causing Allergy and Poisoning (1989) and Sir, You Are Diabetic! (1997) he was a co-author. In the role of a postgraduates' supervisor, he has raised a number of experts in fields of Pharmacognosy and Toxicology of Natural Compounds. In addition, he is the author more than one hundred scientific papers, he gave nearly two hundred lectures, one third of them at international congresses. He is the author of several patents and many popular educational treatises. During his rich professional life, he was awarded a number of awards, *e.g.* the Golden Medal of Charles University, Medal of University of Veterinary and Pharmaceutical Sciences in Brno, Medal of G. J. Camel of Faculty of Pharmacy in Brno, Medal of the Faculty of Pharmacy of Comenius University in Bratislava, Weber price of Slovak Pharmaceutical Society, Price of J. V. Žuffa of Slovak Pharmaceutical Society, Silver Medal of Comenius University in Bratislava and Medal of Rudolf Skarnitzl of Czech Pharmaceutical Society. He is a honorary member of Slovak Pharmaceutical Society and in 2018 he was awarded the honorary title of an Emeritus Professor of the Charles University.

On behalf of the committee of Czech Pharmaceutical Society, colleagues of the Department of Pharmaceutical Botany, Department of Pharmacognosy and other members of the Faculty of Pharmacy we wish to Prof. RNDr. L. Jahodář, CSc. good health, well-being, plenty of time for hobbies and a lot of unceasing enthusiasm for the years to come.

J. Karlíčková, J. Spilková, and M. Doležal

Prof. RNDr. FRANTIŠEK BARTOŠ, DrSc. PASSED AWAY

Prof. RNDr. František Bartoš, DrSc., who worked at the Faculty of Pharmacy of Charles University for many years, died on 14th February 2019 at the age of 95 years.

František Bartoš was born on 21st December 1923 in Nový Bydžov. Later (1932) his family moved to Hradec Králové where he attended State Rašín Grammar School in the period 1935–1943. As he loved nature, he decided to study biology and geography at the Faculty of Science of Charles University (1945–1950). Already during his university studies, he worked as a research assistant at the Anthropological Institute of the university.

After graduation, F. Bartoš started his professional carrier as an assistant at the Department of Biology of the Military Medical Academy, which arose by the transformation of the Faculty of Medicine of Charles University in Hradec Králové in 1951. Seven years later, the academy was abolished, and F. Bartoš continued his work at the re-established Faculty of Medicine of Charles University in Hradec Králové. He devoted himself to studies of cell cultivation and behavior *in vitro* and started to deal with regeneration and healing processes as well. He was particularly interested in intercellular filamentous structures, especially collagen and elastin, and their changes during aging process. Wound healing was also topic of his candidate dissertation thesis, which he successfully defended and obtained CSc. (*candidatus scientiarum*) degree (1962). He became an Associate Professor in the field of Experimental Gerontology in 1967.

Prof. Bartoš became a member of newly established Faculty of Pharmacy of Charles University in Hradec Králové in 1971. He taught the subject General Biology and continued his research activities in the cooperation with the Faculty of Medicine in Hradec Králové and with some clinics of the Teaching Hospital in Hradec Králové. He published more than hundred scientific papers and gave numerous lectures for both scientific and public audience. In 1987, he defended his doctoral dissertation at the Faculty of Science of Charles University in Prague and was awarded the degree DrSc. (*doctor scienciarum*). He

was the head of Department of Pharmacological Propedeutics (nowadays Department of Biological and Medical Science) from 1976 to 1990 and retired in 1991. Due to his political nonconformity with socialist ideology and practice he could be named full Professor only after "Velvet Revolution" in 1993. Prof. Bartoš was a member of Czechoslovak Society of Gerontology and for many years he was also scientific secretary of Research Field Committee of Gerontology at the Ministry of Health.

Pedagogical skills of Prof. Bartoš must also be mentioned. "He passed the knowledge on his students and colleagues in an unforgettable and peculiar way. His lectures were very popular among students because they were full of humor, hyperbole and experience of research work. Professor Bartoš was demanding on himself as well as others. Fairness, honesty and constant humor are the characteristic features of this pedagogue who had the rare gift of passing knowledge on others."[1]

In addition to his scientific interests, Prof. Bartoš was interested in social affairs and culture and was an active painter. He painted for his own pleasure and his work was not publicly known for a long time. On occasion of the exhibition named *František Bartoš and his family in works of art*, he said: "Science is the adventurous discovery of what exists in the field of laws and objects. On the contrary, art creates something new, formerly nonexistent. It creates what attracted the creator as an observing percipient."[2]

Death of Prof. Bartoš means a big loss for his family and all who knew him. Tribute to his memory!

P. Nachtigal, V. Opletalová

1. Jílek, P.: Prof. František Bartoš, DrSc. Is Ninety-Three. Folia Pharm. Univ. Carol., XLVII, 2017, 156.
2. Soukup, J.: Výtvarné dílo profesora Bartoše (Artwork of Professor Bartoš). SCAN, 2007 (2), 23.

Assoc. Prof. MUDr. IVAN TILŠER, CSc. PASSED AWAY

Ivan Tilšer was born into a medical family in the year 1945. He spent his high school years in Mělník, which he always liked to recall. In the period 1963–1969, he studied medicine at the Faculty of Medicine of Charles University in Hradec Králové, and he had stayed in this town ever since. After graduation he devoted himself to research and pedagogical activities in pharmacology. At the beginning, he was included in the then scientific education, so called *aspirantura*, equivalent to today's postgraduate doctoral studies. Later he worked as an Assistant Professor at the Department of Pharmacology of the Faculty of Medicine of Charles University in Hradec Králové until 1996. In his research work he studied predominantly pharmacokinetics and toxicity of drugs on experimental animals and their isolated organs. The degree CSc. (*candidatus scientiarum*) was awarded to him based on the successful defence of his candidate dissertation thesis in 1981. During his employment at the Faculty of Medicine he obtained the attestation in Internal Medicine as well. For several months, he was engaged as a scholarship holder at the University of Sienna and as a research fellow in the Mario Negri Institute for Pharmacological Research in Milano. Simultaneously, he was a clinical pharmacologist in the Teaching Hospital in Hradec Králové for twelve years. He also pioneered the use of computer technology in clinical pharmacology.

In 1997, he became a pharmacologist at the Department of Pharmacology and Toxicology of the Faculty of Pharmacy of Charles University in Hradec Králové. He elaborated and defended his habilitation thesis and was appointed an Associate Professor in Pharmacology in 1999. He continued his research activities, lectured and led seminars till his retirement. As a pensioner, he continued to be involved in teaching. The results of his work were published in dozens of original papers, reviews and textbooks. He co-operated also with the Department of Social and Clinical Pharmacy, is a co-author of the book Clinical Pharmacy I and has been a member of the Board of the doctoral study in the field Clinical Pharmacy since 2011.

Due to some introversion, few people from his neighbourhood knew that Assoc. Prof. Ivan Tilšer had a wide social insight and knowledge in humanities in addition to his professional interests. His favoured activities included to travel and to explore the world with his own eyes. He liked the cross-country skiing and walking in the group of his close friends. He also participated in several high-mountain events in the Tatras, the Caucasus and the Altaji.

His life was characterized by modesty, conciliant behaviour and willingness to help. In private life, he was an exemplary husband and father. In a harmonious family, he and his wife brought up two children. Their son Jan is a physician and their daughter Hana is a psychologist and physiotherapist. In recent years, he has been particularly pleased with his five successfully growing grandchildren.

Assoc. Prof. Ivan Tilšer died on 29th March 2019 at the age of just accomplished 74 years. To the grief of his loved ones and his acquaintances he was torn out from the full life by a malignant disease within a few months.

Tribute to his memory.

Jaroslav Držata and Veronika Opletalová

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 by Charles University (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.

Journals

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

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