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# CONTENTS

# ABSTRACTS

# 7th POSTGRADUAL AND 5th POSTDOCTORAL SCIENTIFIC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ, CHARLES UNIVERSITY, HRADEC KRÁLOVÉ, 7–8 FEBRUARY 2017

## BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

### CHALCONES AND THEIR PYRAZINE ANALOGS AS POTENTIAL AGENTS IN PREVENTION OF LONG-TERM COMPLICATIONS OF DIABETES

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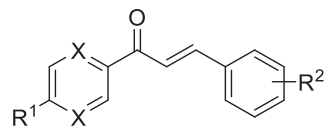
Long-term complications of diabetes have been associated with the distinct activation of polyol pathway enzyme – aldose reductase (AKR1B1) that is responsible for sorbitol production and its accumulation under hyperglycemic conditions which contributes to oxidative stress.[1]

Chalcones and their pyrazine analogs synthesized by Claisen-Schmidt condensation have been tested on inhibition of AKR1B1 isolated from rat eye lenses. The most active compounds exerted $IC_{50}$ within the range 19–40 μM and their interactions with the enzyme have been described in a molecular docking study. Antioxidant activity of some compound have been explored in DPPH assay. Chalcone derivatives did not reach the inhibition activity of reference drug epalrestat in the enzyme assay.




X = CH, N
$R^1$= H, $C_3H_7$, $CH(CH_3)_2$
$R^2$ = 2-OH; 3-OH; 4-OH; 3-$OCH_3$,4-OH; 4-$NO_2$; 4-$OCH_3$

*The study was supported by PRVOUK P40 (Charles University) and by the project SVV 260 291.*

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# A NEW CLASS OF CONSTITUTIVE ANDROSTANE RECEPTOR AGONISTS

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Constitutive androstane receptor (CAR), together with pregnane X receptor (PXR) and aryl hydrocarbon receptor (AHR), are ligand-activated transcription factors that play pivotal roles in xenobiotic clearance. These transcription factors control expression of target genes responsible for synthesis of key phase I and phase II drug metabolizing enzymes and some drug transporters. Currently, there is no specific high-affinity agonist for human CAR. Known to date is CITCO (**1**, Fig. 1), which is a potent human, but not mouse, CAR agonist. However, this unstable compound also activates PXR[1].

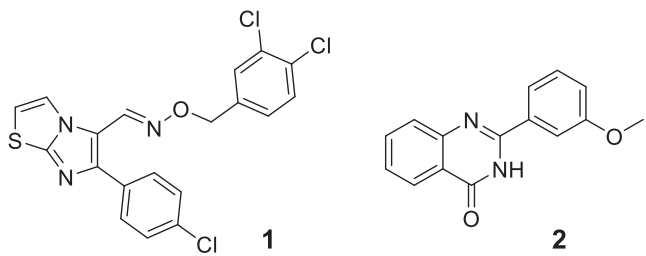
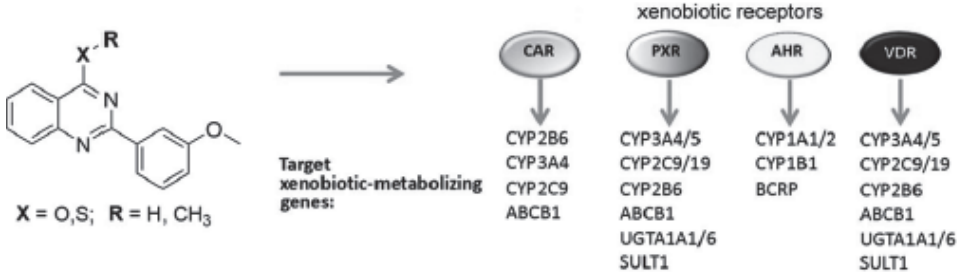



*Fig. 1.*

In this work, a library of compounds previously prepared as potential antituberculotics was subjected to random screening. The screening revealed that 2-(3-methoxyphenyl)-3,4-dihydroquinazolin-4-one (**2**, Fig. 1) displayed promising activation of the CAR receptor comparable to that of CITCO in reporter gene assay. We also observed in the study that some compounds are at the same time highly potent ligands of xenobiotic receptors PXR and AHR.[1] We therefore synthesized sulphur, *S*-alkylated and *O*-alkylated analogues of 2-(3-methoxyphenyl)quinazoline and analyzed their interaction with the human CAR and other receptors.[2] Attempted coupling of iodoanisole or boronic acids into position 2 of quinazolin-4-ol using the direct C-H bond activation failed[3] (Scheme 1).

*The work was supported by Charles University (GAUK 398315), SVV-260-291 and Czech Science Foundation (15-07332S).*




*Scheme 1.*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## GOLD OR CYANO? THAT IS THE QUESTION! – SYNTHETIC APPROACHES TO DIVERSE COMPOUNDS


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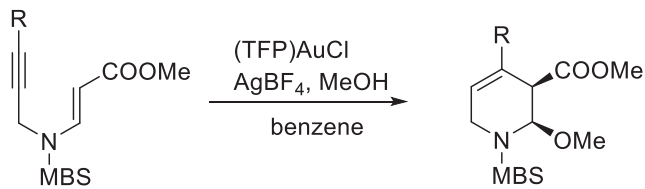
In order to expand our research[1,2] on chemoselective gold(I)-catalyzed cyclization of β-propargylamino acrylic esters, we developed the nucleophile-assisted diastereomeric cyclizations to furnish substituted aminoacetals (Scheme 1).




*Scheme 1.* Nucleophile-assisted cyclization.

Nostotrebin 6 (Fig. 1) is a polyphenolic secondary metabolite isolated from cynobacteria containing the cyclopentenedione moiety. It possesses an antimicrobial activity, and is also an efficient inhibitor of both acetylcholinesterase and butyrylcholinesterase.[3] Synthetic approaches towards key intermediates and derivatives will be discussed.


*This work was supported by Charles University (SVV 260 291 and GAUK 262416) and Czech Science Foundation (Project No. 15-07332S).*

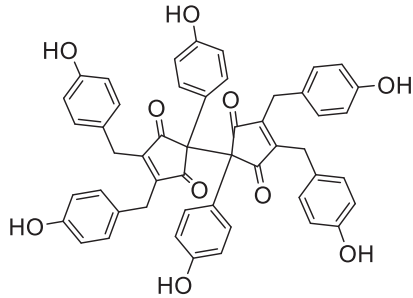



*Fig. 1.* Nostotrebin 6.

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## RED-EMITTING AZAPHTHALOCYANINE SENSORS HIGHLY SELECTIVE TOWARD POTASSIUM CATIONS

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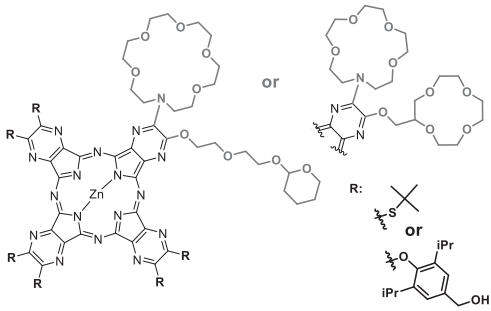
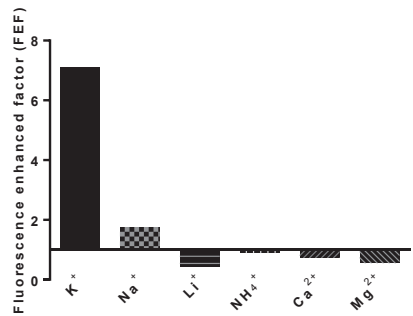
Azaphthalocyanines (AzaPcs) are planar macrocyclic compounds with advantageous fluorescence properties like absorption and emission over 650 nm, high extinction coefficient (approx. 200 000 L $mol^{-1}$ $cm^{-1}$) and high fluorescence quantum yields. Recently, AzaPcs bearing aza-crown on periphery have been shown as promising sensors for metal cations.[1,2] This work is follow up project focused on improvement of sensor selectivity to particular biogenic cations by the attachment of substituent (lariat ether) close to recognition moiety (aza-crown).

Appropriate precursors, i.e. 5,6-disubstituted pyrazine dicarbonitriles, were obtained by nucleophilic substitution. Their cyclotetramerization using template method with zinc acetate in high boiling solvent led to statistical mixture of AzaPc congeners. Required asymmetrical AzaPc congener AAAB was isolated by chromatographic methods. Finally, sensing properties of target AzaPcs were studied by the mean of fluorescence titration experiments. The improved selectivity towards $K^+$ with insensitivity to other cations ($Na^+$, $Li^+$, $NH_4^+$, $Ba^{2+}$, $Ca^{2+}$, $Mg^{2+}$) was observed.

*The study was supported by GA UK 494214 and SVV 260 291.*

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## 3,5-DINITROBENZYL HETEROCYCLES AND THEIR ANALOGUES: SAR STUDY OF NEW, HIGHLY EFFECTIVE ANTITUBERCULAR AGENTS

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According to WHO, tuberculosis belongs to TOP 10 world killers. In 2015, this infectious disease was responsible for 1.4 million deaths and additional 0.4 million deaths among HIV positive patients. Generation of resistant strains of *Mycobacterium tuberculosis* brings even more complications to the treatment, which usually consists of taking two or more drugs for at least 6 months. In the cases of drug-resistant forms of tuberculosis, the therapy is prolonged up to 2 years. This brings many unpleasant side effects to the patients and leaves space for non-compliance, which further enhance rising resistance of mycobacteria. After several decades without any new antitubercular drug, two compounds – delamanid and bedaquiline – were recently approved for the treatment of drug-resistant tuberculosis. Despite this particular success, there is a strong need for new, more effective and better-tolerated drugs for the treatment of tuberculosis.

Our group discovered a new class of compounds with high antimycobacterial activity even against drug-resistant strains and dormant forms of *Mycobacterium tuberculosis*. Lead compounds contain 3,5-dinitrobenzylsulfanyl moiety and five-membered heterocycle (Fig. 1). To confirm our hypothesis that 3,5-dinitrobenzylsulfanyl moiety is responsible for

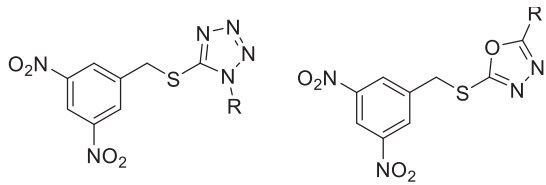

*Fig. 1.*

the antimycobacterial activity, we prepared several series of analogues of lead compounds and studied their structure-activity relationships.

*This work was supported by the Charles University Grant Agency (project 361215/2015) and Charles University (project SVV 260 062).*

## SYNTHESIS OF NEW α,β-DIPHENYL FURANONES AS POTENTIAL ANTITUMOR AND ANTIMICROBIAL AGENTS

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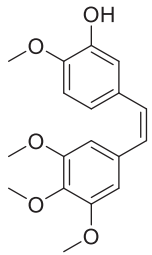
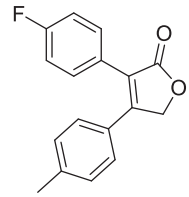
The aim of this project was to synthesize a novel library of α,β-diphenyl furanones as potential antitumor and antimicrobial agents. Model structures were designed as analogues of the stilbenoid combretastatine (**1a**), without oxygenation of the phenyl rings. Our previous results have shown that derivatives with halogen substitution in the *p*-position of the α-phenyl group and alkyl group in *p*-position of the β-phenyl ring possessed high inhibitory activity against malignant cell lines, low toxicity against healthy cells and *Staphylococcus aureus*. To broaden this library of derivatives, we prepared homologues



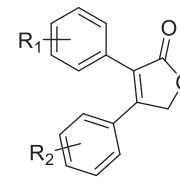
**1a**

Combretastatine CA4
$IC_{50}$ = 1,6 nM SH-SY5Y



**1b**

$ED_{50}$ = 4 μM K526
$ED_{50}$ > 40 μM RPE-1



**2a**: $R_1$= 4-F $R_2$ = 4-$CH_2CH_3$
**2b**: $R_1$= 3,4-diF $R_2$ = 4-$CH_3$
**2c**: $R_1$= 4-F $R_2$ = 4-$SCH_3$
**2d**: $R_1$= 4-F $R_2$ = 4-iBu
**2e**: $R_1$= 4-F $R_2$ = 4-CN

*Fig. 1.* Combretastatine and newly prepared derivatives.

and isosters of compound **1b**. The biological activity of the new compounds **2a**–**e** is currently under evaluation.

*This work was supported by Charles University (GAUK 1906214, SVV 260-291) and Czech Science Foundation (15-07332S).*

## SYNTHESIS OF PHTHALOCYANINES CONTAINING ANIONIC GROUPS

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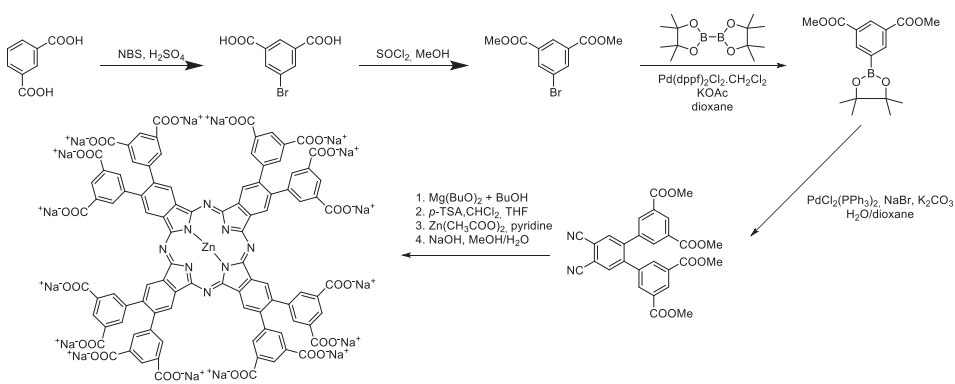
Phthalocyanines (Pc) represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. This work closely follows the previous project[1] that studied azaphthalocyanine bearing carboxylate groups in rigid arrangement. The aim of this work was a synthesis of anionic Pc analogue. The starting compound was isophthalic acid that was brominated, esterified and subsequently converted to boronic acid pinacol ester (see Scheme). 4,5-Disubstituted phthalonitrile, a precursor for Pc, was obtained by Suzuki coupling from boronic adic pinacol ester. Cyclotetramerization using magnesium butoxide as initiator gave magnesium Pc substituted with sixteen alkylcarboxy groups. In next step, magnesium complex was converted to metal-free ligand and then to zinc complex. Ester bonds in this zinc(II) Pc were hydrolyzed by NaOH and the final sodium salt was then purified by size-exclusion chromatography.




*The work was supported by GA UK 1060216 and SVV 260 291.*

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## DERIVATIVES OF PYRAZINE CARBOXYLIC ACID: SYNTHESIS AND ANTI-INFECTIVE EVALUATION

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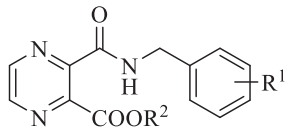
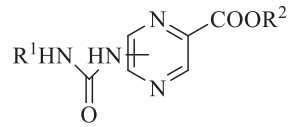
Tuberculosis (TB) is a common infection that had been successfully treated with proper first line anti-TB drugs. Yet this curable infection has become a frequently deadly illness due to the increasing antimicrobial resistance (AMR).[1] This serious issue has driven efforts to find new therapeutic drugs effective against *Mycobacterium tuberculosis*. Ongoing research area is the synthesis and evaluation of pyrazinamide derivatives as potential anti-TB drugs. Benzyl derivatives of 3-pyrazine-2-carboxamide (**1**) and urea-containing pyrazinoic acid derivatives (**2**) are two derivatization approaches this abstract focuses on.[2] In the second series, different aromatic and aliphatic substituents will be used. All synthesized compounds were tested *in vitro* for antimycobacterial activity. The minimum inhibitory concentration was determined for the tested compounds beside isoniazid and pyrazinamide as reference standard. Results of the biological testing and structure activity relationships will be discussed in the presentation.



$R^1$: Different Substituents
$R^2$: H/$CH_2CH_2CH_3$
(1)



$R^1$: Aliphatic/Aromatic Substituents
$R^2$: H/$CH_2CH_2CH_3$
(2)

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## INTRAMOLECULAR TSUJI-TROST REARRANGEMENT

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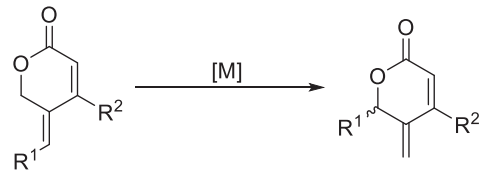
Our group has previously reported an interesting rearrangement of lactones (**1**) prepared by Migita-Stille coupling.[1] The original coupling products rearranged to the isomeric 5,6-dihydro-5-methylene-2*H*-pyran-2-ones (**2**).

After optimization of reaction conditions, we prepared a library of compounds, which show high functional tolerance for this reaction. Second, the rearrangement introduces a new chiral center in the pyranones. We have therefore also performed screening of chiral ligands in order to explore the possibility of asymmetric induction.




**1** **2**

M - rare metal
catalyst

*The study was supported by by Grant Agency of Charles University (project No. 1054216), Czech Science Foundation (project No. 15-07332S) and Faculty of Pharmacy in Hradec Králové (SVV-260-291).*

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## SYNTHESIS OF MODIFIED GANGLIOSIDES

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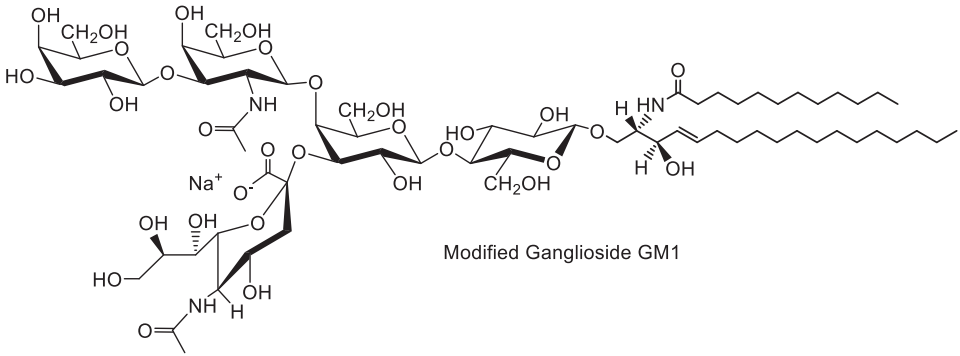
Gangliosides are acidic glycosphingolipids that contain one or more sialic acid residues and are particularly prevalent on neuronal cells. They seem to be involved in cell-to-cell interactions and regulations of activities of receptor tyrosine kinases in the plasma membrane. The regulation of the receptor of epidermal growth factor, nerve growth factor or insulin belong among the most important regulations.[1]

The naturally occurring gangliosides are a very structurally diverse group of molecules. This diversity is based on different sugar sequences, position of sialic acid as well as the variations in ceramide moiety. Gangliosides, which are used as an internal standard for LCMS quantitative analysis, must preserve the properties of naturally occurring gangliosides, but they cannot interfere with the analyzed lipids. For these purposes, the shortening of an acid chain in the ceramide moiety was selected to obtain molecules with lower molecular weight than those occurring naturally.

The first attempt of the shortening of the acid chain was done by enzymatic deacylation using ceramide N-deacylase followed by acylation of the resulting lyso-ganglioside with




N-lauroyloxysuccinimide.[2] This synthetic route showed to be unsuitable due to different chain lengths in the sphingoid part of the starting material.

The desired molecule of ganglioside GM3 was finally obtained by acylation of commercially available lactosylsphingosine with lauric acid in presence of carbodiimide followed by an enzymatic attachment of N-acetylneuraminic acid to the lactosyl ceramide using trans-sialidase. The overall yield of this reaction sequence was approximately 35% and the final ganglioside GM3 was obtained in more than 98% purity.

*This work was supported by the Czech Science Foundation (16-25687J) and Charles University (SVV 260291).*

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## SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL TRICLOSAN DERIVATIVES

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Tuberculosis (TB) represents one of the leading causes of morbidity and mortality worldwide. Development of new potential drugs is essential because of the existence of latent and drug-resistant TB forms. Triclosan (TRC) is a broad-spectrum antimicrobial agent used in household products. TRC has been shown to inhibit essential enoyl acyl carrier protein reductases from many human pathogenic organisms including InhA from *Mycobacterium tuberculosis* (*Mtb*).[1] In contrast to isoniazid (INH), triclosan mechanism

of action does not require a prior enzymatic activation, thus bypassing the most common cause of INH resistance. However, its use is limited, e.g., by an insufficient bioavailability. That is why we have designed novel TRC prodrug forms with potentially improved properties, e.g., a higher activity and/or lower toxicity, enhanced bioavailability and/or absorption.

We prepared 37 TRC-based esters and *N*,*N*-disubstituted carbamates based on various (cyclo)aliphatic, aromatic and heteroaromatic structures. Reactions of TRC with appropriate acyl/carbamoyl chlorides in the presence of a tertiary base or Steglich esterification were used. Pyrazine-2-carboxylate showed the best *in vitro* activity against *Mtb.* $H_{37}Rv$ with minimum inhibitory concentration (MIC) of 16 μM. Isonicotinate exhibited the highest activity against atypical mycobacteria; its MIC values for *M. kansasii* 6509/96 were comparable to INH and even improved for *M. avium* and *M. kansasii* 235/80. Generally, the best activity against bacteria showed esters with propiolic acid (MIC ≥ 0.49 μM against methicillin-resistant *Staphylococcus aureus*) and pyrazine-2-carboxylic acid (MIC ≥ 15.62 μM against extended-spectrum β-lactamases-positive *Klebsiella pneumoniae*). These two esters also exhibited the strongest antifungal action (MIC ≥ 7.81 μM against *Trichophyton mentagrophytes*).

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

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## DESING AND SYNTHESIS OF NOVEL 3,4-DIARYLSUBSTITUTED FURANONES

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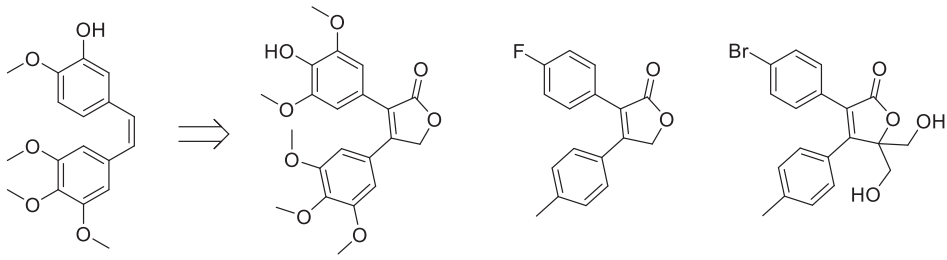
Synthesis, derivatization and biological activity evaluation of 3 libraries of α,β-diaryl furanones have been executed.

The first library was derived from natural Combretastatin. Since the *cis*-stilbene structural pattern as well as 3,4,5-trimethoxy substitution are essential for antitumor activity, the first library of molecules was characterized with high oxygenation of both phenyl rings.

In the second series of compounds, different substituents were attached. Furanones bearing halogen on C3 aromatic core and alkyl or alkoxy group on C4 were found to possess significant antineoplastic activity against human leukemia cancer cell lines. More specifically, several compounds showed no toxicity up to the concentration of 40 μM.

In order to increase the hydrophilicity of our analogs, a third library was developed by the introduction of two hydroxymethyl grous to the structure. Hydroxymethylated molecules showed reasonable antimicrobial effect.




**1** **2** **3** **4**

Combretastatin A4

Example of 1st library
K562 ($IC_{50}$ > 50 μM)

Example of 2nd library
K562 ($IC_{50}$ = 4 μM)

RPE-1 ($IC_{50}$ > 40 μM)

Example of 3rd library
K562 ($IC_{50}$ > 50 μM)

*Staphylococcus aureus*
(ATCC 6538 )
$IC_{95}$ = 0,98 μmol / L

*Fig. 1.*

*This work was supported by Charles University (GAUK 1906214, SVV 260-291) and Czech Science Foundation (15-07332S).*

## AMPHIPHILIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY

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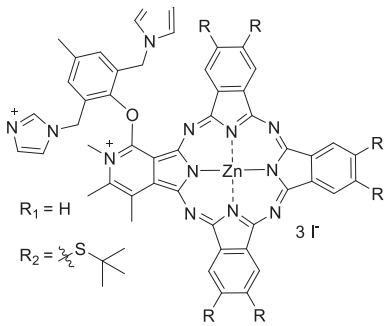
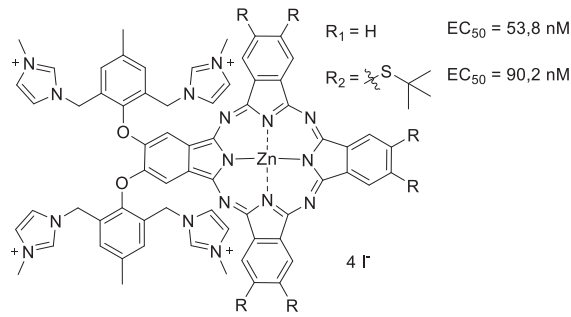
Phthalocyanines (Pcs) are planar macrocyclic compounds, which have ability to produce efficiently singlet oxygen. Singlet oxygen is the cytotoxic species in photodynamic therapy of cancer that besides this agent involves also light and photosensitizer (*i.e.* Pc). Recent results of research of our group indicated that hydrophilic cationic Pcs and azaphthalocyanines might be very efficient photosensitizers.[1] In this project, we prepared amphiphilic cationic Pcs based on the previous successful derivatives.

The synthesis consisted of preparation of precursors (substituted phthalonitriles) with following cyclotetramerization. Peripheral substituents for each precursor were added by nucleophilic substitution. Subsequently, two precursors underwent cyclotetramerization leading to six congeners of which the AAAB type was separated by chromatographic

methods. Following alkylation by methyl iodide lead to final cationic amphiphilic compounds. In case of *tert*-butylsulfanyl substituted Pcs, we observed realkylation by $CH_3I$ and exchange of *t*Bu substituents for methyl. Photodynamic activity of the final four compounds was determined on HeLa cells indicating high activity comparable with clinically approved photosensitizers.




*The study was supported by SVV 260 291.*

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## SECTION OF CLINICAL AND SOCIAL PHARMACY

### THE ANALYSIS OF THE FALLS OF PATIENTS IN A REHABILITATION FACILITY

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The risk of falling rises with the number of used risk drugs and with appearance of other risk factors associated with falls (risk diagnosis, age etc.).

The research objective was to analyze the patients who have fallen during the certain period and assess effects of pharmacotherapy and other factors associated with falls. Moreover, in the study DRPs (drug related problems) not related with falls were revised.

The data of this retrospective cross-sectional observational study were obtained from the patients who have fallen during hospitalization in the health-care facility providing rehabilitation care from 1st of December 2014 to 31st of December 2015. Available data

describing fall, personal and drug anamnesis were analyzed. The patients with missing data were excluded. The analysis of the data was aimed to identify risk factors, diagnosis and other risk factors which could be related with falls. Based on prepared overview of risk drugs the individual and population risks for each patient were determined. The individual risk presents drugs with increased risk of falls either by mechanism of action or by circumstances of the fall where the drug cause might not be excluded. The other DRPs were analyzed and were classified by modified PCNE classification V5.01. The possible effect of pharmacotherapy on the fall probability was classified by Likert scale. The results were described by descriptive statistics.

207 falls distributed between 47% men and 53% women were identified during a revision of data. The median age was 71 ± 13.6 years. The potential risk drugs were 4.55 and individual risk drugs was 0.74 per patient in average. The most frequent risk groups of drugs were hypnotics/anxiolytics, antidepressants, antihypertensives and the most frequent risk diagnosis were arterial hypertension, instability, musculoskeletal diseases.

The presence of a clinical pharmacist can be a tool to eliminate any potential errors in pharmacotherapy that can be one of the reasons leading to falls.

*The study was supported by grant of Charles University (SVV 260 295). Supported by Ministry of Health of the Czech Republic, grant nr. 16-33463A.* 

## JEWISH PHARMACISTS IN THE CZECH LANDS IN THE YEARS 1918–1945

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In my contribution I would like to summarize the position of Jewish pharmacists in pharmacies in the Czech lands in the interwar Czechoslovakia and their fate during the Second World War II. Jews constituted in these countries, ethnic and religious minority They were largely assimilated into the surrounging environment. Most of them identified with the German language, a minority trying to subsume into the Czech environment. A special group was the Zionists.

According the census in 1930 Czech lands had 10,674,386 inhabitants. 37,093 persons (0.35%) claimed allegiance to Jewish nationality and 117,551 persons (1.1%) claimed allegiance to the Jewish religion.

The observed data I compared with the number of pharmacies, the owners (tenants, etc.) and employed pharmacists – for one things from an overall point of view and for another from a number of Jewish pharmacists. The number of Jewish pharmacist included the successful Jewish students (Jewish nationality or religion – or both), who worked in pharmacies. This number I completed names of pharmacist from the lists of victims and survivors the Shoah, who worked before the Second World War in the pharmacies, too.

In the Czech lands existed 1,028 pharmacies in 1937. The Jews owned 26 pharmacies (2.53%). Employed pharmacists were 1064 in 1937. In the Czech lands worked 64 em-

ployed pharmacists (6.02%). I counted Jewish pharmacists from Slovakia and Carpathian Ruthenia.

If the percentage of Jewish faith in total number of inhabitants was 1,1%, percentage Jews among pharmacists was more larger. Pharmacy owners were 2.36 times as much as faithful Jews. Employed pharmacists were even 5.48 times as much as faithful Jews. Because between 1930 to 1937 years number of new Jewish pharmacists increased, we can assume, percentages Jews among pharmacists would have been larger in the case of the further existence of the Czechoslovak Republic. Furthermore, I have identified a further 85 Jewish pharmacists without information on their employment – for instance, they could work in the drugstores, to go abroad, to work in other sectors of pharmacy (production, distribution, education, science).

From all Jewish pharmacists (175 persons) I, meanwhile classified this way: 56 people were murdered during the Shoah, 16 persons survived, 2 persons died before the war and the fate of 99 of them are still unknown. From the 56 murdered, 46 were imprisoned in the Theresienstadt concentration camp. From the 16 survivors 4 persons emigrated, 4 people fought int the1st Czechoslovak Army Corps fighting on the Eastern Front alongside the Soviet Red Army and 6 persons lived to the liberation of the concentration camp Theresienstadt.

*The study was supported by SVV 260 295.*

## DIFFERENTIATED GENERICS – ADDED VALUE FOR PATIENTS AND HEALTHCARE SYSTEMS IN EUROPE: RESEARCH PROJECT FRAME

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Despite a significant role in reducing healthcare expenditures and improving patient access to modern effective medicines, generic medicines are perceived as a copycat or identical drugs with the innovative products, are interchangeable and therapeutically equivalent to them. As me-too copies, generic products normally do not provide any other innovation or added value except their lower price.[1] However, during a development of generic medicines, generic companies are exploring and identifying possible improvements to the reference products which have not been considered by innovators at the first stage of their lengthy and costly development.

Advanced therapies that emerged from launched molecules during their product life-cycle have gained considerable attention as clinical practice provides evidence for additional therapeutic values, patient centric delivery systems show improved therapeutic outcomes or emerging technologies offer efficiency gains in manufacturing.[1]

These products are also referred as added value generics, new therapeutic entities or hybrids, but no unified nomenclature yet exists. Regulatory authorities in EU has already adopted regulatory pathway to enable authorization of those innovative products. In Eu-

rope it was introduced within the Directive 2001/83/EC in November 2001 and in the Regulation (EC) No 726/2004 2.

Despite the attention from all involved stakeholders, market access and pricing is identified as a bottleneck preventing broader use of such innovative products and will be subject of further research.

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## RESPIRATORY QUOTIENT AS A PROGNOSTIC FACTOR OF INTENSIVE CARE UNIT LENGTH OF STAY IN POLYTRAUMA PATIENTS

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The metabolic prognostic factors in critically ill patients have not been well defined. In this study, energy metabolism was characterized during the first week of intensive care unit (ICU) stay (minimum day 3; maximum day 7) and its effect on total length of ICU stay was analyzed. Twenty polytrauma patients were examined. By indirect calorimetry, $O_2$ consumption and $CO_2$ production were measured and from these parameters energy expenditure (EE) and respiratory quotient (RQ) were calculated. Results were obtained after at least 4 hours of nutritional support administration. Subjects were at rest for at least 30 minutes before the assessment. EE of patients was significantly higher than the predicted basal metabolic rate ($P = 0.0097$). The mean of energy expenditure was 111.20 ± 16.60% of Harris-Benedict equation. A correlation analysis showed that RQ ($P = 1.710 \times 10^{-4}$; $r = -0.744$) and non-protein RQ ($P = 0.001$; $r = -0.699$) were significant factors determining length of ICU stay. The length of ICU stay was significantly longer in patients with low RQ (< 0.75) than in patients with RQ ≥ 0.75 ($P = 0.0272$). If the results are confirmed in a larger sample of patients, evaluation of respiratory quotient can be used to predict length of ICU stay in polytrauma patients.

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# POTENTIALLY INAPPROPRIATE MEDICATION USE IN OLDER LONG-TERM CARE RESIDENTS IN THE CZECH REPUBLIC: CROSS-SECTIONAL STUDY IN 10 LONG-TERM CARE FACILITIES PARTICIPATING IN THE EU SHELTER PROJECT

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Use of potentially inappropriate medications (PIMs), non-geriatric dosing and drug-disease interactions create specific areas of inappropriate prescribing in older patients and may lead to frequent adverse drug events particularly in older patients suffering from multiple disorders, using polypharmacy and presenting higher degree of frailty. The EU SHELTER project (Services and Health in the Elderly in Long-TErm care, 7th FP, 2009–2014) aimed to described comprehensive 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 the PIMs prescribing after application of the most known Beers 2012 criteria (1), Czech national consensus (2) and STOPP/START criteria (3) in newly admitted older adults (65+) in 10 Czech (CZ) long-term care facilities during the baseline period of the EU SHELTER project (total N (CZ) = 490, LTCF1: Home Odry (N = 29), 2: Home for Seniors Hortenzie, Prague (N = 36), 3: Home for Seniors Sluníčko, Ostrava (N = 94), 4: Long-term care (LTC), Thomayer's hospital, Prague (N = 45), 5: LTC, Hospital Liberec (N = 31), 6: Nursing home Odry (N = 37), 7: Nursing Home Ostrava (N = 61), 8: Nursing Home Ryjice (N = 62), 9: Nursing Home Plzeň (N = 54), 10: LTC, Hospital Třebotov (N = 41).

The highest prevalence of potentially inappropriate prescribing (62.3%) has been determined by Czech national consensus of potentially inappropriate medications (CNC), then by Beers 2012 criteria (60.2%) and STOPP and START criteria (44.5% and 52.9%, respectively). The most prevalent prescribing problems were (according to CNC): long-term use of benzodiazepines (BZDs) in depressive patients (7.8% in total sample), untreated constipations caused by opioid analgesics (7.4%), long-tern use of BZDs in patients suffering from syncope and falls (6.3%), long-term use of NSAIDs and ACE-I without clinical monitoring (6.1%), use of verapamil in patients with chronic constipation (3.9%) and use of doxazosine 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 suffering from falls (6.3%) and in cognitively impaired patients (5.1%), use of zolpidem in cognitively impared residents (4.3%) and long-term use of ASA or clopidogrel with NSAIDs without specific gastroprotection and monitoring (3.7%). The most frequent problems of undertreatment (according to START criteria) were: no anticoagulation treatment in atrial fibrilation (7.1%), no ACE-I or sartane treatment in chronic heart failure (4.5%) and no antidepressive treatment in patients with moderate to severe depression (3.9%).

Application of Czech national consensus (CNC) of PIMs in older patients yielded higher prevalence of prescribing problems than the use of other separate tools. It is mostly because CNC was summarized from different up-to-date published explicit criteria of PIMs and enables identification and solution of more prescribing problems than other foreign criteria.

*The study was supported by the EU SHELTER project, PRVOUK P40/FAF/2016 program at the Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové (subgroup Aging and Changes in the Therapeutic Value of Medications in the Aged led by Fialová D, PharmDr., PhD) and the EU COST Action IS 1402 "Ageism – a multi-national, interdisciplinary perspective", working subgroup WG1b "Healthy clinical strategies for healthy ageing".*

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# INVOLVEMENT IN MANAGEMENT OF OSTEOPOROSIS: A FOLLOW-UP SURVEY AMONG CZECH GENERAL PRACTITIONERS

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General practitioners (GPs) are key participants in osteoporosis (OP) management. The goal of the study was to evaluate knowledge, steps taken after a fracture and potential barriers in management of OP among Czech GPs and to discuss differences observed in comparison with the baseline survey performed in 2007.

It was conducted a cross-sectional questionnaire study. On behalf of two professional associations 2-round postal survey among randomly selected GPs (>1/4 of all Czech GPs) was performed in 2014. The questionnaire consisted of multiple choice questions and covered areas concerning GP's role in the fight against OP, knowledge about OP, management of OP-related fractures, barriers to the management of OP.

It was received 551 filled questionnaires, overall return-rate was 37%; mean age of the respondents was 53 years (37% men). The knowledge of risk factors was very good, however only 41% and 40% of respondents stated correctly recommended daily intake of calcium and vitamin D, respectively. Three quarters reported active steps after a fracture. Half of the respondents focus on fall prevention. System-related barriers such as lack of possibility to prescribe selected drugs (61%) were most frequently reported.

Knowledge of risk factors and involvement in post-fracture care was relatively high. Compared to baseline survey in 2007, patient-related barriers to the management of OP were more common. Prescribing conditions are still an important issue. Emphasis should be placed on education related to calcium and vitamin D intake, doses, sources, and supplements.

*The study was supported by SVV 260 295.*

## PHARMACOGENETIC APPLICATION FOR PREDICTION OF CARDIOVASCULAR RISKS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is an autoimmune disease with 1% occurrence in general population. RA plays inconsiderable role in patient's morbidity and mortality, mainly in cardiovascular system. Methotrexate (MTX) is anchor drug used in RA. The treatment of rheumatoid arthritis (RA) patients with MTX is connected with the effect on inflammatory activity, which has influence to risk of cardiovascular diseases. The aim of this study is to determine whether folate pathway related single nucleotide polymorphisms (SNPs) might be predictive of increased cardiovascular comorbities in RA patients treated with oral MTX.

We investigate the effect of 677C>T and 1298A>C methylenetetrahydrofolate reductase gene (*MTHFR*) SNPs on occurrence of cardiovascular risk factors and metabolic syndrome in 180 patients with RA. All of whom fulfilled the 1987 RA criteria of the American College of Rheumatology, each patient with history of MTX treatment. Genotyping was performed by quantitative PCR with allelic discrimination using commercial TaqMan (allele-specific) assays (Life Technologies, USA). Using questioners, ECG and laboratory tests of collected patient's blood and urine samples, we will evaluate their cardiovascular diseases and cardiovascular risk factors.

Based on genetic analysis it would be possible to diagnose patients, who are primarily resistant or who have decreased response for the MTX treatment. In consequence, the analysis should be useful tool for patient's individual therapy.

## THE EFFECT OF FLUID INTAKE ON THE FLUID BALANCE IN CRITICALLY ILL PATIENTS

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In critically ill patients, there are many changes in amount and the distribution of body fluids. The stress response to trauma and inflammation lead to microcirculatory dysfunction, capillary leak and thus to the redistribution of body fluids. Finally, fluid management strategies in the intensive care unit also contribute to the fluid accumulation. The exact mechanism and kinetic of this shift in body fluids has not been known. According to studies, fluid overload is independently associated with poor prognosis. Our results also show that positive fluid balance on hospital day 3 correlates with longer length of hospital stay ($p = 0.039$, $r = 0.538$) and duration of mechanical ventilation ($p = 0.026$, $r = 0.571$). Therefore, the aim of this study was to evaluate fluid balance in polytrauma patients focusing on the kinetics of changes in body fluid volumes and the influence of infused fluids to diuresis, total fluid output and fluid balance. The study included 25 polytrauma patients. In total, 70 examinations using bioelectrical impedance spectroscopy for determination the quantities of body fluids were performed. Hospital documentation with data about fluid balance was analyzed too. It was found that a positive fluid balance dominated mainly in the first three days of hospitalization, and then patients already did not have such fluctuations. In these days, infused fluid intake statistically significantly correlated with fluid balance ($p < 0.001$, $r = 0.924$). This correlation will be validated in following study. When these findings were confirmed, it could be used for prediction of administration fluid intake to prevent positive fluid balance, which could be harmful.

*The study was supported by the Faculty of Pharmacy SVV/2016/260295, Charles University GA UK 772216, MH CZ – DRO (UHHK, 00179906), PRVOUK P40.*

## COMPARATIVE STUDY OF EXPLICITE CRITERIA OF POTENTIALLY INAPPROPRIATE MEDICATIONS AND CREATION OF SUMMARIZED TOOL FOR PROSPECTIVE EUROPEAN RESEARCH

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Proportion of older people rapidly increases in the world population. Elderly represents a heterogeneous population more vulnerable to various adverse drug events and "specifically geriatric" adverse drug reactions. Many explicit criteria of potentially inappropriate medications have been developed in different countries in order to improve the quality and safety of geriatric pharmacotherapy. These criteria represent basic tools defining fundamental rules of safer drug choice and drug dosing in the old age. The aim of the work was to compare explicit criteria of potentially inappropriate medications published until Dec 2015 and to summarize list of all until now published potentially inappropriate medications regardless of diagnoses and concomitantly used drugs, applicable in future prospective EU COST Action research.

Based on systematic literature review in PubMed dataset during the period 04/2015–12/2015, all explicit criteria (N = 15) and explicitly-implicit criteria (N = 5) validated by expert panels and published in foreign peer-reviewed or impact factor journals by Dec 2015 have been identified. Methodology of criteria development and their advantages and disadvantages were compared and summarized in comparative tables. Comprehensive list of all known potentially inappropriate medications was created from the first parts of these criteria, including medications potentially inappropriate in older patients regardless of diagnoses or concomitantly used medications.

The majority of criteria identified in the literature review have been validated using 2 round Delphi or modified Delphi technique. They used more or less Beers criteria as the main source. Validation method and content of criteria were substantially different. Summarized list of all potentially inappropriate medications, available in at least 3 explicit criteria, finally contained 125 items. 99 medications were listed in the less than 7 criteria, 7–10 criteria listed 23 medications, and more than 12 criteria listed only 3 medications.

Explicit criteria of potentially inappropriate medications in older patients significantly differ in applied methodology and the content. Due to different availability of drugs on local pharmaceutical markets applicability of individual criteria is limited. This work enabled to compare all existing criteria and helped to create summarized list of PIMs, suitable for cross-national epidemiological research.

*The study was supported by the EU COST Action IS 1402 project "Ageism – a multi-national, interdisciplinary perspective", working subgroup WG1b "Healthy clinical strategies for healthy ageing" and PRVOUK P40/FAF/2016 of the Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové (subgroup Aging and Changes in the Therapeutic Value of Medications in the Aged, chair: Fialová D, PharmDr., PhD).*

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# ANALYSIS OF THE RISKS ASSOCIATED WITH USING DIETARY SUPPLEMENTS BY PATIENTS IN PRE-OPERATIVE PERIOD FUTURE RESEARCH

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Use of complementary and alternative medicine (CAM) is relatively popular among the general population.[1] The prevalence of CAM use is about 30% among presurgical patients.[2] The most popular CAM methods are use of vitamins/minerals and herbal medicines.[1,2,3] Yet, some complications during surgery may arise from the use of CAM (e. g. bleeding, prolonged effects of anaesthesia).[3] The aim of this study is to determine the use of CAM by the patients before surgery and their awareness of the risks associated with the use of CAM.

This will be a cross-sectional survey. The questionnaire will be piloted on a sample of the target population and distributed among 500 patients before surgery from different departments of University Hospital Hradec Králové during a 3-month period. Data on patients´ pharmacotherapy and CAM use will be collected and potential drug-CAM interactions evaluated. Patients' awareness of risks associated with CAM use and doctors' awareness of CAM use by their patients will be assessed as well.

The results of this study could identify potential drug/CAM-related problems in presurgical patients and make recommendations to hospital policy decision-makers in order to increase the quality and safety of perioperative care in the Czech Republic.

*The study was supported by SVV 260 295.*

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# FROM METABOLISM AND DISPOSITION OF DEXRAZOXANE TO PK-GUIDED EXAMINATION OF CARDIOPROTECTIVE EFFECTS OF ITS METABOLITE ADR-925

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Dexrazoxane (DEX) is the only drug with proven efficacy against chronic anthracycline (ANT) cardiotoxicity in both experimental and clinical settings. DEX cardioprotection has been traditionaly explained *via* its iron-chelating metabolite ADR-925 which originates inside cardiomyocytes and suppresses ROS production, but no direct evidence supports this hypothesis. This study aimed to clarify the role of ADR-925 on DEX protection against ANT cardiotoxicity in isolated neonatal rat cardiomyocytes (NCVM) and in rabbits. Firstly, we have determined the PK profiles of ADR-925 *in vitro* in cardiomyocytes and *in vivo* in rabbit plasma and hearts after administration of DEX or ADR-925. We found that administration of ADR-925 is able to achieve same or even higher exposure than those after DEX. These findings allowed us to directly compare cardioprotective effects of ADR-925 on the same models as DEX. Results from rabbits clearly showed that ADR-925 is unable to ameliorate DAU-induced mortality and provide significant cardioprotection regardless dosing schedule as judged by functional, morphological or molecular parameters, which is in sharp contrast to DEX. These findings were corroborated also by *in vitro* data from NVCM. Finally, ADR-925 was not able to interact with topoisomerase II beta (TOP2b) which was recently showed as primary target og ANT in cardiomyocytes. In conclusion our data shows that metal-chelating metabolite ADR-925 is not responsible for DEX cardioprotective properties against ANT cardiotoxicity, and the interaction of DEX with TOP2b deserved further study.

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## THE HAEMODYNAMIC EFFECTS OF FLAVONOID METABOLITE, 3,4-DIHYDROXYPHENYLACETIC ACID IN RAT

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Biological activity of oral flavonoids is, at least partially, related to low molecular weight phenolic acids formed in the colon by intestinal microflora.[1] The aim of this study was to evaluate the biological activity of one of these metabolites, 3,4-dihydroxyphenylacetic acid (3,4-DHPA). A series of *in vitro* tests were carried out on isolated rat aortic rings to study the effects of 3,4-DHPA and to elucidate its mechanism of action. The results showed vasodilatory activity which seems to be endothelium NO-based. Subsequent *in vivo* experiments confirmed the peripheral vasodilatory activity of 3,4-DHPA on both normotensive and spontaneously hypertensive rats, and excluded an effect on the heart.

In conclusion, similar to our previous report on 3-(3-hydroxyphenyl)propionic acid, the formation of 3,4-DHPA contributes to the *in vivo* effect of orally administered flavonoids.

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## LEFLUNOMIDE AND ITS METABOLITE TERIFLUNOMIDE ARE HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) LIGANDS THAT MAY INFLUENCE GLUCOSE METABOLISM

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Disease modifying antirheumatic drugs (DMARD) are compounds used in clinical treatment of rheumatoid arthritis (RA). Leflunomide is one of the most relevant drugs and its metabolite, teriflunomide, is also active in the treatment of this condition through inhibition of the dihydroorate dehydrogenase mitochondrial enzyme.

Constitutive Androstane receptor (CAR) is a transcription factor found mainly in the cytoplasm of hepatic cells but also in other tissues such as kidneys, intestines or brain. Activation of CAR has influence on the metabolism of drugs and xenobiotics as it is a regulator of phase I and II enzymes. It is more and more evident that CAR has also

a significant role in regulation of endogenous metabolism such as in the homeostasis of glucose and energy. For instance, CAR activation is able to attenuate the glucose levels in the organism, improving the profile for diabetic patients, by down-regulation of several genes involved in gluconeogenesis.

In our laboratory, we identified that leflunomide and teriflunomide activate CAR and therefore, it is possible to assume a potential to down-regulate glucose levels in patients being treated with these compounds. This outcome could be of great relevance and could have clinical applications since many of these patients suffering from RA are adult people, close to senescent age, in which the incidence of diabetes mellitus type II (DM II) is sensibly higher.

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## NBMPR, AN ESTABLISHED SPECIFIC INHIBITOR OF EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS, INHIBITS ABCG2 EFFLUX TRANSPORTER

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S-(4-Nitrobenzyl)-6-thioinosin (NBMPR) is a chemical substance that has been for decades considered as a specific inhibitor of equilibrative nucleoside transporters (ENTs, *SLC29A*); revealing higher inhibitory potency to ENT1 compared to ENT2. However, in our experimental pharmacokinetic models expressing ATP-binding cassette (ABC) efflux transporters, P-glycoprotein (ABCB1) or Breast Cancer Resistance Protein (ABCG2), we observed that NBMPR changed transmembrane permeation of non-nucleoside drugs. Therefore, we aimed to investigate whether NBMPR might affect activity of either ABCB1, ABCG2 or both.

At first, we performed accumulation of ABCB1/ABCG2 model substrate hoechst 33342 in MDCKII cells overexpressing ABCB1 or ABCG2. We observed that NBMPR increased hoechst 33342 accumulation in MDCKII-ABCG2, but not in MDCKII-ABCB1 and parental control cells. NBMPR at a concentration $\geq$ 50 μM exhibited a comparable effect to Ko143 or elacridar. The calculated inhibition concentration ($IC_{50}$) in this experimental system was 52.87 μM. Subsequently, we showed that NBMPR inhibited ABCG2-mediated permeation of clinically relevant ABCG2 substrate glyburide across the MDCKII-ABCG2 monolayer. Furthermore, using *in situ* dual perfusion of rat term placenta we demonstrated that NBMPR decreased active fetal-to-maternal transport of glyburide *in vivo*.

In summary, our data suggest that NBMPR is not the inhibitor selective for ENTs, but in concentrations commonly used in routine experimental setups, it also inhibits rat and human ABCG2. This information should be borne in mind when interpreting studies of ENTs mediated transport.

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## NON-QUATERNARY OXIME REACTIVATORS IN THE TREATMENT OF ORGANOPHOSPHORUS POISONING – NEW HOPE OR A BLID WAY?

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Organophosphorus (OP) nerve agents and pesticides represent permanent threat to the population in both armed conflicts and civilian sphere. OP inhibitors mechanism of action involves irreversible inhibition of acetylcholinesterase that physiologically plays a crucial role in neurotransmission. Current therapy of OP intoxication combines parasympatholytic agent (e.g. atropine), oxime reactivator and anticonvulsant drug (e.g. diazepam).

However, currently used quaternary oxime reactivators (HI-6, obidoxime) possess several drawbacks. Quaternary oximes have only a limited permeability to the brain, so AChE in the central compartment cannot be reactivated. That's why a concept of non-quaternary oxime reactivators have been proposed. The main idea of the non-quaternary reactivators concept is to reach a higher concentration in the brain. However, from the practical point of view, several drawbacks of physico-chemical, pharmacological and toxicological origin can be expected. A higher lipophilicity, leads to a low solubility in hydrophilic media worsening any *in vivo* methodology. Form the pharmacological aspects, it is known that non-quaternary compounds do not reach the reactivation potency of quaternary reactivators. Furthermore, the lipophilic character of these molecules completely changes the pharmacokinetic profile known for hydrophilic quaternary reactivators. Finally, from the toxicological point of view, we must consider the fact that all AChE reactivators are known as weak inhibitors of AChE. However, if high concentration of reactivator occurs in the

brain, such is supposed for the non-quaternary compound, toxic effect based on the AChE inhibition can be expected. Its higher persistence in the system increases the toxicity too.

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## THE ANTI-CANCER EFFECT OF AN ANTHELMINTIC AGENT FLUBENDAZOLE IN MALIGNANT MELANOMA CELL LINES

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Malignant melanoma (MM) is generally one of the most dangerous and aggressive tumors with rapidly increasing incidence worldwide. The incessantly increasing incidence and fast development of resistance to current treatment of MM lead to searching for new therapeutic approaches both finding very new anti-cancer agents and repositioning of drugs commonly used in the other indications. As the result of so called drug repurposing (using existing drugs in new indications) the benzimidazole anthelmintic, flubendazole (FLU), has been recently found for its promising anti-cancer activity due to changing the structure of microtubules and inhibition of β-tubulin polymerization. We studied the effect of FLU on three different MM cell lines – A375 cell line, BOWES cell line and RPMI-7951 cell line. The IC50s for all above mentioned cell lines were measured *via* xcelligence real time cell analysis. The values of IC50 were determined as 0.966 μM for A375 cell line, as 0.90 μM for BOWES cell line and as 0.25 μM for RPMI-7951. Cell proliferation was tested using WST-1 reagent. The structure changes were captured by both phase contrast microscopy for two FLU concentrations (1 μM and 5 μM) in three time intervals – at 0 h, 24 and 48 hours after treatment of FLU, and fluorescent microscopy, staining for β-actin, β-tubulin and DAPI (FLU 5 μM, treatment for 24 h). In order to get more detailed overview about changes on molecular level and to determine the way of cell death, western blot analysis of following proteins was done – p53 and its phosphorylated forms (ser15 and ser46), p21, BAX, BCL2, β-tubulin, p38MAPK, PARP, Caspase 2 and 3, using β-actin as a housekeep gene, with significant changes in protein levels of p53 throughout all the cell lines.

## INFLUENCE OF RADIOLABELING METHOD ON BINDING ABILITY OF ANTI-VEGFR2 MONOCLONAL ANTIBODY RAMUCIRUMAB

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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 greater affinity than its natural ligands and selectively inhibits its function. Several types of cancer are known to overexpress VEGFR2. Therefore, RAM with proper labeling could be potentially used for scintigraphic visualization or targeted radiotherapy. The aim of this work was to compare selected methods of radiolabeling in terms of binding ability to VEGFR2.

The three selected radiolabeling methods were as follows: direct $^{99m}$Tc labeling based on the reduction of disulfide bridges, $^{131}$I direct labeling according to chloramine-T protocol and indirect $^{99m}$Tc labeling with HYNIC molecule as a chelator. Radiochemical purity (ITLC-SG), stability (SE-HPLC) and receptor-ligand affinity (real-time radioimmunoassay performed on LigandTracer Yellow instrument) were tested for each selected method. Two VEGFR2 expressing cell lines were used in the binding study.

All introduced methods enabled effective labeling of RAM with either $^{99m}$Tc or $^{131}$I. Direct $^{99m}$Tc labeling provided slightly lower radiochemical purity than the other two methods. Both $^{99m}$Tc labeling methods provided better stability under the used conditions (storage at 4 °C in phosphate buffer). However, the both direct labeling methods negatively influenced RAM binding ability and only indirectly labeled $^{99m}$Tc-HYNIC-RAM maintained the binding ability to the VEGFR2 receptors.

Based on the obtained results we can conclude that the direct labeling methods with $^{99m}$Tc and $^{131}$I are inappropriate for RAM labeling as they impair the ability of RAM to bind VEGFR2. On the other hand, the indirect labeling using HYNIC chelator seems to be useful for follow up experimental studies.

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## ANTIPLATELET POTENTIAL OF A SERIES OF 9-PHENYL-2,6,7-TRIHYDROXY-XANTHENE-3-ONES DERIVATIVES

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The number of current oral antiplatelet drugs is limited and associated with a risk of treatment failure or pharmacokinetics interactions,[1] thus, the research of novel substances in this area is desired. A series of newly synthesized 9-phenyl-xanthene-3-ones were tested at different levels of platelet aggregation in human blood.

The initial screening in the whole blood has shown that most of the tested derivatives possessed some inhibition effect on platelet aggregation induced by arachidonic acid. None of the compounds was able to block cyclooxygenase 1 or thromboxane synthase, but the mechanism of the most potent compound, 9-(4′-dimethylaminophenyl)-2,6,7-trihydroxy-xanthene-3-one, seemed to be based on antagonism of thromboxane $A_2$ receptors. In addition, this compound had better potential to block collagen induced platelet aggregation than clinically used acetylsalicylic acid.

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## HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1, NOTCH3 AND MICRO RNA-21 CAN PREDICT GEMCITABINE EFFECTS IN PATIENTS WITH PANCREACTIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with very poor prognosis and response for treatment. In 10–20% of patients with pancreatic cancer can be surgically resected and then gemcitabine is a recommended standard first-line adjuvant chemotherapy.[1] Unfortunately, many patients do not show any benefit from gemcitabine treatment due to pharmacoresistance. This is supposed to be caused by altered expression of mechanisms involved in gemcitabine transport or in cell apoptosis such as equilibrative nucleoside transporter 1 (hENT1)[2], Notch3[3] and miR-21[4]. RNA

for immunohistochemical or PCR expression analysis was extracted from biopsies proposed that decreased levels of ENT1 or increased Notch3 and miR-21 have suggested these molecules as predictive biomarkers of patient's response to gemcitabine treatment.

In this study we used method of absolute quantitative analysis of these potential predictors in formalin fixed paraffin embedded (FFPE) samples and we also compared levels of hENT1, Notch3 and miR-21 expression in tumor and healthy pancreatic tissue. Out of 64 samples, we selected patients group with highest and lowest expression of hENT1, Notch3 and miR-21. Subsequently we performed overall survival (OS) analysis using Kaplan-Meier method.

We detected no significant differences in OS between gemcitabine-treated patients with low and high mRNA expression levels of all the genes examined. However, we observed higher median survival in patients with low expression of Notch3 and miR-21 that did not reach statistical significance.

We investigated whether tumor levels of biomarkers hENT1, Notch3 and miR-21 were associated with efficacy of gemcitabine therapy. Multivariate analysis of hENT1 will be further performed and compared to the results obtained from immunohistochemical analysis. This information may thus broaden the knowledge about molecules predicting the effectiveness of treatment with gemcitabine in pancreatic cancer patients.

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## COMPARISON OF *IN VITRO* VASCULAR EFFECTS OF ISOFLAVONOIDS AND THEIR METABOLITES FORMED BY HUMAN MICROFLORA

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Isoflavonoids are naturally occurring polyphenols with some documented positive effects on the human cardiovascular system.[1,2] Although their bioavailability is low following oral administration, absorbable metabolites are formed by intestinal bacteria.[3] Some isoflavonoids, as well as their metabolites, are able to dilate human arteries *in vivo*.[4] For

the purpose of this study, several isoflavonoid glycosides and aglycones, as well as their two metabolites formed by human microflora, were tested on isolated rat aortic rings. Their *in vitro* vasodilatory effect was measured against noradrenaline pre-contraction. The responses were dose dependent, however preliminary results showed that for most of them the effect seems to be low in plasma achievable concentrations. Glycitein appeared to be the most active, while glycosides failed to cause potent vasorelaxation. Interestingly, one of the intestinal metabolites, O-desmethylangolensin, possessed a higher effect than the majority of isoflavonoids. Future tests will be aimed at confirmation of these results on smaller vessels using an already established method for rat mesenteric artery.

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## OPTIMIZATION OF *IN VITRO* AND *EX VIVO* METHODS TO STUDY ROLE OF ABCB1 AND ABCG2 TRANSPORTERS FOR INTESTINAL ABSORPTION

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P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) decrease absorption from intestine of orally administered drugs that are their substrates. Drug-drug interactions (DDIs) on these transporters may result in elevated levels of plasma concentrations of a victim drug thus improving efficacy of pharmacotherapy or increasing the risk of side effects. As DDIs on intestinal ABC transporters cannot be investigated directly in living humans, it is essential to collect experimental data using surrogate techniques. In our project, we aimed to establish complex array of *in vitro* and *ex vivo* experimental techniques to investigate DDIs on intestinal ABC transporters. As an entry step, using model fluorescent substrate of ABCB1/ABCG2, rhodamine123, we validated the Caco-2 intestinal *in vitro* model in our lab. In transport experiments rhodamine123 reached efflux ratio ≈ 3. This was completely abolished with elacridar, dual inhibitor of ABCB1/ABCG2 transporters, confirming involvement of ABCB1/ABCG2 in its transepithelial transport. Moreover, rhodamine123 was used to optimize a unique *ex vivo* model based on accumulation of a drug in precision cut slices resected from rat intestine. Among particular segments of the rat intestine (duodenum, jejunum, ileum, and colon), we observed highest uptake of rhodamine123 uptake in the ileum. We also showed that elacridar and antiviral drug ritonavir, but not lopinavir, significantly increased intestinal rhodamine123 accumulation in the ileum, showing suitability of this experimental model for DDIs detection.

Subsequently, we tested clinically relevant ABCB1/ABCG2 substrate, tenofovir disoproxil fumarate (TDF). We confirmed ABCB1/ABCG2-mediated transport across the Caco2 cell monolayer that was inhibited by lopinavir. In conclusion, we partly succeeded to establish *in vitro* and *ex vivo* methods to study role of ABCB1/ABCG2 in drug intestinal absorption. To finish goal of this study, we will test suitability of the *ex vivo* technique for DDIs detection of clinically relevant ABCB/ABCG2 substrates (e.g. TDF). Moreover, to increase complexity of our research we also plan to introduce accumulation technique in precision cut slices of human intestine into our research.

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## THE HAEMODYNAMIC EFFECTS OF FLAVONOID METABOLITE 4-METHYLCATECHOL

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The 4-methylcatechol is a metabolite of quercetin formed by bacterial microflora in the colon.[1] Vasorelaxant effect of 4-methylcatechol has already been demonstrated *in vitro* experiments by us.[2] In this study, we have focused attention on its haemodynamic effects *in vivo*.

Male Wistar: Han rats were anaesthetized with urethane (1.2 g $kg^{-1}$). Arterial blood pressure and heart rate were measured by a pressure transducer linked to the left common iliac artery. A volume-pressure catheter was inserted *via* right common carotid artery into the left ventricle for analysis of cardiac contraction and relaxation. The increasing doses of 4-methylcatechol ranging from 0.2 to 25 mg $kg^{-1}$ dissolved in physiological solution were administered *via* vena saphena. The antihypertensive effect of 4-methylcatechol was also tested on spontaneously hypertensive rats (SHR).

Both systolic and diastolic arterial blood pressure significantly decreased from a dose of 2.5 mg $kg^{-1}$. Neither heart rate nor parameters of cardiac contractility and relaxation were significantly affected with the exception of the highest dose. These data were confirmed in SHR where the infusion at rate of 5 mg per kg per min significantly decreased both the systolic and diastolic blood pressures.

In conclusion, the decrease of blood pressure observed after administration of 4-methylcatechol is caused by peripheral vasodilatation.

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## THE EARLY DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN MICE IS ASSOCIATED WITH CHANGES OF MEMBRANE AND SOLUBLE ENDOGLIN

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Endothelial dysfunction is considered as the first and key step in the development of atherosclerosis. Our previous studies suggested potential role of endoglin in endothelial function and dysfunction. Aortic endoglin was suggested to regulate eNOS expression and soluble endoglin (sEng) was demonstrated to be cleaved from membrane endoglin in various cardiovascular pathologies. Increased levels of sEng were found in patients with preeclampsia, type II diabetes, hypertension and hypercholesterolemia. The aim of this study was to evaluate the changes in membrane endoglin expression in aorta and sEng levels in blood during early development of endothelial dysfunction in mice.

Two-month-old female mice double knockout ApoE/LDLR$^{-/-}$ mice and age-matched female mice C57BL/6J (control mice) were fed chow diet for 2 months. Western blot analysis of aorta and Luminex analysis of inflammatory and oxidative stress markers in blood were performed.

The membrane aortic expression of endoglin was significantly reduced in ApoE/LDLR$^{-/-}$ group as compared to control group. The same reduced expression was also demonstrated for p-eNOS (active form of eNOS) mediating NO-dependent vasodilation and pSmad2/3, which was shown to regulate eNOS expression. In addition, levels of sEng and soluble P-selectin levels (marker of inflammation) in blood were significantly higher in ApoE/LDLR$^{-/-}$ group.

Our results suggest that early development of endothelial dysfunction is accompanied by reduced expression of aortic endoglin and increased levels of sEng. Prospective studies are now focused on the potential impact of reduced endoglin expression on the development of endothelial dysfunction and the mechanism of sEng cleavage from aorta.

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## SMALL MOLECULE PROTEIN KINASE INHIBITORS REVERSE CANCER MULTIDRUG RESISTANCE

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Multidrug resistance (MDR) of tumors to structurally unrelated cytotoxic drugs is a phenomenon caused by different molecular mechanisms such as increased drug metabolism or efflux of the drug out of cancer cells by ATP-binding cassette (ABC) transporters. As a result, anticancer chemotherapy fails to reach its goal of complete remission and patient survival. In this study we evaluated the ability of several protein kinase inhibitors (PKI) to inhibit ABC transporters and overcome the transporter-mediated MDR *in vitro*. Six investigational or recently approved PKI acting as cyclin dependent kinase inhibitors (CDKI) or tyrosin kinase inhibitors (TKI) were involved in this work.

Using accumulation-efflux assay in MDCK-ABCB1 and MDCK-ABCG2 cells, we identified palbociclib and ribociclib, the CDK4/6 inhibitors approved in 2015 and 2016, respectively, for treatment of breast cancer as inhibitors of ABCB1 and ABCG2. Similarly, inhibitory effect to ABCB1 and ABCG2 was demonstrated in AZD5438 and R547, but at concentrations approaching their respective cytotoxic $IC_{50}$ values. Antiproliferative XTT assays further demonstrated that ribociclib and palbociclib are able to reverse MDR in the transporter-expressing human breast cancer cell lines and synergize with concomitantly administered conventional anticancer drugs that are ABCB1 and/or ABCG2 substrates. Additionally we showed that MEK inhibitor cobimetinib and B-raf inhibitor dabrafenib, drugs recently approved for treatment of melanoma, inhibit both, ABCB1 and ABCG2 transporters, suggesting also ability to overcome MDR in resistant cancer cell lines.

In conclusion, our study demostrates that several PKI could help overcome ABC transporter-mediated MDR *in vitro*. These findings might be exploited when optimizing anticancer therapeutic regimens and introducing the PKI into combination therapy schemes against resistant non-responding tumors inducing survival benefit to patients.

*The study was supported by GAUK 344315/C/2015, GACR 1626849S and SVV 2016/260-293.*

## HUMAN INDUCED HEPATOCYTE-LIKE CELLS AS AN *IN VITRO* LIVER CELL MODEL FOR THE ASSESSMENT OF HERB-INDUCED LIVER INJURY

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Herb-induced liver injury (HILI) is one of the most occurred safety concerns attributed to the administration of herbal medicines. Since traditional herbal medicines gain an increasing popularity around the world, the development of competent *in vitro* models enabling to evaluate a risk of HILI are urgently needed. Pluripotent cells are a promising source of human hepatocytes. In this study, we aimed to assess a capability of induced pluripotent stem cell-derived hepatocytes-like (iHep) cells to predict a hepatotoxic effect of model phytochemicals known to cause liver injury.

The off-the-shelf iHep cells were analysed by a confocal microscopy and qRT-PCR with regards to their phenotypical traits. The effect of plant-derived hepatotoxins were evaluated using MTS and LDH assays. For our work, a couple of hepatotoxic phytochemicals (saikosaponin D, monocrotaline, deoxycalyciphylline B, and triptolide) with different modes of hepatoxicity were chosen.

As shown by a confocal microscopy, iHep cells disposed of typical mature hepatocyte markers such as CYP3A4, HNF4α, and albumin. Nevertheless, the further analysis revealed lower mRNA levels of these genes in iHep cells in comparison to human hepatocytes.

The iHep cells also produced some immature hepatocyte markers including α-fetoprotein and cytokeratin 19. Toxicological screening showed iHep cells to be more sensitive to sakosaponin D ($IC_{50}$ = 0.96 μM; 48 h; MTS) and triptolide ($IC_{50}$ = 18.98 nM; 48 h; MTS) than the reference hepatic HepG2 cells.

To best of our knowledge, this is first work dealing with iHep cells as *in vitro* model for toxicological assessment of phytochemicals inducing HILI.

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## MARAVIROC IS A SUBSTRATE BUT NOT INHIBITOR OF ABCB1

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The chemokine receptor 5 (CCR5) antagonist maraviroc belongs among the HIV entry inhibitors used in combination antiretroviral therapy (cART). Concomitant administration of two or more drugs during cART, however, bear the risk of drug-drug interactions (DDI) affecting therapy safety and efficacy. Here we aimed to evaluate interactions of maraviroc with ATP-binding cassette (ABC) drug efflux transporter ABCB1, which is physiologically expressed in several human tissues and affects pharmacokinetics of high variety of structurally unrelated drugs. In particular, we aimed to investigate, whether (i) maraviroc is a substrate and/or inhibitor of ABCB1 *in vitro* and (ii) ABCB1 affects transplacental transport of the drug.

Three methods were employed in this study: a) *in vitro* accumulation assay in MDCKII cell line stably expressing ABCB1 and MDCKII parental cells using Hoechst 33342 as a model fluorescent ABCB1 substrate, b) *in vitro* transport assay across monolayers of MDCKII-ABCB1 and parental cells and c) an *in situ* dually perfused rat term placenta.

Our data showed that in contrast to the effect of control inhibitors (zosuquidar, elacridar), maraviroc was not able to inhibit ABCB1-mediated efflux of Hoechst 33342 in MDCKII-ABCB1 cells. *In vitro* transport assay in MDCKII-ABCB1 monolayer indicated that maraviroc transport across cell monolayer is mediated by ABCB1. However, similar results were observed in MDCKII-parental cells suggesting maraviroc as a substrate of endogenous canine Abcb1in the MDCKII cell lines. Nevertheless, a significant asymmetry

in maraviroc transplacental clearences was revealed, showing acceleration in the fetus-to-mother transport when compared to the mother-to-fetus direction. In addition, placental transport of maraviroc was saturable and reduced in presence of several ABCB1 inhibitors (elacridar, zosuquidar and ritonavir). To conclude, our data suggest involvement of ABCB1 in maraviroc transplacental passage. This information might be of significant importance, when optimizing therapeutic regimens absent of DDI in HIV-positive pregnant women.

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## INTERACTIONS OF STEVIOL AND STEVIOSIDE WITH NUCLEAR RECEPTORS AND BIOTRANSFORMATION ENZYMES

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*Stevia rebaudiana Bertoni* is a herb known for high content of natural sweeteners in the leaves. It has been used as a treatment of diabetes in Paraguay and Brazil by the origins.[1] Nowadays, it is very modern to use as a sweetener and a substitution for sugar, because its main secondary metabolites stevioside and its aglycone steviol are 300 times sweeter than glucose. Extracts from the leaves of *stevia* have been used to treat diabetes because it has insulinotropic and antihyperglycaemic effect.[2] Nuclear receptors control expression of a wide range of genes of transporters and drug metabolizing enzymes, such as cytochrome P450. CYP3A4, CYP2C9, CYP2B6, CYP1A1,CYP1B1 and CYP1A2 are just some of them, although, these isoforms are of a massive importance as 90% of clinicaly used drugs are substrates of these and thus, their metabolism and excretion can be influenced by compounds that act like agonists/antagonists of the nuclear receptors, or direct inducers/inhibitors of the CYP enzymes. This could lead to severe drug-drug or herb-drug interactions. In this study we tested interactions of steviol and stevioside with various nuclear receptors and with some of the enzymes that are controlled by them. We found out some important interactions of steviol and stevioside that may indicate food-drug interaction of the sweetener.

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# RILPIVIRINE INHIBITS MDR1 AND BCRP TRANSPORTERS AND INCREASES ORAL ABSORPTION OF ABACAVIR

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Rilpivirine (TMC278), is a highly potent, second generation non-nucleoside reverse transcriptase inhibitor (NNRTI), which represents a new effective component of combination antiretroviral therapy (cART) of wild-type and nevirapine or efavirenz resistant strains of HIV. Nowadays, rilpivirine is co-administered with abacavir and lamivudine as an effective, safe and cost-effective cART treatment option. However, many antiretroviral drugs are substrates of drug transporters and, therefore, prone to pharmacokinetic drug-drug interactions (DDIs). Since abacavir is known substrate of MDR1 (ABCB1) and BCRP (ABCG2) membrane transporters and lamivudine efflux is driven by OCT (SLC22A) and MATE (SLC47) transporters, the aim of our study was to evaluate potential inhibition of those ATP-dependent (ABC) and solute carrier (SLC) transporters with rilpivirine and assess for the potential of developing DDIs *in vivo*.

Using accumulation assays in MDCK cells overexpressing selected ABC or SLC drug transporters, we revealed rilpivirine as a potent inhibitor of MDR1 and BCRP, but not MRP2, OCT1, OCT2 or MATE1. Transport experiments across monolayers of MDCKII-MDR1, MDCKII-BCRP and human intestinal Caco-2 cells demonstrated that rilpivirine inhibits MDR1- and BCRP-mediated efflux of abacavir and increases its transmembrane transport. *In vivo* experiments confirmed inhibition of Mdr1/Bcrp in the small intestine, when rilpivirne was intraduodenally co-administered with abacavir to male Wistar rats. A result of this transporter-mediated DDI we could observe significant increase in oral bioavailability of abacavir.

In conclusion, rilpivirine inhibits MDR1 and BCRP transporters and may, therefore, affect pharmacokinetic behavior of concomitantly administered drugs, which are substrates of these ABC drug transporters.

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## PHARMACEUTICAL ANALYSIS SECTION

### DEVELOPMENT OF SAMPLE PREPARATION STEP FOR UHPLC-MS/MS ANALYSIS OF *CANDIDA ALBICANS* QUORUM-SENSING MOLECULES

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*Candida albicans* belongs to commensal fungi as a member of a gastrointestinal, oropharyngeal and female genital indigenous microbiota. However, it is also a pathogen causing life-threatening diseases in immunodeficient individuals. *C. albicans* can grow as a commensal budding yeast and invasive hyphae or pseudohyphae. The morphological conversion of these forms is affected by physico-chemical factors together with the effect of quorum-sensing molecules (QSMs). QSms are extracellular signals which can regulate virulent, morphological and physiological properties through activation of proper genes. The QSMs identified in *C. Albicans* are farnesol and tyrosol.

High-throughput approach based on solid phase extraction (SPE) using pipette tips (μ-SPE-PT) and microextraction by packed sorbent (MEPS) were developed for the isolation and quantification of farnesol and tyrosol in vaginal washing samples. The extracts were analysed by fast, selective and senstive UHPLC-MS/MS method. The analytes were separated on Acquity BEH C18 (2.1 × 50 mm, 1.7 μm) analytical column with gradient elution using 0.075% formic acid and acetonitrile with 0.075% formic acid at flow-rate 0.2 mL $min^{-1}$. Quantification of analytes was performed by selected reaction monitoring (SRM) using the precursor ions $[M+H-H_2O]^+$ and the corresponding product ions.

Both methods were validated in terms of precision, accuracy, range, linearity, limit of detection, limit of quantification and matrix effects according to European Medicine Agency Guideline on bioanalytical method validation.

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### NANOFIBER POLYMERS AS SORBENTS FOR SOLID PHASE EXTRACTION IN ON-LINE HPLC SYSTEMS

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Sample pretreatment is mostly the essential part of sample analysis. Recent trends in sample pretreatment are focused on finding new possibilities in extraction technology and on increasing the speed of the analysis. Solid phase extraction (SPE) is one of the most popular sample pretreatment technique. The aim of the presented work is to demonstrate the possibilities of nanofibers as novel sorbents for SPE with high potential to be also used in on-line extraction coupled into chromatography systems (HPLC).

Nanofibers have great sorption area thanks to a small fiber size and due to this fact could have potential as great sorbents in solid phase extraction. Four available nanofiber polymers (polyamide 6 in two forms, ε-polycaprolacton and polystyrene) were tested and their extraction efficiencies were compared with a reversed-phase C-18 sorbent. Tested analytes were chosen from the group of pyrethroids and carbamates. The conditions for on-line nanofibrous SPE-HPLC (valve switching time, sample washing step, HPLC mobile phase composition) were optimized. The system suitability parameters, extraction efficiency, sorbent reuse and linearity were tested. Stability of the nanofibers before and after all analyses was evaluated and shape-shifts were observed by scanning electron microscope.

In this study, polyamide 6 appeared to be a sorbent for lipophilic analytes comparable with the C-18 sorbent and provided a similar retention and extraction efficiency for all of the target analytes.

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## UHPLC-MS/MS DETERMINATION OF URINARY RETINOL

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Vitamin A plays a crucial role in many biological processes such as reproduction, vision, growth and development as well as immunity. Its antioxidant activity is also essential.[1] Renal proximal tubule cells are very important for the metabolism and homeostasis of vitamins (especially vitamins A, D, B12). Healthy humans have no detectable quantities of retinol in urine. In contrast, urinary retinol can be detected in patients with kidney pathology, liver disease, diabetic nephropathy, neoplasm and possibly other conditions.[2,3]

Studies show, that urinary retinol might be use as an early state biomarker for detection of kidney failure in contrast with currently used markers.

To date, there is no clinically useful chromatographic method for measurement urinary retinol and creatinine, therefore the new UHPLC-MS/MS method for determination of retinol and creatinine has been developed and will be discussed.

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## EVALUATION OF EQUIVALENCE OF POLYSACCHARIDE STATIONARY PHASES FOR SUPERCRITICAL FLUID CHROMATOGRAPHY

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Enantiomeric purity control is one of major concerns in pharmaceutical industry. Enantioselectivity between LC methods and SFC changes, due to different solvent interactions. In this study the evaluation of selected chiral stationary phases from different manufacturers based on tris(3,5-dimethylphenylcarbamate) of cellulose and amylose was performed using UHPSFC. Four pairs of immobilized and coated stationary phases were evaluated based on created evalutation model using 37 pairs of enantiomers including API, their intermediates and metabolites. Evaluated parameters were qualitative characteristics of peak ($A_s$, $w_{50}$) and the quality of separation itself (k, $R_s$). Gradient elution with column temperature set to 40 °C, ABPR pressure of 12 MPa and flow-rate of 2.0 ml/min was used. For primary screening 10 mobile phases with additives were chosen and screened on 2 representative chiral stationary phases, one based on amylose and one on cellulose. Three best performing mobile phases were used for all of the tested columns. An equivalence of columns with the same chiral selector was not confirmed. The best performing column based on success rate of enantioseparation (22 out of 37) was Chiralcel OD-3 with modifier MeOH + 0.1% TFA/DEA, thus being the most generic conditions. For separation of all enantiomers the least combination of columns and their respective mobile phases were 5. Pharmacopeias descriptive parameters of chiral columns include the chiral selector and particle size. Considering the chiral recognition mechanism, its complexity and non-equivalence of stationary phases, these descriptive parameters are not sufficient for successful introduction of chiral SFC pharmacopeia articles.

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## STUDY OF "LAB-IN-SYRINGE" ANALYSIS FOR THE AUTOMATION OF MICROEXTRACTION PROCEDURES

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The "Lab-In-Syringe" (LIS) technique is a versatile automation technique, able to speed up liquid-liquid microextraction. It uses the void of a syringe pump of a sequential injection analysis system as extraction vessel. Low consumption of extraction solvent is in good agreement with the objectives of green chemistry.

For the first time dispersive liquid-liquid extraction followed by dispersive back-extraction using the automation technique Lab-In-Syringe has been studied for the determination of Cd, Cu and Pb by inductive coupled plasma atomic emission spectrometry (ICP-AES). Ammonium pyrrolidinedithiocarbamate was used as chelating reagent and toluene as an extraction solvent for the metal complexes. In a first step, the extraction method was optimized using a Box-Behnken design. In a second step and system configuration, turning the syringe pump up-side down, in-system automatic back-extraction was performed immediately after extraction. Finally, on-line coupling to ICP-AES was studied on various sample types yielding quantitative recovery.

In the current work, a novel automation approach and alternative to previous solvent dispersion-based extraction and backextraction is developed. For this, a stirring bar, coated with a porous, solvent-impregnated, hollow fiber (HF) membrane tube, has been used for automatic sample clean-up for spectrophotometric determination of nitrophenols following recently proposed "Magnetic Solvent Bar Microextraction".[1] Extraction into the solvent filled HF pores is carried out from acidified standards followed by backextraction in alkaline medium. Materials for stirring bar coating, type of extraction solvent, and automation of HF soaking with extraction solvent have been studied so far. Optimization of the extraction parameters, measurement of the real samples and eventually separation of nitrophenols are planned.

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## LOVASTATIN DETERMINATION IN DIETARY SUPPLEMENTS BY A FULLY AUTOMATED MIP-SPE PROCEDURE

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Lovastatin, also known as Monacoline K, is a drug used in reduction of cholesterol levels. It is also naturally occurring in red yeast rice, which is used as a component of dietary supplements with effect on cardiovascular system and control of cholesterol blood level.

The aim of this work was to apply a laboratory-prepared molecularly imprinted polymer (MIP) as a sorbent for pre-concentration of lovastatin by solid-phase extraction (SPE) prior its determination by UHPLC-MS method. After preliminary studies of MIP sorbent properties performed off-line, automation of this MIP-SPE procedure was carried out using low-pressure sequential injection chromatography system (SIChrom$^{TM}$) composed of a robust bidirectional syringe pump, an 8-port selection valve, an SPE mini-column with MIP sorbent (MIP-SPE), a flow cell and a UV detector. Such system enabled automation of the MIP-SPE procedure with some benefits. Compared to the off-line performed MIP-SPE procedure, automated one shows faster and more robust performance (due to lower volumes of solutions and sample needed and programming of flow-rate in each step), and also enabled on-line pre-treatment directly followed by UV detection in one closed system.

The experiments concerning an optimization of aspirated volumes of sample and solvents and corresponding flow-rates in each step were performed. The optimized MIP-SPE procedure was validated and applied to analysis of four dietary supplements containing red yeast rice to prove that this procedure can be applied to analysis of real samples. The obtained results and the comparison of lovastatin content with manufacturer's data will be presented.

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## APPLICATION OF CHARGED AEROSOL DETECTOR IN ANALYSIS OF BIOLOGICALLY ACTIVE SUBSTANCES IN FOOD SUPPLEMENTS

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Detection of any nonvolatile or semivolatile analyte without a chromophore or a fluorophore in its molecule, gradient compatibility and very good sensitivity are main advantages of the universal evaporative Corona-charged aerosol detector (CAD).[1] This type of detector is very suitable alternative to routinely used UV (PDA) detector for analysis of biologically active substances with low or no response in UV spectrum contained in food supplements, such as plant sterols and stanols.

Phytosterols and their esters are natural steroids that are important structural components of plant membranes. Most phytosterols contain 28 or 29 carbons and one or two carbon–carbon double bonds. Phytostanols are a fully-saturated subgroup of phytosterols (contain no double bonds). In addition to the free form, both phytosterols and phytostanols occur as conjugates in which the 3 –OH group is esterified to a fatty acid.[2]

In our study it was proved that sensitivity of CAD is approximately three-times better than UV under 210 nm, and that CAD is capable of a detection of compounds not detectable in the UV spectrum. The developed method provided a faster analysis with higher sensitivity, the separation of more compounds and a wider calibration range than earlier described methods employing evaporative detectors (ELSD). In the most GC methods (except that with MS detection) which are commonly used for analysis of phytosterols a derivatization of free sterols after saponification had to be done prior to the analyses. With the use of CAD, no derivatization is needed, which saves the labor-time and yield of the extraction.

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## STUDY OF CHROMATOGRAPHIC BEHAVIOR OF SELECTED ANALYTES ON THE MIXED MODE STATIONARY PHASES

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Charged Surface Hybrid (CSH) columns are mixed mode stationary phases, which combine reverse phase particles and anion exchange mechanism. CSH technology should provide symmetric peak shapes, high efficiency and loading capacity for basic compounds when using acidic mobile phases with low ionic strength. Three CSH columns were used for the screening: CSH C18, CSH Phenyl-Hexyl, CSH Fluoro-Phenyl (2.1 × 100 mm; 1.7 μm).

Pharmaceutically important compounds with basic properties (Cytosine, Uracil, Pindolol, Acebutolol, Celiprolol, Desipramine, Imipramine, Amplodipine, Vortioxetine) as well as with acidic and neutral properties (Paracetamol, Risperidon, Telmisartan, Prednisolone, Triamciolone, Losartan, Propylparaben, Ketoprofen, Flurbiprofen, Indometacin, Diclofenac) were used in this study.

The study was conducted on UHPLC system Acquity UPLC with PDA detection at 254 nm. The separations were performed using gradient elution with mobile phases consisting of acetonitrile and buffers with different pH ($HClO_4$ + $Na_2SO_4$, HCl + $Na_2SO_4$, $H_3PO_4$ + $Na_2SO_4$, $KH_2PO_4$, $NH_4H_2PO_4$) or acidic solutions (formic acid, hydrochloric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, perchloric acid). The influence of the concentration of acidic solutions (0.01–0.5%) and the effect of pH (1.0–3.1) was

tested. The comparisons of efficiency and peak shapes as well as the comparison of retention times as a function of pH of selected acidic mobile phases were made.

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## UHPLC-MS/MS METHOD AS A POWERFUL TOOL FOR PERSONALIZATION OF VANCOMYCIN THERAPY

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Several guidelines and clinical studies for optimal vancomycin dosing and monitoring were published. However, in some special clinical situation, the recommended dosage of vancomycin could be inadequate. This problem can occur in patients where severe changes in liquid volume in the "third space" accompany some clinical states.

This part of work was focused on the development and validation of the method for determination of vancomycin in human serum, urine and peritoneal/pleural effusion using a Nexera® UHPLC system with Triple Quadrupole Mass Spectrometer LCMS 8030 (Shimadzu, Japan). Combination of YMC Meteoric Core C18 BIO column, 2.7 µm particle size, 100 × 4.6 mm (YMC Europe GmbH, Germany) and water/acetonitrile with 0.1% (v/v) FA mixture in gradient mode provided the best results of chromatographic separation. Simple protein precipitation and sample dilution were applied for sample pre-treatment. Only 50 µL of sample was required. Vancomycin and teicoplanin (IS) were determined by using multiple reaction monitoring (MRM) transitions. This UHPLC-MS/MS method, same for all listed matrices, represents a powerful approach for vancomycin dose optimization clinical study.

*The work was co-financed by IGA NT14089-3/2013 and SVV 260 292.*

## OPTIMIZATION AND PREPARATION OF FLAT SHEET POLYSULFONE MEMBRANE DEVELOPED FOR BIOLOMECULES SEPARATION

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Polysulfone (PSf) porous membranes are nowadays used in wide range of separation methods for various types of molecules. Hemodialysis (HD) is one of the fields, where PSf plays very important role as a key synthetic polymer used for production of dialysis membranes. PSf membranes have gained their major position due to their mechanical stability, possibility to control pore size and feasibility to introduce the hydrophilic character by additives.[1] Despite the increase in membrane biocompatibility, the long term contact of HD patients with artificial material causes consistent presence of chronic inflammation and systemic oxidative stress by the patients. Therefore, the trends in membranes improvement are focused on diminishment of these negative impacts of HD procedure.[2]

In the present work, the developed flat sheet membrane was composed by PSf as a polymer, N-methylene-2-pyrrolidone as a solvent, PVP/PEG as an additive and was obtained by spin coating and phase inversion technique. The effect of amount of additives and coagulation bath characteristics were evaluated relating to the membrane structure and solute removal ability.

The membrane prepared with PVP showed better results concerning to solute removal (urea, lysozyme, albumin) when compared to PEG. Moreover, the temperature of coagulation bath (ultrapure water) showed important effect on the removal characteristic. In conclusion, both, the type of additive in casting solution as well as the process of phase inversion significantly influence the biomolecules permeability adequate for hemodialysis.

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## MOLECULARLY IMPRINTED SOLID PHASE EXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY – COMPARISON OF ON-LINE AND OFF-LINE EXTRACTION APPROACH

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Molecularly imprinted polymers (MIPs) belong to highly selective group of sorbents for solid phase extraction (SPE). Similar to immunoaffinity sorbents, MIPs are prepared with cavities for selective recognition of analyte by polymerization with desired template (analyte itself or its analogue). Molecularly imprinted solid phase extraction (MISPE) includes usual steps – conditioning of the sorbent, loading of sample, washing out interferences and finally elution of the extracted compounds. Automation of the tedious process would be beneficial, only the optimization is more challenging.

In presented study, we connected MISPE to HPLC using column-switching technique. The main steps or choice of solvents were kept as was proposed for the off-line method to maintain MIP selectivity, but for example incompatible washing solvent or evaporation had to be dismissed totally. For that reason, clean-up of the matrix interferences and preconcentration was not as efficient as with off-line method.

Both HPLC methods, using off-line MISPE and on-line column-switching approach, were validated and compared to each other. Although automation enables significant time saving, less human errors and handling with toxic sample, it was reached lower concentrations with off-line method due to preconcentration and better clean-up.

Troubles and compromises during optimization of on-line MISPE-HPLC and critical evaluation are going to be presented in detail on method for determination of mycotoxin patulin.

*The study was supported by the project of specific research, no. SVV 260 292, and by the Charles University Grant Agency, project no. 726 316.*

## UHPLC-MS/MS METHOD FOR EVALUATION OF THE EFFECT OF FLAVOURING ADDITIVES ON THE CONTENT OF CATECHINS IN TEA SAMPLES AND ON MATRIX EFFECTS

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Catechins belong to the group of flavanols (derivatives of flavan-3-ol), and are major components of green tea. This work is based on the findings that were previously published[1] where the incidence of matrix effects in several samples of flavored tea infusions was published. The catechin content in flavored teas was found to be lower than in non-flavored forms. One possible explanation for this phenomenon is the presence of matrix effects or other flavorings influence on their content.

In this study commercially available teas and mixtures prepared in our laboratory were analyzed. The aim of this project was the measurement of the catechin content and the occurrence of matrix effects, which can negatively or positively influence the analysis. Matrix effects were evaluated using the method of comparison of the calibration curve slopes. From the perspective of the structure of the analytes, it was necessary to perform UHPLC-MS/MS analysis, which was able to distinguish between individual catechin epimers and gallocatechins. The analyzed catechins were found in only three flavors (rosehip, cranberries, cinnamon bark). Flavors with the most positive influence on catechins content were ginger root (+26.8%), cranberry fruit (+21.3%) and mint leaf (+21%). Significant matrix effects (greater than 15%) were observed only for the cranberry fruit and rosehips flavors. Cranberry fruit flavor caused the positive matrix effect in all studied catechins, but the significant matrix effects were observed only for GCG (+36%) This study showed the independence of matrix effects on pH and positive effect of flavors on the level of catechins

in the freshly prepared mixtures. This addition was not caused by matrix effects or by adding flavors. On the other hand, in commercial mixtures the lower level of catechins could be caused by different composition of the tea. More complex mixtures showed the combined effect. Our findings suggest that the added flavors, influenced the level of catechins in another way than by the matrix effects. The observed matrix effects were generally lower than we expected.

*The study was supported by SVV 260292/2016*

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## DEVELOPMENT OF CE METHOD FOR THE SEPARATION OF FLAVONOLIGNANS OCCURING IN MILK THISTLE (*SILYBUM MARIANUM*)

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The project deals with the development of micellar electrokinetic chromatographic (MEKC) method for the determination of structurally similar flavonolignans silybin A (SBA), silybin B (SBB), isosilybin A (ISBA), isosilybin B (ISBB), silychristin (SCH), silydianin (SD) and their precursor taxifolin (TX) in *Silybum marianum*.

The separation was carried out in a fused silica capillary (internal diameter 50 μm, total length 48.5 cm and effective length 40 cm), with UV detection at 200 and 320 nm. The capillary temperature was maintained at 25 °C. The method for separation of all main flavonolignans (including the diastereomers SBA/SBB, and ISBA/ISBB) was optimized by examining a number of experimental conditions, such as concentration of boric acid, concentration of sodium dodecyl sulfate (SDS), concentration of cyclodextrins (CD), volume fraction of organic modifier and the applied voltage. The separation of diastereomers SBA/SBB was achieved only by the addition of SDS as pseudostationary phase, while the separation of ISBA/ISBB was possible only with the addition of cyclodextrins. Optimal conditions for the separation of all the flavonolignans were: 140 mM SDS, 5 mM 2-hydroxypropyl-β-CD, 100 mM boric acid (pH 9.0 adjusted with 1M NaOH), 10% (v/v) MeOH, and applied voltage 25.0 kV. Such separation conditions found by univariate optimization did not provide base-line separation of all flavonolignans (the resolution $R_S$ between the SBA/SBB and ISBA/ISBB diastereomers was 1.22 and 1.40, respectively).

Chemometric approach (design of experiments) will be applied as future step for optimizing the CD-MEKC separation conditions to achieve base-line separation of all main flavonolignans.

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## DEVELOPMENT OF UHPSFC-UV-MS ACHIRAL SCREENING APPROACH FOR IMPURITY PROFILING

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Ultra-high performance supercritical fluid chromatography has become important complementary technique in separation science. Its use in impurity profiling has been limited until recently. However, the interest in SFC separations of API and its achiral impurities increased. In this study, 10 various real case QC 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. Over all, about seventy different pharmaceuticals were analyzed by SFC-UV-MS and evaluated. The separations were accomplished using eight different stationary phases (Torus DIOL, Torus DEA, Torus 2-PIC, Torus 1-AA, BEH 2-EP, BEH, CSH PFP and HSS C18). The influence of 3 modifiers (MeOH, EtOH, IPA) and 3 modifier blends (MeOH/ACN, MeOH/EtOH, EtOH/ACN) and 5 additives in methanol (0.1% formic acid, 10 mM ammonium formate, 10 mM ammonium acetate, 0.1% ammonium hydroxide and 2% water) was tested.

Resulting chromatograms were evaluated in 6 parameters: number of eluted peaks, number of separated peaks, resolution between API and following impurity, peak symmetry, peak width at 50%, and selectivity. The mobile phases as well as stationary phases were compared and the best conditions for each application were selected. Based on the balanced representation of neutral and acidic/basic compounds and the use of real case pharmaceutical mixtures of API and impurities, some recommendation for method development using screening approach were made. Volatile additives in SFC mobile phase (ammonium formate, ammonium acetate, ammonium hydroxide) provided generic approach enabling to obtain very good chromatographic performance in impurity profiling. Torus DIOL seemed to be the most generic stationary phase and column HSS C18 offered unique complementary selectivity.

*The study was supported by SVV 260292/2016.*

## UHPLC-MS/MS METHOD FOR STABILITY STUDY OF SOBUZOXANE IN BIOLOGICAL MATRICES

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Pro-drug sobuzoxane (SBZ) was synthetized to improve bioavailability of an anticancer agent – bis-dioxopiperazine ICRF-154 belonging to a group of topoisomerase II inhibitors. SBZ is probably immediately conversed to ICRF-154, that may be further metabolised to a strongly chelating agent – EDTA-diamide. Nevertheless, there is no method capable of simultaneous analysis of SBZ and its metabolites (ICRF-154, EDTA-diamide) in relevant biological materials that could be used for comprehensive bioactivation study.

Hence, the aim of this work was to develop such method and utilize it for a pilot investigation of stability and bioactivation of SBZ in plasma and cell culture medium. Plasma and DMEM cell medium were incubated with SBZ (100 μM and 30 μM, 37°C) up to 24 hours. Plasma was precipitated with ice-cold methanol or methanol with addition of 0.1% formic acid. Cell medium was simply diluted with the same solvent. All analyses were performed on Nexera UHPLC system coupled with LCMS-8030 triple quadrupole mass spectrometer with ESI ion source (Shimadzu). Zorbax SB-Aq chromatographic column (3 × 100 mm, 1.8 μm, Agilent) and mobile phase composed of 1 mM ammonium formate and methanol in a gradient mode provided the best separation. The stability/bioactivation study showed that SBZ is quickly converted in plasma to ICRF-154 that is further changed to EDTA-diamide. Already after 8 minutes of incubation SBZ was not detectable and appropriate gain of ICRF-154 and EDTA-diamide was determined. In DMEM medium ICRF-154 and EDTA-diamide were not found up to 24 hours.

Developed method will be further modified and utilized for analysis of samples from *in vitro* study on cardiac cells and *in vivo* study on rabbits.

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## TESTING OF SEVERAL STATIONARY PHASES IN THE ANALYSIS OF B VITAMINS

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Water-soluble vitamins, especially B-group vitamins, are well studied compounds that are essential and play key metabolic functions in cellular energy metabolism. One of the least stable and frequently overlookd water-soluble B-group vitamin is vitamin $B_1$ (Thiamine) and its biologically active esterified forms. Discrepancies in thiamine status may cause serious health problems in patients with long term intensive care.[1,2]

Application of chromatographic methods especially on biological samples brings benefits for the separation and quantitation of water soluble vitamins in terms of sensitivity, time and economical demands. Several types of modern reversed phase columns and conditions have been assessed in this work to analyze whole blood concentration of thiamine and its derivatives. Better overall performance characteristics and improved peak capaci-

ties were observed with new type of core-shell particle columns Meteoric core-BIO. It helped to shorten analysis time, decrease solvent consumption and allow the detection of smaller concentrations of blood thiamine derivatives. In sample preparation it was possible to test new approaches without the loss in sensitivity during chromatographic analysis. Unique core-shell properties, wide pores designed for high throughput analysis of biological samples and stability under wide pH range ensured longer column lifetime and proved its suitability for clinical applications.

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## SECTION OF PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS

### BIOLOGICAL ACTIVITIES OF *PAPAVER RHOEAS* L. ALKALOIDS

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Aerial parts of *Papaver rhoeas* L. (Papaveraceae) were extracted with ethanol and fractionated using light petrol and chloroform on alumina column. Subsequently, additional column chromatography and repeated preparative TLC led to the isolation eight isoquinoline alkaloids belonging to rhoeadine, protopine, protoberberine and benzyltetrahydroisoquinoline structural types. Chemical structures of the isolated alkaloids were elucidated by optical rotation, spectroscopic and spectrometric analysis (NMR, MS) and comparison with literature data. Compounds isolated in sufficient amounts were tested on human blood acetylcholinesterase (AChE), human plasma butyrylcholinesterase (BChE), recombinant prolyl oligopeptidase (POP) inhibitory activities, and cytotoxic effects against selected carcinoma cell lines. Alkaloids inhibited enzymes in a dose-depend manner, and the most cholinesterase inhibitory activity demonstrated cheilanthifoline and glaucamine with the $IC_{50}$ values of 139.9 ± 26.0 μM (AChE) and 53.7 ± 4.8 μM (BChE), and the $IC_{50}$ values of 181.9 ± 17.4 μM (AChE) and 73.3 ± 7.8 μM (BChE), respectively. Reference cholinesterase compounds were galanthamine, eserine and huperzine A. Furthermore,

glaucamine and pseudocodamine showed the best POP inhibition with $IC_{50}$ values of 237 ± 4.0 μM and 298 ± 11 μM, respectively. *Z*-Pro-prolinal and berberine were used as POP standards. All isolated alkaloids were considered inactive against selected carcinoma cell lines in comparison with the reference standard doxorubicine.

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## EXTRACTION, ISOLATION, IDENTIFICATION, AND BIOLOGICAL ASSESSMENT OF ASTAXANTHIN ESTERS FROM MICROALGAE *HAEMATOCOCCUS PLUVIALIS*

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Astaxanthin (AXT), the most powerful antioxidant found on nature, is a red pigment that has been attracting a considerable interest from the scientific, biotechnological and commercial sectors due to its noticeable and varied bio-functional properties of great significance in human health and nutrition. The microalgae *Haematococcus pluvialis* is considered to be the major AXT natural source, where AXT occurs mainly as monoesters (70%) and diesters (25%) along with a low content of non-esterified AXT (5%).[1] The esterified forms have been recently shown to exert better benefits than the non-esterified AXT.[2] However, no scalable isolation methods have been yet developed for obtaining these carotenoids from their natural source. Moreover, their bioactivity has not been extensively investigated. In the present proposal, conventional and supercritical fluid extraction (SFE) methods will be integrated with countercurrent chromatography (CCC) technology for obtaining pure AXT esters from *H. pluvialis* biomass. The resulting isolated AXT esters will be assessed for their antioxidant, antiaggregant, vasodilatory, immunomodulatory and anti-tyrosinase capacity.

*The study was supported by SVV 260294.*

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## ALKALOIDS FROM *NARCISSUS* CV. PROFESSOR EINSTEIN (*AMARYLLIDACEAE*) – ISOLATION AND BIOLOGICAL ACTIVITY

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More than 500 *Amaryllidaceae* alkaloids (AmA) have been detected in plants of many different species belonging to the *Amaryllidaceae* family. These alkaloids showed wide range of biological activities. They are isolated from plant material and tested for their possible use in treatment of various illnesses. The most important AmA is galanthamin which is already used in the treatment of Alzheimer's disease as an inhibitor of human erythrocytic acetylcholinesterase (HuAChE; $IC_{50,HuAChE} = 1.5 \pm 0.2$ μM).[1] Some alkaloids display more biologic activities together. Active AmA serve as a template for a synthesis of series of semisynthetic analogues. Among the most widely used template AmA belong lycorine and haemanthamine.

Summary alkaloidal extract has been prepared from ca 34 kilograms of fresh bulbs of *Narcissus* cv. PROFESSOR EINSTEIN (*Amaryllidaceae*) and separated by column chromatography ($Al_2O_3$). Almost five hundred fractions were collected and, based on analytical TLC, pooled into 27 subfractions. Fourteen substances were already obtained in pure form so far and identified as eugenine, epimaritidine, 8-*O*-demethylmaritidine, ismine, haemanthamine, hippeastrine, homolycorine, 9-*O*-demethylhomolycorine, lycorine, lycoraminone, massonine, narwedine, norpluviine and tazettine. Lycorine, haemanthamine and hippeastrine are currently used for the preparation of their semisynthetic analogues. All substances which have been obtained are successively tested for their biologic activities e. g. inhibition of HuAChE and HuBuChE (human butyrylcholinesterase), POP (prolyl oligopeptidase), GSK 3β (glycogen synthase kinase-3β) and AKR1C3 (aldo-keto reductase 1C3); antineoplastic, antimicrobial and antimalarial activity.

*The study has been supported by SVV 260 292.*

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## THE IDENTIFICATION OF THE ISOLATED ALKALOIDS FROM *GLAUCIUM FLAVUM* EMPLOYING NMR SPECTROSCOPY

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Two presented alkaloids were isolated from *Glaucim flavum (Papaveraceae)* at the Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Hradec Králové. Papaveraceae family is rich in specific alkaloids, mainly in isoquinoline alkaloids.

The isolated substances were characterized employing basic $^{1}H$ and $^{13}C$ NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC, gCOSY and NOESY.

Both of these isolated compounds have been already described in the literature as chelidonine (Fig. 1) and norchelidonine (Fig. 2).

The isolated alkaloids were subjected to the screening of their biological activities on acetylcholinesterase and butyrylcholinesterase. The compounds possessed inhibitory effects. Other biological activities will be investigated.

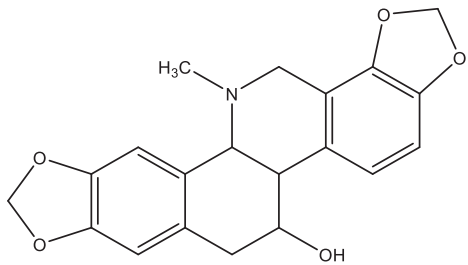


*Fig. 1.*

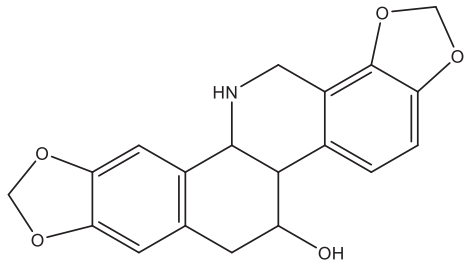


*Fig. 2.*

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## *IN VITRO* INTERACTIONS OF DIHYDROXYCOUMARINS AND THEIR CLOSE DERIVATIVES WITH COPPER

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Coumarins belong to plant secondary metabolites commonly occurred in many natural sources. They possess many potentially interesting biological properties including ability to chelate metals.[1]

The aim of the study was to test a series of coumarins of natural and synthetic origin[2] for interaction with copper at (patho)physiologically relevant pH conditions by use of our previously published approaches.[3,4] Tested compounds included mostly dihydroxycoumarins but also their close derivatives for the analysis of structure-activity relationship.[2]

Compounds with *o*-dihydroxyl group had the largest copper chelating (hematoxylin method) and reducing properties. There was little difference between 6,7- and 7,8-dihydroxycoumarins including side chain modifications of the latter. Monohydroxy-, hydroxymethyl- or dimethoxycoumarins seemed to be almost free of chelating properties but were able to reduce cupric ions. Beyond *o*-dihydroxycoumarins, only 7,8-diacetoxycoumarin chelated copper ions. Interestingly, in a more competitive ambient (bathocuproine assay), none of coumarins chelated significantly copper.

In conclusion, coumarins are active copper reductants and although *o*-dihydroxycoumarins are able to chelate copper, their chelating properties are lost upon competition with a more potent chelator.

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## ALKALOIDS FROM *MAGNOLIA* × *SOULANGEANA* AND *GLAUCIUM FLAVUM* AND THEIR BIOLOGICAL ACTIVITY

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Alzheimer's disease (AD) is neurodegenerative disease with specific neuropathological changes with impact on cholinergic system. Natural products are source of potentially active compounds with neuroprotective effects.[1]

The species *Magnolia* × *soulangeana* (saucer magnolia) and *Glaucium flavum* (*yellow horned poppy*) have been chosen as source of alkaloids for *in vitro* studies.

The primary extracts was acquired from dried magnolia flowers (30 kg) or dried aerial parts of *yellow horned poppy* (0.8 kg) by extraction with ethanol and then was treated by liquid extraction with different pH. Single alkaloid extracts were treated by standard chromatographic methods. Alkaloid structures were determined by spectroscopic methods (MS, NMR) and optical rotatory was ascertained. All isolated alkaloids were subsequently tested for their inhibition activity in term of human erythrocytary acetylcholinesterase (HuAChE), human-serum butyrylcholinesterase (HuBuChE) and prolyl oligopeptidase (POP).

Different types of alkaloids have been isolated: protopine type alkaloids (+)-protopine, allocryptopine; aporphine type alkaloids liriodenine, (+)-glaucine, dehydroglaucine, (+)-cataline, (+)-isocorydine and (+)-*N*-methyllaurotetanine; morphinane type alkaloid (–)-pallidine; benzylisoquinolines (+)-coclaurine, (+)-*N*-methylcoclaurine, (+)-armepavine, (+)-*N*-norarmepavine and (+)-reticuline; and benzophenanthridine type alkaloids chelidonine and (−)-norchelidonine. The benzophenanthridine type alkaloids were potent inhibitors of HuAChE with $IC_{50}$ approximately 32 μM. Potent inhibitors of HuBuChE were benzylisoquinoline alkaloids *N*-methylcoclaurine and reticuline with $IC_{50}$ 15.02 ± 1.35 μM and 33.85 ± 4.99 μM respectively. Other isolated alkaloids were considered to be inactive ($IC_{50}$ > 100 μM). Any alkaloid did not show significant POP inhibition activity; alkaloids were considered to be inactive ($IC_{50}$ > 100 μM).

Alkaloids isolated from saucer magnolia flowers and aerial part of *yellow horned poppy* were not potent compounds for AD treatment, although *N*-methylcoclaurine, reticuline, chelidonine and norchelidonine could serve as lead structures for preparation of semi-synthetic cholinesterase inhibitors.

*This study was supported by SVV 260 292 and PRVOUK-P40.*

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## PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

### *IN VITRO* CARDIOPROTECTIVE ACTIVITY OF NOVEL DEXRAZOXANE ANALOGS JR-311 AND JAS-2

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Dexrazoxane (DEX) is so far the only approved and clinically available cardioprotective compound for the prevention of the cardiomyopathy caused by anthracyclines (ANT). Unfortunately, the precise mechanism of DEX cardioprotection or structure-activity relationship of DEX or other bis-dioxopiperazines have not been fully resolved.

The aim of our work is the study of the biological activity of the newly synthesized compounds regarding their cardioprotective activity in the *in vitro* model of ANT cardiotoxicity on isolated neonatal rat cardiomyocytes, antiproliferative activity using human promyelocytic leukaemia cell line HL-60, their ability to chelate free catalytically active iron ions and also for their ability to inhibit the catalytic activity of topoisomerase II or modulate its content in isolated neonatal cardiomyocytes. The two novel analogues share the ability to inhibit the activity of purified human topoisomerase II in solution, but differ in the cardioprotective activity. Further studies revealed a stability issue in JR-311 with its rapid metabolization. Restoration of cardioprotection was observed after a modification of the dosing scheme.

These results help in elucidating the mechanisms of the DEX-afforded protection of the ANT-induced cardiomyopathy.

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## SULFORAPHANE AND ITS ABILITY TO AFFECT BIOTRANSFORMATION ENZYMES IN RAT

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A diet-based isothiocyanate, sulforaphane (SF), is found especially in the cruciferous vegetables (*Brassicaceae*) such as broccoli or cabbage. SF has many beneficial effects on human health such as antioxidant, anti-inflammatory, and anticancer.[1] Its effect on the enzymes of the phase I and II biotransformation has been investigated in various human and animal cell cultures, subcellular fractions and *in vivo*, but little is known about its effect on non-cancer cells, e.g. hepatocytes.

The aim of our study was to evaluate an effect of SF on the activities of the selected biotransformation enzymes in rat hepatocytes and rat liver subcellular fractions. The enzymes include cytochromes P450 (CYP), carbonyl-reducing, and conjugation enzymes. Except the enzyme activities, the mRNA gene expression of the tested enzymes was studied as well.

We found out that SF affects several detoxification enzymes in rat hepatocytes, but some of them only after inhibition or induction. SF increases an activity of glutathione S-transferase (GST), sulfotransferase (SULT) and aldoketoreductase 1C (AKR1C) after a primary inhibition by β-naphthoflavone (β-NF) in the hepatocytes. On the other hand, SF inhibits β-NF-increased CYP1A activity in the hepatocytes. There is an opposite effect of SF in NAD(P)H:quinone oxidoreductase 1 (NQO1) activity in the hepatocytes and

the subcellular fractions. The activity of CBR rapidly increased after SF-treatment in the hepatocytes. SF does not affect most of the enzymes in rat liver subcellular fractions. The mRNA levels of the tested enzymes were affected by SF as well but does not correlate accurately to the activity levels.

The obtained results show that SF affects the activities and expression of several phase I and II biotransformation enzymes.

*The study was supported by Czech Science Foundation, Grant No. P303/12/G163.*

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## MODULATION OF ACTIVITY AND RNA LEVELS OF CONJUGATION ENZYMES BY PRENYLATED FLAVONOIDS

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Prenylated flavonoids are a unique class of naturally occurring flavonoids and hops (flowers of *Humulus lupulus* L.), one of the beer components, are their main dietary source. They have been intensively studied mainly for their estrogenic properties. As the main route of administration of these agents is oral, we decided to study effect of xanthohumol (XH) and its active metabolites isoxanthohumol (IXH), 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN) on conjugation enzymes in intestine. Caco-2 cell line in proliferative (represent cancerous cells; P-cells) and differentiated (enterocyte-like cells, D-cells) form was chosen as model system.

We observed significant decrease in glutathione S-transferase (GST) mRNA levels in treated P-cells and D-cells after 72 h. However, GST activity did not differ from control in P-cells but it was increased in 6PN and 8PN treated D-cells. The inhibition of sulfotransferase (SULT) 1A1/3 mRNA was observed in D-cells after 72 h and the same effect was observed in SULT activity. Significant induction of catechol-O-methyl transferase (COMT) activity was observed in D-cells after 72 h treatment with all four prenylated flavonoids. Nevertheless, this induction was not found at mRNA level. COMT mRNA levels were decreased in XH and 6PN treated D-cells after 72 h of incubation. The activity of UDP-glucuronosyltransferase was not detected in microsomal fraction of all types of Caco-2 cells. UGT1A6 mRNA levels were detected in D-cells only, comparable in treated and control cells.

Based on the results, low concentrations of xanthohumol and its metabolites seems to be safe for human health, as the changes in the activity and mRNA expression of phase II enzymes were none or mild.

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## EFFECT OF SELECTED SESQUITERPENES TO DOXORUBICIN EFFICACY IN SENSITIVE AND RESISTANT CANCER CELL LINE

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Sesquiterpenes are secondary metabolites of plants, fungi, bacteria and marine invertebrates. The sesquiterpenes β-caryophyllene oxid (CAO), α-humulene (HUM), *trans*-nerolidol (NER) and valencene (VAL) are main components of *Myrica rubra* essential oil.[1] In our previous studies, we described the antiproliferative effect of this essential oil and its components and their ability to increase efficacy and accumulation of cytostatic drug doxorubicin in colon cancer cells.[2]

Present study has been focused on sesquiterpenes effects in lymphoblast cell lines with aim to compare their ability to affect doxorubicin efficacy in cell lines with different sensitivity to doxorubicin. Doxorubicin-sensitive lymphoblast cell line CCRF/CEM and its resistant subline CEM/ADR5000 were used for this purpose. In cell line CCRF, NER and CAO seemed to have synergistic effects, while HUM and VAL had additive effects to DOX treatment. In doxorubicin resistant cell line, HUM, CAO and NER had no effect, while VAL decreased efficacy of doxorubicin. The test with rhodamine 123 showed that all sesquiterpenes tested have ability to inhibit drug transporter P-glycoprotein, which is mainly responsible to doxorubicin resistance.

In conclusion, sesquiterpenes CAO and NER could potentiate efficacy of doxorubicin in doxorubicin-sensitive but not in doxorubicin-resistant cancer cells, even if they inhibit P-glycoprotein.

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## THE KEY VIRULENCE FACTORS OF *CANDIDA ALBICANS* ARE EXPORTED TO EXTRACELLULAR MILIEU *VIA* EXTRACELLULAR VESICLES PRODUCTION

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The release of effector proteins into extracellular space in fungi is essential for a number of physiological and pathophysiological events, including nutrition, intercellular communication, biofilm formation, adhesion, invasion to host cells or activation of host immune cells.

*C. albicans* is exqusively sensitive to changes in concentration of glucose and other nutrients in the environment.[1] In our study, we have focused on the impact of nutrient limiting condition in *C. albicans* extracellular vesicles EVs release and the EVs protein cargo analysis.

In our study, we have revealed statistically significant differences in EVs protein yields, in EVs released under stress condition. Especially, EVs of *C. albicans* clinical isolate strain carry considerable number of the well-studied *C. albicans* key virulence factors such as secreted aspartyl proteinases, enolase, or cell surface mannoprotein and some putative virulence factors as well.

*The study was supported by Fund of Dean and PRVOUK P40.*

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## REGULATION OF GLUTATHIONE PEROXIDASE 7 BY microRNAs

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The aerobic reactions in organisms lead to the formation of reactive oxygen species (ROS). Excessive accumulation of ROS causes cell damage and may contribute to the development of various diseases. In connection with that, the organisms created enzymatic and non-enzymatic systems for the detoxification of these compounds.

We specifically focus on Glutathione peroxidase 7 (GPx7), one of eight members of the glutathione peroxidase family. GPx7 is monomeric enzyme with antioxidant effect. It plays an important role as an intracellular stress sensor/transmitter, participates in maintaining redox homeostasis and protein folding and probably may act as an classical tumor marker. Lower expression or inactivation of GPx7 may lead to cancer development and may contribute to the development of obesity. GPx7 is highly expressed in preadipocytes and deficiency of GPx7 facilitated preadipocytes to differentiate to adipocytes and promoted both adipocyte hypertrophy and hyperplasia. MicroRNAs (miRNAs) are small noncoding RNAs responsible for the posttranscriptional regulation of a variety of human genes by binding mainly to their 3´untranslated region (3´UTR). To date, their involvement in the

regulation of GPx7 is unknown. This study reports the identification of several miRNAs and their involvement in the regulation of human GPx7. A negative correlation was identified between GPx7 and three miRNAs in two colorectal cancer cell lines (Caco2 and HT29).

We have prepared the plasmids with ligated 3´UTR region of GPx7 containing the miRNA binding sites. Simultaneously we prepared also several mutants with mutated recognition sites for each tested microRNA. Luciferase reporter assay showed that miR-29b and miR-137 directly targeted GPx7. Furthermore, overexpression of miR-29b and miR-137 impaired GPx7 expression in sw480 cell line.

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# PHARMACEUTICAL TECHNOLOGY SECTION

## PROLINE DERIVATIVES AS SKIN PERMEATION ENHANCERS


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Transdermal drug delivery possess many advantages, compared to conventional routes, however skin itself is admirable barrier protecting our body from excess water loss and from entering of pollutants and pathogens. So if we want to administer drugs into human body *via* skin we have to overcome this barrier, for example by the use of permeation enhancers; it means substances of both chemical and natural origin, which can reversibly decrease skin barrier properties for a short time, so drug can get into human body. For example, derivatives of amino acids, such as dodecylester of *N*-acetyl proline (L-Pro2), are potent enhancers.[1]

In this study, we modified structure of the enhancer L-Pro2, which is potent non-toxic substance with low dermal irritation,[1] and studied the effect of this structure modification on the permeation activity of the prepared substances.

First, we prepared analogues of L-Pro2 with acyl chain length ranging from 3 to 8 carbons (Pro3 to Pro8) and derivatives of 4-hydroxy- and 5-oxoproline with acyl chain length of 2 to 5 carbons. Afterwards we studied their permeation-enhancing activity *in vitro*, using human skin and different model drugs- theophylline (TH), diclofenac (DC) and hydrocortisone (HC).

Pro2, Pro3 and Pro4 enhanced flux of TH 26.6, 28.9 and 22.7 times, respectively and DC 6.4, 7.8 and 9.3 times, respectively, compared to control. With further prolongation of the acyl, the enhancement activity decreased, for example, flux of HC was enhanced only by Pro2.

Analogues derived from hydroxyproline enhanced permeation of both TH and HC, however none of such derivatives exceed the effect of Pro2; oxoproline derivatives showed only poor activity in enhancement of TH flux.

TEWL measurement on skin treated by selected enhancer Pro4 confirmed its reversible effect on skin barrier properties within 24 hours.

Cellular toxicity of Pro3 and Pro4 was studied on two cell lines (human keratinocytes-HaCaT and mouse embryonic fibroblasts 3T3). Toxicity of Pro3 and Pro4 increases with the acyl chain length on both cell lines, but is still comparable to Pro2.

Results of infrared spectroscopy of stratum corneum treated by Pro3 and Pro4 suggest, that mode of action of both enhancers could be based on their interaction with skin barrier lipids.

We found two new potent enhancers (Pro3, Pro4) with reversible action by modification of L-Pro2 structure. Both of them warrant further investigation, so we are going to study their mode of action and enhancing effect on flux of other perspective drugs through human skin.

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## RATIONAL DEVELOPMENT OF ORODISPERSIBLE TABLET FORMULATION: SORBITOL INCORPORATION

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Orodispersible tablets (ODT) are tablets designed to disintegrate within 3 minutes in patient's mouth.[1] Rapid disintegration, sufficient mechanical strength, and the taste are key parameters of ODT.[2]

In this study, effect of sorbitol addition to the previous formulation, composed of potato starch acting as filler, and crosscarmellose acting as superdisintegrant, was examined.

Sorbitol was added to the formulation as a filler, a sweetening agent, and to the povidone binding solution in weight ratios of 10, 20, 30, 40%. After fluid bed granulation, granules were evaluated for their particle size distribution, flow and compressibility prop-

erties and particle density. Then, magnesium stearate (0.5%) was added as a glidant and tablets of 7 mm in diameter were compressed using the different compression forces to achieve the starting radial strenght of 1 MPa. The diameter and the height of tablets was measured as well as the crushing strenght and the disintegration time.

The results proved, that out of the tested combinations, the granules containing 30% of sorbitol served the best flow and tablet properties. The granules had satisfactory particle size distribution and the mean particle size $X_{50}$ (350 μm), the appropriate flow rate (4.77 g/s) as well as the compresibility index (22.00%). Tablets had sufficient radial strenght (0.86 MPa), pleasant taste and disintegrated within two minutes (98 s).

*The study was supported by GAUK No. 322315/2015, SVV 260 291 and PRVOUK programme.*

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# NOVEL POLYAMIDOAMINO-DENDRIMERIC STRUCTURES: DESIGN, SYNTHETIC STRATEGIES AND POTENTIAL APPLICATIONS

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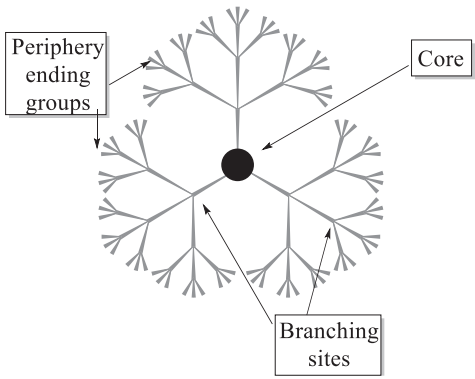
Highly branched, multivalent and monodisperse polymeric molecules, generally known as dendrimers (Scheme 1), have received continuous interest in recent years because of the global demand for new nanomolecules that are useful in advanced technology and medicine.[1]




*Scheme 1:* General structure of dendrimers.

The present work is summarizing our recent attempts to develop new structures of dendrimers possessing repeated amide and amine branching sites as well as their potential to be used in specific biological applications.

*The study was supported by the Faculty of Pharmacy in Hradec Králové (PRVOUK, 2016).*

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## EVALUATION OF COMPRESSIBILITY OF LIQUISOLID POWDERS

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Liquisolid systems preparation represents one of the most promising and innovative techniques for improving *in vivo* bioavailability of poorly soluble drugs. The functional principle of these formulations is conversion of the drug in the liquid phase (liquid drug; drug solution, suspension or emulsion) into an apparently dry powder by its blending with specific carriers. The carrier particles are subsequently coated with a material having high absorptive properties and large specific surface area giving the liquisolid system the desirable flow and compression characteristics.[1] The main benefit of the liquisolid systems represents their enhanced dissolution rate and hence improved *in vivo* bioavailability of poorly soluble drugs. Moreover, liquisolid systems show further advantages in comparison to conventional dosage forms including lower production costs; similar final processing as tablets or hard capsules and minimized pH influence on dissolution rate.[2]

However, it was established that powder material can retain only a limited amount of liquid while maintaining acceptable flow and compression properties. Therefore, the presented work aimed at the characterization of the flowable liquid retention potential and compressible liquid retention potential of the mixture of Neusilin® US2 (carrier) and polyethylene glycol 400 (solvent). The obtained results showed that 1 g of Neusilin® US2 can retain up to 1.16 g of polyethylene glycol 400, while maintaining acceptable flow properties. Moreover, it was observed that value of compressible liquid retention potential of Neusilin® US2 for polyethylene glycol 400 is 0.55.

*The study was supported by SVV 260 291.*

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## MONOLAYER LIPID MODEL OF B-GLUCOCEREBROSIDASE DEFICIENCY IN THE SKIN

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Ceramides (Cer) together with free fatty acids and cholesterol form the intercellular space of the uppermost skin layer, the *stratum corneum* (SC). This lipid matrix represents the skin barrier, which protects mammalian organisms against environmental factors (endogenous substances, physical radiation) and prevents body from water loss. Cer are synthetized from their polar precursors, glucosylated Cer (GlcCer) and sphingomyelins, by removing the polar part by hydrolytic enzymes βglucocerebrosidase (GlcCerase) and sphingomyelinase, respectively. A lack of these enzymes leads to accumulation of precursors and a disturbed skin barrier function. The goal of this work was to study the GlcCerase defects by monolayer lipid models of SC. The control monolayers contained Cer, free fatty acids and cholesterol. In the diseased models Cer were gradually (25%, 50%, 75%, 100%) replaced by GlcCer. The impact of GlcCer quantity on the lipid organisation was evaluated by several techniques (Langmuir monolayers at the air-liquid interface, Brewster angle microscopy and atomic force microscopy).

At the air-liquid interface, the mixtures with GlcCer do not organise spontaneously at low surface pressures (1.5 mN $m^{-1}$). However, with increasing surface pressure (20 mN $m^{-1}$) the area per molecule of the mixtures with GlcCer is lower than in the sample without the precursor. The lipid mixtures containing both Cer and GlcCer are more compressible, while compressibility of mixtures containing only Cer or GlcCer is low. GlcCer does not prevent lipids from forming domains, but they are smaller and at higher surface pressures some lipids can flip over into bilayers. It seems that GlcCer does not disturb the tight organization of lipids in the monolayer SC model but the presence of polar head influences the mutual interactions between lipids during the formation of lipid membranes.

*The study was supported by the Czech Science Foundation (13-23891S) and the Charles University (SVV 260 291).*

## BRANCHED POLYESTERS AS A PROSPECTIVE DRUG CARRIERS

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Poly lactic-*co*-glycolic acid (PLGA) branched on polyhydric alcohols or oligomeric substances are characterized by low degree of swelling, and continuous degradation by nonenzymatic hydrolysis.[1] They have been among the most attractive candidates for drug delivery systems and tissue engineering applications beacause of biodegradability, biocompatibility, wide range of erosion time, tunable physico-chemical properties and harmless potential.[2]

Our work deals with formulation of the solid dispersions based on PLGA branched with tripentaerythritol, thermal properties, rheological behavior, and release of incorporated drugs. Incorporation of active substances (salicylic acid, miconazole) were employed by i) dissolution of both drug and polyester in ethylmethylketone and evaporation of the solvent, or ii) melting and subsequent plasticization of polyester by methylsalicylate, and dispersing of the drug particles.

The results show that systems exhibit Newtonian behavior thus viscosity can be optimize with concentration of plasticizer, and temperature. Methyl salicylate as plasticizer is miscible in all proportions with polyesters and significantly decreases the glass transition temperature. Thermal analysis of the solid dispersions demonstrated the absence of an insoluble fraction of the drug in the polymeric matrix. We conclude that the drug suprisingly forms molecular dispersion with the branched polymer. Drug dissolution profiles reveal prolonged release of the incorporated drugs.

*The study was supported by SVV 260 291.*

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## DEVELOPMENT OF A NOVEL BIORELEVANT *IN-VITRO* RELEASE TESTING METHOD FOR CONTROLLED RELEASE PARENTERAL PREPARATIONS

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To simulate the environment surrounding implantable devices at the site of administration, use of hydrogels have been suggested.[1,2] In our proposed novel setup, the controlled release device is incorporated in a thin agarose hydrogel and placed in phosphate buffer (pH 7,4). Similarly as in extracelular matrix in tissue, the released drug has to overcome the gel environment by diffusion before it reaches buffer medium. The possibility of sampling from buffer and use of conventional analysis methods is retained. As a gel forming polymer, agarose have been used because of its long-term stability in testing conditions.[3]

Drug release from PLGA films loaded either with flurbiprofen or lidocaine have been compared – in our proposed hydrogel-based metod and conventional method in phosphate buffer. Depending on the PLGA polymer grade, the studied films swell, shrink or remain unchanged during the drug release test. The swelling or shrinkage behaviour of same formulation batch have been observed to be different when incorporated in gel than when the films were placed freely in buffer. This led to different drug release and polymer degradation in the two compared drug-release testing methods.


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## EVALUATION OF COMPRESSIBILITY OF MICROCRYSTALLINE CELLULOSE PELLETS


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This study deals with the properties and the tablet preparation of two types of microcrystalline cellulose pellets (Cellets 100 and Cellets 200) and compares them with the compressibility and properties of tablets made of two types of powder microcrystalline cellulose (Comprecel 102 and Avicel PH-200). The most important properties of these materials and the compaction process were analysed in this work. The flowability, the angle of repose, the moisture content, the particle size distribution, the bulk and tapped density and the Hausner ratio[1] were evaluated. Furthermore, the compressibility was described by the force displacement method[2] and by the three-exponential compaction equation.[3] Finally, the radial strength[4] and the friability were used to describe the properties of the prepared tablets.

The results showed that both types of Cellets have much better flow properties than microcrystalline celluloses. This is caused by the higher bulk and tapped density, the narrow particle size distribution and the conspicuously smoother surface. However, these properties influenced their compressibility described by the parameters of compaction equation and the force-displacement method and resulted in tablets of lower quality. The radial strength of tablets prepared of Cellets was low and the friability was too high. Due to these results we can expect a significant fragmentation of pellets during the compaction process.[5]


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# POLYESTER NANOPARTICLES PREPARATION PROTOCOL OPTIMIZATION

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Poly(DL-lactide-co-glycolide) (PLGA) was used as a material for nanoparticles intended as drug carriers for site-specific distribution.[1] Adopted diffusion-solvent evaporation method was the first protocol employed in this study. Nanoprecipitation method was elected as second protocol employed by this study. The aim of this study was to assess various formulation parameters and their effect on quantity and quality of prepared nanoparticles. Composition of solvent system consisting of organic solvents, a type and concentration of aqueous phase stabilizer were the variable parameters of nanoparticles formulation. Particle size, polydispersity index and zeta potential were parameters of nanoparticles assessed. Moreover, the efficacy of nanoparticulation process expressed by % yield and drug loading efficacy were investigated.[2]

The modified nanoprecipitation method provided a good yield of PLGA, better polydispersity and was suitable for model drug encapsulation compared to solvent evaporation protocol. Solvent evaporation method further suffers from drawbacks such as use of chlorinated organic solvents. Nanoparticles prepared by nanoprecipitation over a wide range of organic solvents and using various stabilizers in various concentrations showed desired size distribution.

This study has demonstrated that formulation variables can be exploited to prepare the drug loaded PLGA nanoparticles by the nanoprecipitation technique. Nanoparticles prepared were of satisfactory quality and yield for pharmaceutical purposes.

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## SECTION OF BIOLOGICAL SCIENCES

### ALKALOIDS FROM THE HERB OF *GLAUCIUM FLAVUM* CRANTZ AND THEIR IMPACT ON HUMAN CHOLINESTERASES

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Alzheimer's disease, the most widespread neurodegenerative disease, causes decrease of cognitive functions and dementia. The most effective therapeutic approach is the application of central cholinesterases inhibitors, which alleviate cholinergic deficit in brain and thus improve memory.[1] Currently, intensive investigation of new active compounds including natural substances is carried on.[2]

Within the preliminary testing, alkaloid extract from *Glaucium flavum* Crantz herb showed promising inhibition of human cholinesterases, so it was selected for further examination.

The primary alkaloid extract was acquired from dried flowering herb by extraction with ethanol and subsequent liquid extraction with different pH. This extract was treated by preparative thin layer chromatography. The structure of alkaloids was determined by spectroscopic methods (GC-MS, NMR) and their optical rotation was ascertained.

Four alkaloids were obtained, in yellow horn poppy previously detected isoquinoline alkaloids protopine and (−)-norchelidonine and aporphine alkaloids (+)-cataline[3] and (+)-*N*-methyllaurotetatin.[4]

Subsequently, each alkaloid was tested *in vitro* for their inhibition of human acetylcholinesterase and butyrylcholinesterase by modified spectrophotometric Ellman's method.[5]

(−)-Norchelidonine was evaluated as the most potent inhibitor of AChE (IC50 = 35.1 ± 3.9 μM), however, its activity is not significant enough for further investigation. Other isolated alkaloids were considered to be inactive (IC50 > 100 μM).

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# PREPARATION OF HAEMANTHAMINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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The plants of the Amaryllidaceae family are known to contain a specific type of compound, namely the Amaryllidaceae alkaloids. One of them is haemanthamine (1), 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 was also tested (IC50 HuAChE, HuBuChE > 1000 μM).

In the present work, selective modification on the structure of 1 were carried out to study the relationship structure and biological activity. Fifteen derivatives of 1 were prepared and purified using analytic and preparative TLC. The obtained substances were subjected to structural analysis (NMR, MS methods, optical rotatory). Most of prepared compounds were tested on its possibility to inhibit human erythrocytic acetylcholinesterase (HuAChE) and human serum butyrylcholinesterase (HuBuChE). Some of the analogues were also tested for their cytotoxicity against various cancer cell lines. The most promising compound in HuAChE assay was 11-*O*-(3-fluorobenzoyl)-haemanthamine (IC50 AChE = 79 ± 1 μM), and in HuBuChE assay 11-*O*-(3-methylbenzoyl)-haemanthamine (IC50 BuChE = 25.9 ± 2.6 μM). The results suggest that some haemanthamine analogues provide a useful starting point for future experiments.

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## ISOLATION AND BIOLOGICAL ACTIVITIES OF ALKALOIDS FROM *NARCISSUS* CV. PROFESSOR EINSTEIN

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The plant cultivar *Narcissus* cv. PROFESSOR EINSTEIN has been chosen for phytochemical study based on result of previous experiments. Twelve alkaloids have been detected by GC/MS and ten of them identified (e.g.: lycoramine, pluviine, haemanthamine, pancracine, homolycorine etc.). Due to the diversity of alkaloids and the fact that summary alkaloidal extract showed interesting human serum butyrylcholinesterase inhibitory activity (IC50 = 49.99 ± 5.38 μg $ml^{-1}$), this cultivar has been chosen for isolation of Amaryllidaceae alkaloids in pure form and the study of their biological activity.

Alkaloidal extract has been prepared from 34.3 kg of fresh bulbs. Separation was initiated by column chromatography and extract was divided into almost 500 fractions some of them were put together based on TLC analysis and finally 27 subfractions were formed. Subfraction 26 was selected for isolation of pure alkaloids. The subfraction was repeatedly divided by preparative TLC to obtain 9-O-demethylhomolycorine; alkaloid of homolycorine structural type. The isolated compound was tested for its acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity against p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

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## PRODUCTION OF SECONDARY METABOLITES IN PLANT TISSUE CULTURES

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The goal of this study is to determine the influence of sodium molybdate and sodium tungstate as elicitors on production of scopoletin in cell suspension culture of *Angelica archangelica L.* The culture was grown in a liquid culture medium Murashige and Skoog on a roller apparatus in the dark and light. The content of scopoletin was in cells and in the culture medium determined by high performance liquid chromatography with fluorometric detection. The results show that sodium molybdate served as an elicitor to the production of scopoletin positively, application of sodium tungstate did not increase scopoletin production in any case. The highest production of scopoletin after application of sodium molybdate *versus* the control cells was reached in the suspension culture of *Angelica archangelica L*. cultured in the dark at a concentration of 25.50 mg/l. Scopoletin content increased by 166.7%. After application of sodium tungstate the production always decreased, in the medium of a suspension culture of *Angelica archangelica L*. cultivated in the light at a concentration of 66.00 mg/l, the production decrease was by up 62.5%.

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## ALKALOIDS ISOLATED FROM *NARCISSUS* CV. PROFESSOR EINSTEIN

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Within the Amaryllidaceae family, the genus *Narcissus L*. comprises around a hundred wild species with a center of diversity in the Iberian Peninsula and North Africa. In adition to their ornamental value, *Narcissus* plants have been found to contain many alkaloids with pharmacological activity. Most of the species can hybridize, and a large number of cultivars have been developer with ornamental purposes, with over 27,000 names of *Narcissus* cultivars now registered in the International Register. The *Narcissus* cultivars have advantages for commercial alkaloid production, since they are available in large quantities, but only a few studies on alkaloid profile and content in ornamental *Narcissus* cultivars have been published.[1]

Summary alkaloidal extract obtained from 34 kg of fresh bulbs of *Narcissus* cv. Professor Einstein was separated by column chromatography and gave 27 fractions. For isolation of alkaloids in pure form has been used fraction 6. Preparative TLC and crystallization

have been used for further separation process and two alkaloids of lycorine-structural type were isolated, namely caranine and pluviine. Both compounds were tested for their acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity of both alkaloids agains p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

*The study was supported by SVV 260 412.*

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## THE SELENIUM EFFECT ON SECONDARY METABOLITES PRODUCTION IN *IN VITRO* CULTURES OF MEDICINAL PLANTS

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The subject of this study is the evaluation of secondary metabolites production in *Fagopyrum esculentum* variety Spacinska cultures *in vitro* after elicitor treatment. The aim was to determine if selenium as an abiotic elicitor[1] increases rutin production in *F. esculentum* var. Spacinska cultures *in vitro*. The experiment was carried out in callus and suspension cultures of *F. esculentum* using Murashige and Skoog[2] nutrient medium supplemented with 1 mg $l^{-1}$ 2,4-dichlorophenoxyacetic acid as growth regulator. The elicitor was added in the form of a solution of 3 different concentrations (c1 = $9.012 \times 10^3$ mol $1^{-1}$, c2 = $9.012 \times 10^4$ mol $l^{-1}$, c3 = $9.012 \times 10^5$ mol $1^{-1}$), and it was affecting the culture for 6, 12, 24, 48, 72 and 168 hours. The release of secondary metabolites into the nutrient medium was studied as well. The content of rutin was determined by HPLC.

The increasing rutin production after elicitor application was observed in both callus and suspension cultures. However, there were higher levels of rutin content detected in callus culture. The maximum rutin content (0.6 mg $g^{-1}$ DW) was reached in callus culture after 12 h of elicitor treatment of c2 concentration. The maximum rutin production in suspension culture (0.1 mg $g^{-1}$ DW) was detected after 6 and 48 h of elicitor application of c3 concentration. The rutin release into the nutrient medium was not observed. The elicitor selenium is able to increase rutin production in *Fagopyrum esculentum* variety Spacinska cultures *in vitro.*

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## ALKALOIDS OF AMARYLLIDACEAE FAMILY: ISOLATION, STRUCTURAL IDENTIFICATION, BIOLOGICAL ACTIVITY III.

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The family of Amaryllidaceae plants consist of about 75 genera, whose 1100 species are widely distributed in tropic and warm temperature region of the world. In history plants of this family have been used in traditional herbal medicine. The family contain significant substances called Amaryllidaceae alkaloids, which was created *via* specialized metabolism. These alkaloids are known to show a wide range of biological activities, included acetylcholinesterase inhibitoty, cytotoxic, antiviral, antibacterial, antigungal, antimalarial and analgesic activity.[1] The aim of the research was to isolate Amaryllidaceae alkaloids from fresh bulbs of *Narcissus* cv. Professor Einstein, and to evaluate their biological activity connecting with Alzheimer's disease and cytotoxicity. Summary alkaloidal extract was prepared from 34 kg of fresh bulbs and separated by column chromatography. Preparative TLC and crystallization were used for the isolation of substances from subfraction 6. Two pure alkaloids (lycoraminone and narwedine) of galanthamine structural type were obtained and tested for their acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity of both alkaloids agains p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

*The study was supported by SVV 260 412.*

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## IRON-CHELATING PROPERTIES OF FRUIT EXTRACTS OF VARIOUS ELDERBERRIES

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Inorganic iron is the major food source of iron in humans. It plays role in many biochemical reactions. Thus, iron metabolism disorders can lead to different diseases as-

sociated with lack of iron or iron overload. One of the possible treatment modalities for the latter represents the administration of iron chelators.

Interest in anthocyanins has increased immensely during the past decade. Anthocyanins may play an important role in health promotion in terms of obesity prevention, cardiovascular health, anti-inflammatory and anti-cancer effects. Elderberry, *Sambucus nigra L.* (*Adoxaceae*), has been used in traditional medicine. The fruits of elderberry are a rich source of cyanidin-based anthocyanins as the main component. There are important differences, both in chemical and physical properties between several cultivars of elderberry. Anthocyanins might interact with metals in the gastrointestinal tract by formation of chelates. However, data on metal interactions with anthocyanins are sparse. The main aim of this study was to perform the analysis of interaction of iron with elderberry fruit extract as a rich and cheap source of anthocyanins with cyanidin as the aglycon.

In this *in vitro* study ten purified and standardized ethanolic elderberry fruit extracts were tested for iron chelating activities under different (patho)physiologically relevant pH conditions. Spectrophotometric method based on ferrozine as an indicator was used for the quantitative comparison.

All extracts were able to chelate iron, however, there were marked differences between extracts from different varieties which might be transformed in dissimilar biological effect. It was found that chelation activity of all tested extracts was increased with increasing pH. The extract of 'Haschberg' was the most potent iron chelator, both of ferrous and ferric ions.

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## ANALYSIS OF GENOMIC REGIONS BOUND AND REGULATED BY ATAXIN-3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is dominantly inherited polyglutamine neurodegenerative disease. In SCA3, the disease protein ataxin-3 (ATXN3) contains an abnormally long polyglutamine (polyQ) tract encoded by CAG repeat expansion. The ATXN3 binds DNA and interacts with transcription regulators pointing toward a direct role for ATXN3 in transcription. It is conceivable that mutant ATXN3 triggers multiple, interconnected pathogenic cascades leading to neurotoxicity, however, the principal molecular mechanism remains elusive. PCR analyses of 16 ATXN3-bound genomic regions were performed. CCAAT/enhancer binding protein delta (CEBPD), period circadian clock-2 (PER2), phosphatase and tensin homolog (PTEN), alpha 2 antiplasmin (SERPINF2) and thrombospondin-1 (THBS1) were selected for further study. To investigate the putative regulatory effect of the ATXN3 on subcloned genomic regions, luciferase reporter constructs were generated. Subsequently, wild type (WT) and hetero-

zygous ATXN3-knockout human neuroblastoma cell line (SH-SY5Y) were transfected and luciferase assays were performed. To further analyze the effect mediated by ATXN3, the luciferase reporter constructs were co-transfected with expression plasmids encoding human full length normal (Q13) and mutant (Q77) ATXN3 into the WT SH-SY5Y. Differences in ATXN3-dependent luciferase activity were observed in CEBPD and THBS1 suggesting the repressor effect of ATXN3 in both cases.

The analysis of normal and mutant ATXN3 regulatory effect on subcloned regions showed differences between luciferase activity in CEBPD, THBS1 and SERPINF2 genomic regions revealing potential connection with SCA3 disease.

## STUDY ON THE ROLE OF ABC EFFLUX TRANSPORTERS IN CELLULAR RESISTENCE TO BRAF INHIBITORS COBIMETINIB AND DABRAFENIB

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ABCB1 (P-glycoprotein, MDR1) and ABCG2 are well-known members of ATP-binding cassette (ABC) transporter family. Overexpressed in cancer cells they efflux a wide variety of structuraly unrelated anticancer drugs out of the cells and thereby represents one of the tumor deffence mechanisms against anticancer therapeutics leading to the development of multidrug resistance (MDR) and treatment failure.

BRAF protein plays an important role in MAPK/ERK pathway affecting cell division, differentiation and secretion. Mutations of BRAF lead to overactivity in MAPK/ERK pathway in many cancer cells and can be therefore targeted by anticancer therapy.

Cobimetinib and dabrafenib are used in melanoma treatment with BRAF mutations. Cobimetinib targets MEK kinase, a component of MAPK/ERK pathway, while dabrafenib inhibits directly BRAF kinase.

The aim of this project was to investigate whether the efflux transporters ABCB1 and ABCG2 could confer MDR to cobimetinib and dabrafenib. Using the XTT assay, we studied the antiproliferative effect of these drugs to MDCKII cell lines overexpressing ABCB1 and ABCG2 and to the transporter-expressing human epithelial A431 cells. Antiproliferative IC50 values in transporter expressing cells were determined and compared with the results from control cell lines.

Our results indicate that presence of ABCB1 can play a role in the cellular resistance to cobimetinib in A431 cell line, nevertheless, the effect could not be observed in the canine MDCKII-ABCB1 cells. Expression of ABCG2 did not affect proliferation of the ABCG2-expressing MDCKII or A431 cells when compared to their respective controls, indicating that ABCG2 is not able to confer MDR to cobimetinib. Interestingly all the cell lines showed high resistance to dabrafenib exhibited no antiproliferative effect in tested concentration scale ($IC50 > 50\ \mu M$). To conclude, ABCB1, but not ABCG2 might slightly affect sensitivity of cancer cells to cobimetinib. Further studies would be needed to evaluate clinical significance of this finding.

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## DEVELOPMENT OF CRISPR-CAS9 BASED TECHNOLOGY FOR GENETIC MODIFICATION OF *LACTOCOCCUS LACTIS*

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*Lactococcus lactis subsp. cremoris*, also known as Lactic Acid Bacteria, is an important microorganism widely used in fermentation of cheese products, but it also became a first genetically modified microorganism used alive for therapeutic reasons.[1] The aim of this study is to develop technology that allows modifying *Lactococcus lactis'* genome using Clustered Regularly Interspased Palindromic Repeats – Cas9 system, that will become faster, easier and relatively cheap tool for genetic engineering of this bacterium.

First part of the project is designed to test cells containing two plasmids, and how efficiently Cas9 expressed from one plasmid is cutting a targeted gene on another plasmid. For this I implemented the erythromycin resistance gene and designed CRISPR-Cas9 system aimed to disable this gene and measured activity of Cas9 protein by growing cells with designed plasmids in different medium (with or without antibiotic) and comparing their optical density.

The second part of the project was based on genetic modification of cell's chromosome using homologous recombination with the fragment on the plasmid and then applied CRISPR-Cas9 as a tool for eliminating cells which remained unchanged.

For both experiments, I used Nisin Controlled gene Expression system.[2] Plasmid genes expression was induced by nisin added into the growth medium.

Experiments showed some promising results although the genetic design of plasmids and the protocols of cell growth still require some further changes and adjustments.

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## PHARMACOLOGICAL CHARACTERIZATION OF NOVEL P2X3 RECEPTOR'S LIGANDS

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P2X3 receptor takes a part in transferring painful signals through the neuronal and non – neuronal cells along the neuraxis. ATP, the agonist of P2X3 receptor, is released from afferent neurons or from the damaged cells and non-neuronal peripheral tissue by stimuli. Before ATP is being degraded, it may activate P2X3 receptor at nociceptive cells endings and stimulate pain pathway. So, it seems to be hopeful to discover potential P2X3 receptor's antagonists which may help in future in treatment of relieving severe pain in cancer or in chronic pain disorders. Therefore, completely new allosteric antagonists were synthesized and tested *in vitro* at human astrocytoma cell line 1321N1 expressing human P2X3 receptor. Some of them have shown promising activity.

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## EFFECT OF PRENYLATED FLAVONOIDS ON BIOTRANSFORMATION ENZYMES *IN VITRO*

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Prenylated flavonoids xanthohumol (XH), isoxanthohumol (IXH), 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN) are flavanones and chalcones occurring in hops and their characteristic feature is prenyl chain present on A ring.[1] Prenylation, addition of isoprenoid functional group, significantly increases their estrogenic and anticancer activity as well as bioavailability in organism. The aim of our study was to find out, whether XH, IXH, 6PN, and 8PN have any impact on cytosolic carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C subfamily (AKR1C). Viability test revealed that prenylated flavonoids in lower concentrations do not affect or even increase the viability of primary rat hepatocytes, but higher concentrations are toxic. CBR1 and AKR1C activity was not affected after 24 h treatment but an increase in expression of AKR1C3 in IXH and 6PN treated samples was observed using immunoblotting, decrease in XH and 8PN samples. Expression of CBR1 was at the detection limit. Using qRT-PCR was found that XH caused significant increase in gene expression of CBR1, and significant decrease in AKR1c14 expression in rat hepatocytes.

Based on the results, prenylated flavonoids XH, IXH, 6PN and 8PN do affect the activity of CBR1 and AKR1C subfamily, gene expression of enzymes is significantly affected only by XH.

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## THE EFFECT OF A PARASITE ON THE ACTIVITY OF SELECTED INTESTINAL ENZYMES OF THE HOST

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*Hymenolepis diminuta*, known as a rat tapeworm, is commonly used in science as a model of Cestoda for studying physiology, biochemistry and drug metabolism.[1] Recently, *H. diminuta* has been studied for helminth-based therapy for inflammatory bowel disease.[2] The aim of our study was to determine how *H. diminuta* influences the activity of detoxification enzymes of the host. At first 6 male rats (Wistar breed) were infected by cysticercoids of *H. diminuta* previously isolated from the beetles *Tenebrio molitor* (intermediate host).[3] At the same time the physiological saline solution was administered for the control group of 6 male rats. All the rats were housed for 2 months in air-conditioned animal quarters with a 12 h light/dark cycle. Thereafter, the tapeworms were removed from the intestines. Intestinal mucosa containing metabolic active enzymes was isolated. Subsequently, the subcelullar fractions were prepared and used for *in vitro* experiment. The activity of enzymes was measured by spectrophotometry and spectrofluorimetry. The results show that *H. diminuta* is able to affect the activity of biotransformation enzymes. It can be assumed that the activity of reductases or some isoforms of cytochrome P 450 can differ. The activity of conjugation enzymes seems to be higher in intestine infected by *H. diminuta*. Concerning the enzymes of oxidative stress, the activity of catalase was increased apparently.

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## EFFECT OF NANOPARTICLES ON PLANT PROTEOME

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The environment is contaminated by increasing amounts of different types of xenobiotics. One of the potential polutants are nanoparticles of heavy metals. Effective method used for decontamination of the environment is phytoremediation which is based on the deposition of contaminants in plants. The plant is suitable for phytoremediation when it can absorb, metabolise and store contaminants without impact on its function. Influence on the function and construction of the plant can be detected at the proteome level.

In our research we focus on effect of nanoparticles on plant proteome in a model organism, which is *Arabidopsis thaliana*. A few weeks old plants were exposed to various types of copper particles (nanoparticles, bulk and salt) for different times. As a next step, proteins were isolated from plant material and electrophoresis and analysis was realised. The changes in the amount of structural proteins, proteins involved in photosynthesis, energy metabolism, carbohydrate metabolism and plant defence were detected. Modulation of the amount of protein was characterized by modified intensity of the spot on protein map. Toxic effect on plants is manifested for example by reducing the amount of structural proteins or proteins involved in photosynthesis. Conversely, increase in the content of proteins involved in stress response may indicate that the plant is able to fight against xenobiotics. One day treatment by bulk copper oxide caused decrease of intensity of six protein spots and four spots were increased.

Proteome influenced by copper oxide nanoparticles shows decrease of eight spots and increase of only three spots. Longer influence of both copper oxide nanoparticles and bulk form caused a decrease of almost all spots. After short acting of copper salt a lot of protein spots were increased. On proteome exposed to copper salt for four days were detected only a few spots with increased intensity, because the proteins were damaged by effect of copper ions. By comparing the results with literature, it was found that the copper ions are the most toxic for plant. They are followed by nanoparticles of copper oxide and the least toxic is bulk form of copper oxide.

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## DETERMINATION OF SELECTED microRNA – POTENTIAL CARDIOTOXICITY BIOMARKERS

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Cardiotoxicity is adverse reaction of chemotherapy that causes damage to the heart. Monitoring of potential biomarkers of cardiotoxicity could have a positive effect on the elimination of drug's toxicity on heart tissue. Therefore, at present, interest in the potential microRNA (miRNA) as biomarkers for cardiotoxicity is rising. MiRNA is very stable short noncoding RNA that has the ability to post-transcriptionally regulate gene expression. From bioinformatic analyses miRNAs are able to regulate more than half of human genes. Various studies have shown miRNAs to be much more specific and rapid diagnostic biomarkers in comparison with troponins. MiRNAs as biomarkers are not established in

routine clinical practice yet. So far, all studies of miRNAs are in the process of search and methods optimization.

In my project selected miRNAs for detection of doxorubicin (DOX) cardiotoxicity *in vivo* in mouse cardiac tissue samples and *in vitro* on rat cardiomyocytes were investigated. RNA isolation from biological samples and reverse transcription, using Stem-loop RT primer, was performed. Primers were designed for the quantitative determination of selected miRNAs using real-time PCR. The levels of expression of monitored miRNAs were compared to a control group of samples that were not affected by DOX. Significant changes in expression of selected miRNAs were detected. When comparing murine and rat samples elevated expression of various miRNAs after DOX treatment were found.

## THE EFFECT OF FLUBENDAZOLE AND MEBENDAZOLE ON EPITHELIAL-MESENCHYMAL TRANSITION

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Epithelial-mesenchymal transition (EMT) is a process in which a non-motile epithelial cell switches to motile mesenchymal phenotype. This phenomenon is typical for multiple biological processes including cancer metastases. It could be induced by transforming growth factor-β (TGF-β) which is produced by cancer cells and which regulates its key transcription factors including SNAIL, ZEB and TWIST. EMT involves a series of defined events mediated by microtubules, including changes in cell shape and migration into the surrounding tissue. Based on these findings we suppose that the inhibition of tubulin polymerization could prevent cell migration, therefore the EMT process and also cancer metastases. In our experiments, this effect was triggered by anthelminitic drugs flubendazole (FLU) and mebendazole (MBZ) in dysplastic oral keratinocytes *in vitro*.

Expression of molecules involved in EMT process was examined on microRNA and mRNA level using RT-PCR. The protein level of EMT markers was determined using western blot analysis.

FLU treatment significantly decreased mRNA levels of some mesenchymal markers such as N-cadherin, MMP2, MMP9 and TWIST, in comparison with TGFβ treated cells. Also microRNA from miR-200 family, miR200b and miR200c, involved in EMT processes was considerably increased after FLU, in comparison with TGFβ. The level of miR21, responsible for cancer progression, was significantly decreased after FLU treatment. MBZ was not significantly effective at any tested concentrations.

In total, FLU significantly inhibited expressions of mesenchymal markers in EMT induced cells, which could possibly prevent cancer metastatic process.

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## CHARACTERISATION OF THE METASTATIC AND NON-METASTATIC BREAST CANCER CELL

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Cancer is the second most frequent cause of death worldwide, while breast cancer is the most common type of cancer in women. According to Institute of Health information and statistic of Czech Republic, more than seven thousand new cases of breast cancer is diagnosed every year and approximately two thousand women die every year because of this type of cancer. The big problem is the treatment of the metastatic breast cancer. The goal of this study was characterization of metastatic and non-metastatic breast cancer cells.

Three breast cancer cell lines were used for the experiment, specifically non-metastatic MCF-7 cells, and two metastatic cell lines – MDA-MB-231 and BT474. The mRNA level was determined using RT-PCR and the protein level using western blot analysis. There was also examined the ability to migration of cell lines using the real time analysis, by the system X-celligence. As well as the effect of cytostatic paclitaxel (PTX) on antiproliferative activity of the cells was determined using the method WST-1.

Especially, in metastatic cancer cell lines BT474 were observed higher level of mesenchymal markers MMP-2, MMP-9 and N-cadherin as well. On the other hand E-cadherin as the suppressor for metastasis was observed in lower levels in MDA-MB-231 and BT474 metastatic cell lines. The highest migration potential was found in MDA-MB-231 cells. On the other hand, as expected, MCF-7 cells were not able to migrate. Testing of cell proliferation revealed MDA-MB-231 as a most sensitive cells to PTX treatment; in the BT474 cells was the effect weakest. In total, significantly higher metastatic potential was found in BT-474 cells, as well as reduced sensitivity to PTX treatment, in comparison with MCF7 and MDA-MB-231 cells.

*The study was supported by the program PRVOUK 37/01.*

## $CO^{2+}$ LOADED BLOCK COPOLYMER MICELLES: PREPARATION AND THEIR UPTAKE INTO MACROPHAGES

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Cobalt is a biogenic trace element, however in its inorganic form may cause immunological reactions in the human body. The immune response can be inflammatory or anti-inflammatory and both are responsible for specific actions, regarding macrophages activation and cytokines release.

The aim of this study was to provoke such response in macrophages and therefore to possibly control the inflammatory process. Nevertheless, free cobalt ions in certain concentrations may be toxic. In order to find the suitable and safe way of cobalt administration, the triblock terpolymer PEO-b-PAGECOOH-b-PtBGE was synthesized and formed into micelles in water.[1] Eventually the micelles were loaded with cobalt chloride. The properties of these nanoparticles were further studied and their structure, size, shape, appearance and net charge were determined, as well as the amount of cobalt inside.

After the synthesis and characterisation, the micelle uptake into macrophages was investigated and it was found out, that the uptake was increased with increasing micelle concentration in the cell culture medium and that the process was probably not carried out by receptor-mediated endocytosis. Further assessment revealed that the vitality of the cells was not significantly affected by the micelles. The cytokine release measurement suggested that the macrophages could have been activated into M2 anti-inflammatory state. The difference in release of IL-10 between cobalt loaded micelles and $CoCl_2$ solution proves that the micelles are potentially suitable drug delivery system.

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# BASIC CHARACTERIZATION OF HUMAN ENZYMES DHRS7B AND DHRS7C

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Human enzymes of the short-chain dehydrogenase/reductase (SDR) superfamily play important roles in the biochemical pathways.[1] They are involved in metabolism of lipids, saccharides, amino acids, steroid hormones, retinoids and prostaglandins etc. Besides physiological processes they take part in development of several serious diseases, e.g. hormonedependent cancer, metabolic syndrome, diabetes mellitus. Moreover, SDR enzymes contribute to the biotransformation and therefore to the detoxification of xenobiotics. Unfortunately, 30% of SDRs remain completely uncharacterized. Dehydrogenase/reductase SDR family members 7B (DHRS7B) and 7C (DHRS7C) are poorly characterized members of the SDR superfamily.[2] According to in silico predictions both are membrane bound enzymes and involved in reductive reactions. The aim of this study was to determine their basic biochemical properties and verify above mentioned predictions.

The results show that both enzymes interact with membrane of endoplasmic reticulum. DHRS7B faces cytosol whereas DHRS7C is oriented to the lumen of endoplasmic reticu-

lum. Reducing activity was detected towards e.g. estrone, prednisone, glucose, ketotifen or 1,2-naphtoquinone for both enzymes.


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## EVALUATION OF ANTIMICROBIAL EFFECT OF QUATERNARY AMMONIUM SALTS


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Nosocomial infections in healthcare facilities lead to high morbidity and mortality in devoloped countries. Some type of quaternary ammonium salts (QAS) are already used as disinfactants in practice (benzalkonium, benzoxonium etc.). Structure of these chemical compounds is quite similar to the cell membrane. So, they influence the permeability of the cell membrane and cause it's disrupt. We have tested three homologues according the side alkyl chain length (12, 14 or 16 carbons). We have performed microdilution broth method to prove their antimicrobial effect. Tested substances were dissolved and diluted into the binary dilution. The method was optimized according to the used strains. Several nosocomial strains of gram positive and negative bacteria (obtained from Faculty Hospital Hradec Králové) were tested. Benzalkonium salts were used as a standard for comparison of efficacy. QAS show sufficient effect againts nosocomial bacteria. Their microbicidal effect was better against gram positive bacteria as expected. They were still effective against gram negative bacteria, however in higher concentrations compare to gram positive.

In general, longer carbon chains show higher antimicrobial effect. There was no significant antimicrobial effect compare to the benzalkonium standard.


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## VALIDATION STUDY OF PREDICTIVE EQUATION OF RESTING ENERGY EXPENDITURE DURING PREGNANCY


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Nowadays, there are a few methods used for expression of resting energy expenditure (REE) during pregnancy: First, REE measurement by indirect calorimetry, very precise, but expensive and time-consuming method. Second, Harris-Benedict equation, which uses a formula to determine the REE, but is aimed to men/non-pregnant women, therefore it is not precise for pregnant women. None of methods present in the field of medicine, predicts the REE in pregnant women with sufficient precision and simplicity. One equation[1] was found and published in 2009: P REE = 346.43943 + 13.962564 · W + 2.700416 · H – 6.826376 · A.

The goal of this study was to verify its validity after almost eight years. A total of 70 randomly recruited healthy pregnant Czech women (non-smokers, not users of chronic medication or abusers of alcohol or drugs, normoglycemic, euthyroid and not anaemic) were divided into three cohorts by the length of gestation and measured by indirect calorimetry. Their REE was also calculated by Harris-Benedict equation and predicted by P REE equation. The results of these measurements were statistically analyzed by correlation analysis and Blend-Altman test. It turned out there was no significant difference between measured and calculated REE.

Results of validation study to confirm possibility of clinical application of P REE predictive equation for exact REE prediction without the need of using expensive technology or invasive examination that are highly important for the settings of proper nutrition during pregnancy.

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## GNOTOBIOTIC MICE MODEL AND EXPERIMENTAL INFECTION WITH *FRANCISELLA TULARENSI*

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*Francisella tularensis*, the causative agent of a disease called tularemia, is a facultative intracellular Gram-negative bacterium. Because of its very high virulency and mortality rate (if untreated) it is included in the Category A of bioterrorism agents by the Centers for Disease Control and Prevention, USA. Since there is no vaccination available for the general public yet, the research nowadays focuses mostly on a vaccine development. However, the experimental infection by *F. tularensis* also plays a significant role in understanding the host-pathogen interactions, serving as an important model of an infection caused by intracellular bacteria.

The aim of this study is to examine the virulence mechanisms of *Francisella tularensis* using the gnotobiotic mice model. The term “gnotobiotic” comes from the Greek words

"gnotos" and "bios", meaning "known life", indicating the limited presence (or absence) of microorganisms in such animal. The gnotobiotic animal model, as a strictly defined system, minimizes the influence of the organism's microbiota on the results of the study. It is, therefore, widely used in immunology and other biomedical sciences, providing great options for vaccine development and studies of the immune system, especially the host-pathogen relationship.

Our long-term goal is to compare the innate immune response of germ-free and specific pathogen free mice after intraperitoneal infection with two different strains of *Francisella tularensis*. By protein fractionation, we have prepared various bacterial samples suitable for the recognition of its immunoreactive proteins and the detection of specific antibodies found in the mice serum – using 2D SDS-PAGE electrophoresis, Western blotting and immunodetection. The different infectivity of the used strains, together with the dissemination of *F. tularensis* into the lungs, spleen and liver of the infected mice were observed as well.

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## THE INFLUENCE OF ANAESTHESIA ON THE DEGREE OF DNA OXIDATIVE DAMAGE

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Oxidative damage is one of the most frequent types of cell components damage leading to oxidation of lipids, proteins and the molecule of DNA. As a consequence, there is a higher occurrence of several pathologies such as atherosclerosis, neurodegenerative diseases, cancer, and diabetes.[1]

In our study, influence of whole body anaesthesia during minor surgery on the level of DNA damage was examined using comet assay technique. The basic principle of this method is fixing the cells (lymphocytes) in agarose, their lysis for the removal of membranes, incubation with the specific enzymes and electrophoresis of the released cell nuclei.[2] During the electrophoresis, free low-molecular weight and negatively charged fragments of DNA move towards anode which causes the formation of the typical comet cell shape. Finally, the gels are stained by ethidium bromide (DNA intercalating dye) and visualized.[3] We have observed single strand breakages (SSBs) and, with the use of modified assay using specific enzymes for detection of specific lesions, also oxidized purines and pyrimidines. The extent of DNA damage as determined by the intensity of the tail of the

comet was quantified using LUCIA Comet Assay (Laboratory Imaging, Czech Republic) software for image analysis. The results were used for the comparison of DNA damage before and after the anaesthesia of the patients. Statistical evaluation was performed in SigmaStat 3.5 (Systat Software, USA).

Results showed a statistically significant increase of DNA damage caused by anaesthesia during minor surgery. These findings imply further investigations, namely evaluation of the changes in the capacity of the affected lymphocytes to repair DNA damage.

*The study was supported by MH CZ – DRO (UHHK, 00179906) and Department of Research and Development, University Hospital Hradec Králové.*

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## SECTION OF CHEMICAL SCIENCES

### DEVELOPMENT OF UHPSFC-PDA METHOD FOR IMPURITY PROFILING IN ACTIVE PRINCIPLE INGREDIENT ATOMOXETINE

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The aim of this project was an optimization of UHPSFC method for determination of atomoxetine and its impurities mandelic acid, o-cresol, phenol, phenoxyatomoxetine, benzyl atomoxetine and atomoxetine carbamate. Atomoxetine is used as centrally acting sympathomimetic agent for the treatment of hyperkinetic disorders such as Attention Deficit Hyperactivity Disorder (ADHD).

Measurements were carried out on the UHPSFC system Aquity UPC2 with PDA detector and column Torus Diol 1.7 µm (3.0 × 100 mm). ABPR pressure was optimized at 2000 psi and the column temperature at 40 °C. Flow rate of mobile phase was 1.5 ml/min. Additional optimization parameters were the mobile phase composition, gradient elution conditions (initial composition of mobile phase, gradient slope, gradient time, gradient curves) and the effect of analysis time on the resolution of critical peak pairs. PDA detector parameters were examined, including comparison of data acquisition in 3 D and 2 D mode, selection of detection wavelength, resolution, sampling rate, filter time constant and mode selected for data acquisition, in order to obtain the maximum sensitivity of the method.

Optimal conditions for the impurity profiling in the drug substance atomoxetine were chosen as folows: $CO_2$/MeOH + 0.1% $NH_4OH$ as mobile phase with a gradient from 1% to

40% in 14 minutes. Detection was made in the 3 D of compensated mode at 215 nm with a resolution of 4.8, with a sample rate of 20 points/sec, the filtering time constant in the normal mode during a 15 minute analysis. The method was validated properly including SST (retention time, peak area, resolution, peak symmetry, peak width at half height) and determination of parameters of precision, accuracy, linearity, selectivity and robustness of the method.

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## DETERMINATION OF PHENOLIC COMPOUNDS IN APPLES

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This work deals with a development and optimization of an HPLC method for determination of selected phenolic compounds including gallic acid, chlorogenic acid, caffeic acid, catechin, epicatechin, rutin, quercetin, quercitrin, phloretin and phloridzin.

In the optimization step several types of stationary phase (C18, phenyl-hexyl, biphenyl, amino, cyano and monolithic column), gradients of the mobile phase and other separation and extractionconditions (temperature, extraction, solvent) were tested and a partial validation of the method was carried out.

Separation of the selected compounds was obtained using a precolumn Ascentis Express C18 (5 × 4.6 mm × 5 µm) and the chromatographic column Kinetex C18 column (150 × 4.6 mm × 5 µm). The detection was performed by a DAD spectrophotometric detector at wavelengths of 255, 280, 320 and 365 nm. Column temperature of 30 °C and gradient elution with mobile phase composed of acetonitrile and water (pH adjusted to 2.8 with acetic acid) was used. Injection volume was 10 µl and the flow rate 1 ml/min. During the optimization real apple extracts of the pulp and peel were also tested. The evalueted validation parameters included the chromatographic system suitability test (peak resolution, symmetry factor, capacity factor and repeatability), linearity and robustness.

The developed method is intended for determination of the selected phenolic compounds in different varieties of apples during their storage under various conditions (low temperature, controlled atmosphere).

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## MODIFICATION OF THE CAPILLARY WALL FOR THE SEPARATION PURPOSE

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Capillary electrophoresis is a separation method for the analysis of charged molecules. Interest on this method in recent years is growing along with it develops further electromigration methods, such as capillary electrochromatography. In terms of improvement separation efficiency offers modification of these methods. One option is the modification of the inner wall of the capillary in order to influence the electroosmotic flow, which contributes to the mechanism of separation largely.

This work deals with modification of the inner capillary wall and subsequently testing the prepared capillaries. One possibility is the chemically coating. The second method of coating is Layer-by-layer and the layered different modifiers. On the surface of the capillary is linked polyelectrolyte poly(diallyldimethylammoniumchlorid)e and graphene dispersion together in several layers. The choice of graphene is very advantageous because it has excellent adsorption properties due to its morphological configuration. For the analysis and subsequent optimization of separation conditions were selected mixture of parabens. They were tested for various optimization parameters such as the influence of the nature of the electrolyte (pH, concentration) and temperature on separation efficiency and speed of analysis. During measurement sacrificing efficiency, in particular capillary coated by chemical means. This phenomenon has been attributed to the gradual washout layers, which were accompanied by changes in electroosmotic mobility.

Method of coating Layer-by-layer was selected to be more appropriate procedure modifications as provided stable conditions for the reproducible analysis of mixtures of parabens.

## SPECTROPHOTOMETRIC DETERMINATION OF CHOLINESTERASE ACTIVITY USING CARBAMATE INHIBITORS

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Nowadays, two types of cholinesterases are known. The first one is acetylcholinesterase (AChE, EC 3.1.1.7), whose function is the cleavage of acetylcholine in the central and peripheral nervous system. The second type is butyrylcholinesterase (BChE, EC 3.1.1.8), sometimes called plasmatic cholinesterase. Whole significance of BChE is yet not fully

understood. Carbamates dominate among reversible cholinesterase inhibitors. Their effect can be decreased from the reason of (possible) hydrolyzation to inactive compounds.[1]

The goal of this experimental work was retrieval of kinetic parameters of AChE and BChE, and optimization of the method for determination of selected carbamate inhibitors – carbofuran (a representative of pesticides) and physostigmine/eserine (a pattern natural compound for obtain the effect of carbamates).[2] Determination of cholinesterase activity was carried with Ellman method. The mentioned enzymes were used for cleavage of thioesters (acetylthiocholin for AChE, butyrylthiocholine for BChE) to acetic, resp. butyric acid, and thiocholine. Thiocholine reacts with a 5,5′-disulfanediylbis(2-nitrobenzoic acid) – DTNB. Thus releasing 5-sulfanyl-2-nitrobenzen (TNB) can be detected spectrophotometrically at 412 nm.[3]

It turned out, firstly, that both inhibitors act very similarly, secondly, that BChE is more resistant to named inhibitors. Reversibility of inhibition will be even further investigated.

*The study was supported by SVV 206 401.*

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## NMR SPECTROSCOPY: A POWERFUL TOOL EMPLOYED IN ELUCIDATION OF UNKNOWN CHEMICAL STRUCTURES

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Nuclear magnetic resonance spectroscopy is a powerful analytical tool used to elucidate unknown chemical structures. Employing the behaviour of nuclei with odd nucleon number under appropriate conditions in a magnetic field with radio frequency ranged pulses applied, various useful data about the individual nuclei and their surroundings can be obtained.

Our task was to determine the structures of samples MC-SN-1 and MC-27, both of which are disubstituted tetrazoles. We ran standard 1H, 13C and 15N 1D experiments, followed by advanced 2D homocorrelated and heterocorrelated experiments: gCOSY, zCOSY, NOESY, gHSQC, gHMBC, 15N gHMBC, which provided us with conclusive proof of their structural arrangement.

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## SYNTHESIS OF NEW ORGANIC COMPOUNDS CONTAINING CHALCOGENS

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The focus of this thesis is on the synthesis of new organic compounds containing chalcogens, particularly sulfur and selenium.

New compound containing selenium was prepared by Huisgen 1,3-dipolar cycloaddition of alkyne and azide, known as the click reaction. Another three compounds contain both selenium and sulphur and had been prepared by substituting the SeCN fragment. These compounds are scheduled to be tested for biological activity in the near future. Due to the presence of chalcogen, they are expected to show antioxidant, anticancer, antifungal or antibacterial effects or their combination.

Additionally, two more compounds had been synthetised by Huisgen 1,3-dipolar cycloaddition of allyl thiocyanate and allyl selenocyanate with sodium azide. The final products are, however, highly unstable and prone to rapid degradation and are therefore unsuitable for further testing.

## SYNTHESIS OF CHIRAL IONIC LIQUIDS

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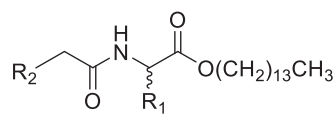
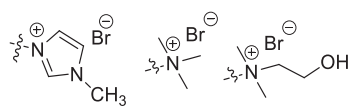
The aim of this project was to synthesize series of chiral ionic liquids, which would differ at the chiral centre, leading to three possible libraries,[1] either derived from L-alanine, D-phenylglycine or L-serine. The polar edge of the final products resulted from the reaction of α-bromoacetyl intermediates with their nucleophilic counterparts (Scheme 1).



$R_1$ = Me, Ph, $CH_2OH$

$R_2$ =



*Scheme 1.*

The products underwent a screening for the capability as additives to background electrolytes in capillary electrophoresis leading to a possible chiral recognition ability.[2]

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# STUDY OF TRANSALKYLATION REACTION OF ALKYL ARYL SULFIDES

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Recently, an atypical transalkylation reaction was observed during synthesis of unsymmetrical quaternized phthalocyanines (Pc) containing quaternizable imidazole moieties and tert-butylsulfanyl susbtituents. The tert-butylsulfanyl substituents that were present on the Pc ring were replaced by methylsulfanyl groups that most likely originated from the alkylating agent – methyl iodide. In a preliminary study, similar transalkylation occurred also in tert-butylsulfanylphthalonitrile, a starting material to Pc. This reaction is unprecedented in literature, so we decided to study this reaction in more detail.

The main objective of this experiment is to present how different parameters (temperature, time, type and excess of the alkylating agent) influence the conversion and the yield of reaction. The subjects of research was primarily simple tert-butylphenylsulfide which underwent transalkylation reactions with different alkylating reagents (methyl iodide, ethyl iodide, dimethyl sulfate, dimethyl carbonate, butyl iodide). The reaction temperature was varied from room temperature to 160 °C with time ranging from 30 minutes to 72 hours. It was observed that the percent of conversion is strictly connected with the time of reaction and the reaction temperature (Graph A). The experiments indicated that methyl iodide is the most efficient alkylating agent for this reaction (Graph B), and that dimethylcarbonate did not induce any change even at 160 °C. Decomposition of the material was observed upon longer reaction times with dimethylsulfate.

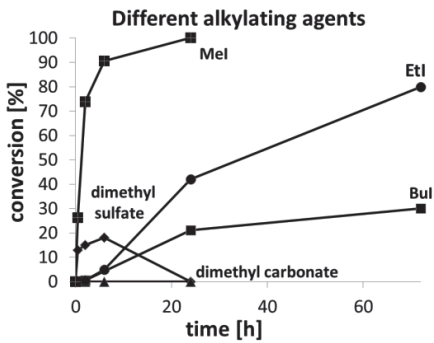


*Fig. 1.*

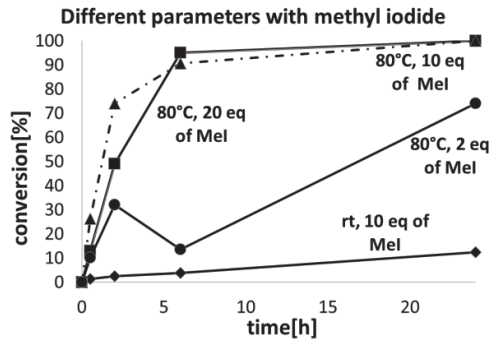


Fig. 2.

# SYNTHESIS AND STUDY OF PHOTODYNAMIC PROPERTIES OF SULFONATED AZAPHTHALOCYANINES

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Photodynamic therapy is one of the methods used for destruction of undesirable cells. It combines three essentially nontoxic components: light, oxygen and a photosensitizer. Azaphthalocyanines (AzaPc) are promising compounds with photosensitizing properties. Their major disadvantage is their low water solubility and significant aggregation that decrease their photodynamic activity.

The aim of this work was to synthesise an anionic derivative of AzaPc substituted with sulfonic groups on periphery 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 hydroxide (Fig. 1). The green coloured product was then purified by gel chromatography using Superdex® as stationary phase and by short preparative reverse phase column. Synthesised compound is soluble in water but according to absorption spectra it is partially aggregated. The tests for photodynamic activity were performed on HeLa cells using serum-free medium (phototoxicity $EC_{50} = 0.833 \pm 0.312$ μM, dark toxicity $TC_{50} = 534 \pm 27$ μM). It was practically inactive in serum-containing medium due to its binding to plasma proteins.

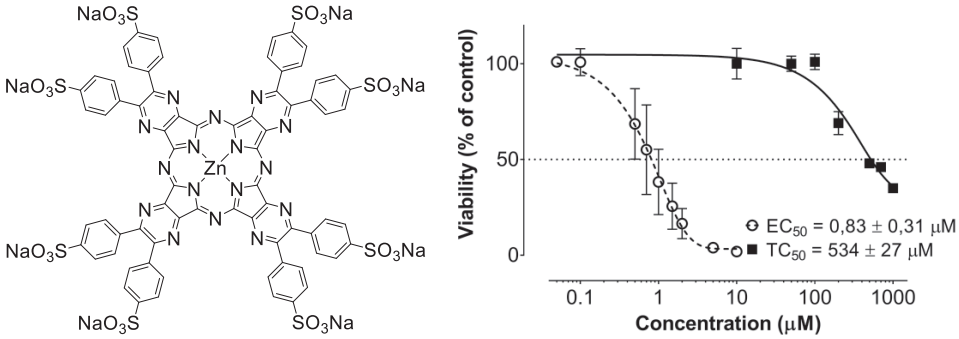


*Fig. 1.*

*The study was supported by Charles University, project SVV 260 401.*

# PHENOL-SUBSTITUTED AZAPHTALOCYANINES: pH SENSORS WITH TUNABLE PKa

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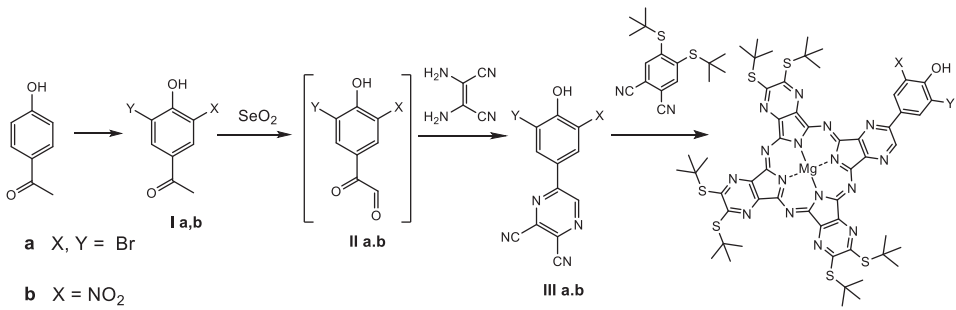
Azaphthalocyanines (AzaPc) are macrocyclic compounds containing a large system of conjugated double bonds which enables them to absorb light in the red part of the spectrum that is promising in biological applications. They are characterized by intense red fluorescence as one of the relaxation pathways of the excited state after absorbing photon. The fluorescence of AzaPc substituted with a phenol on the periphery can be switched ON/OFF depending on the pH of the environment and the pKa of the phenolic group. In basic medium the molecule occurs as phenolate and undergoes so-called intramolecular charge transfer (ICT) between the phenolate group serving as a donor and the electron-deficient nitrogen-rich macrocyclic core serving as an acceptor. As a consequence of this process, the fluorescence is quenched. Switching between ON/OFF states in phenol-substituted AzaPc is dependent on the proton concentration and thus can be utilized in sensing pH.[1]

The aim of this work was to synthesize phenol-substituted AzaPc and to study the effect of different substituents on the pKa of the phenolic group. The synthesis (Fig.1) started with preparation of appropriate precursors (i.e. substituted 5-(4′-hydroxyphenyl) pyrazine-2,3-dicarbonitriles). Electrophilic substitution of commercially available 4-hydroxyacetophenone (bromination in substance Ia and nitration in Ib) was performed. The products were treated with selenium dioxide affording corresponding ketoaldehydes that were not isolated but directly reacted in a condensation reaction with diaminomaleonitrile. To obtain unsymmetrical AzaPc, a mixed cyclotetramerization (statistical condensation) of these precursors (A) with 5,6-bis(tert-butylsulfanyl)pyrazine-2,3-dicarbonitrile (B) was performed using magnesium butoxide as an initiator. Resulting mixture contained six different congeners (i.e. AAAA, AAAB, AABB, ABAB, ABBB, BBBB) from which the required congener (ABBB) was separated using column chromatography. Substance IVa was incorporated to lipophilic particles (microemulsion) in water and the fluorescence changes were investigated as a function of pH of the buffer. Dependence of fluorescence on pH allowed determination of pKa value (see the picture below).



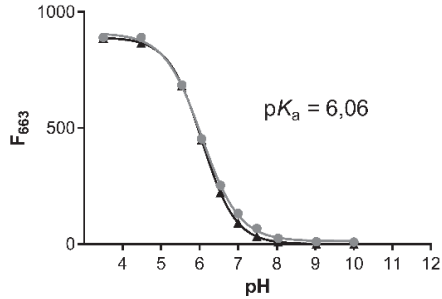



*Fig. 1.*

*The study was supported by SVV 260 401.*

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## DESIGN AND SYNTHESIS OF NOVEL HYBRID MOLECULES MODULATING ACTIVITY OF M1 ACETYLCHOLINE RECEPTORS WITH ACETYLCHOLINESTERASE INHIBITION PROPERTIES

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Alzheimer's disease (AD) is a neurodegenerative disorder of complex etiology, with insidious progression and fatal consequences. The worldwide incidence of AD is very high. AD affects primarily aging population manifesting as progressive decline of cognitive and intellectual functions. Histopathological hallmarks include the presence of β-amyloid neuritic plaques, neurofibrillary tangles composed of hyperphosphorylated τ protein and atrophy of brain tissue. Neurotransmitter levels are decreased in case of acetylcholine (ACh) while glutamate levels are elevated. Nowadays, there are two pharmacological groups employed in the treatment of AD; acetylcholinesterase inhibitors (AChEIs) and antagonist of N-methyl-D-aspartate receptors (NMDARs) – memantine. Both groups act only symptomatically, lacking disease-modifying effect.

The aim of our study was preparation of a series of novel hybrid molecules combining AChEIs, namely tacrine, 7-methoxytacrine and 6-chlorotacrine with molecule BQCA – positive allosteric modulator of M1 subtype of muscarinic ACh receptors (mAChRs). Inhibitory effectiveness of the newly synthetized compounds against cholinesterases (ChEs) was determined *in vitro* by the Ellman's colorimetric method and expressed as IC50. Effect on mAChRs was determined by measurement of intracellular calcium concentration using fluorescent indicator. The results demonstrated the ability of newly

synthesized molecules to inhibit ChEs in the micromolar and sub-micromolar IC50 values, with antagonist activity towards mAChRs.

*The study was supported by SVV 260 401 (Faculty of Pharmacy in Hradec Králové, Charles University) and Long-term development plan (Faculty of Military Health Sciences).*

## SYNTHESIS OF PURPUREALIDIN-INSPIRED BROMOTYRAMINES

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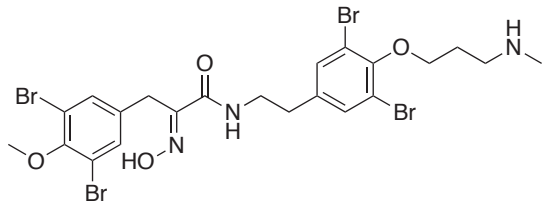
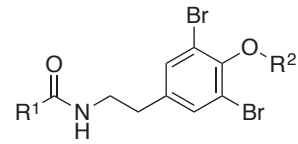
Biodiversity in the sea is a valuable source of lead structures and potential drug candidates.[1] Two bromotyrosine alkaloids purpurealidin I and J were isolated from the marine sponge *Pseudoceratina (Psammaplysilla) purpurea* by the National Institute of Oceanography, Goa, India in 2012.[2]

In this study, simplified dispyrin-like[3] bromotyramine analogs **1** of purpurealidin I were synthesised *via* purpurealidin E. Five of the novel compounds were tested against a hepatitis C virus (HCV) replicon cell model showing activity but also high cytotoxicity. Further research focus has therefore shifted onto studying prospective anticancer effects. A well-established synthetic route allowed the synthesis of variously modified structures. The previous together with improved purification methods fostered a creation of a library of derivatives studied for their activity towards hEAG $K^+$ channels and melanoma cell lines. Both synthetic approaches and selected biological results will be discussed.




**Purpurealidin I**




**1**

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## NUCLEOPHILE ASSISTED GOLD(I) CATALYZED CYCLIZATIONS

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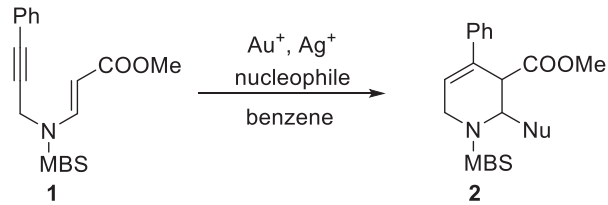
We have developed cyclizations of substitued enynes using gold(I) catalyst turnishing substituted dihydropyridines.[1] In the next step, we focused on gold(I)-catalyzed cyclizations of 1 in the presence of a nucleophile (e.g. methanol) which give substituted dihydropyridines.[2] The formal addition of nucleophile generates a new stereogenic center, whose relative configuration was determined by advanced NMR experiments. Screening of various gold catalysts was performed and the cyclization step optimized.




*Scheme 1.* Nucleophile-assisted cyclizations.

*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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## SYNTHESIS OF DEXRAZOXANE ANALOGUES AS POTENTIAL CARDIOPROTECTIVE AGENTS

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Anthracycline antibiotics (ANTs) such as doxorubicin or daunorubicin belong to anticancer drugs widely used to treat diverse forms of cancer. A high risk of the cardiotoxity represents the most serious side effect connected with the administration of ANTs. Dexra-

zoxane (DEX) has been the only compound capable of the protection against the ANT cardiotoxicity so far. However, the mechanism of its cardioprotective effect is still unknown. The aim of this study was to prepare, evaluate and optimize the preparation of four DEX analogues, in particular *meso*-dimethyl analogue ICRF-193 (Fig. 1).

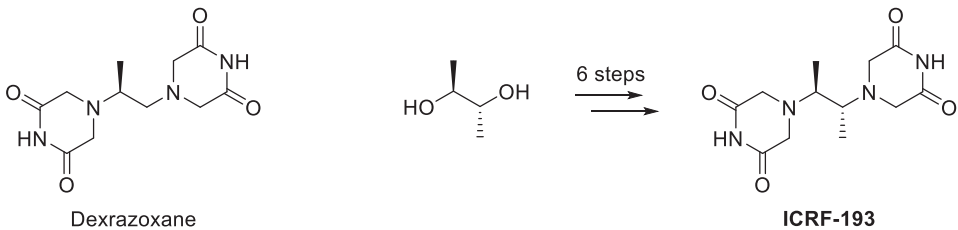


*Fig. 1.* Structure of DEX and its *meso*-dimethyl analogue ICRF-193.

The synthesis of ICRF-193 was accomplished *via* six-step procedure starting with the mesylation of *meso*-2,3-butanediol and subsequent substitution with sodium azide. The obtained *meso*-2,3-diazidobutane was reduced in order to prepare *meso*-2,3-diaminobutane which was subsequently converted to the corresponding diminobutane tetraacetic acid in two-steps. Its cyclization in formamide provided ICRF-193, which was studied for *in vitro* protection against ANT cardiotoxicy. In the pilot experiment ICRF-193 showed higher *in vitro* cardioprotective action than DEX.

*The study was supported by the Czech Science Foundation (13-15008S) and Charles University (SVV 260 401).*

## SYNTHESIS AND *IN VITRO* EVALUATION OF NOVEL IRON CHELATORS BASED ON SALICYLALDEHYDE ISONICOTINOYL HYDRAZONE

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Iron (Fe) is an element essential to all living cells. However, this transition metal may also catalyze the Fenton reaction which results in the formation of toxic reactive oxygen species (ROS), such as hydroxyl radicals.

Salicylaldehyde isonicotinoyl hydrazone (SIH) is a tridental chelator selectively forming complexes with Fe ions. As a result of its low molecular weight and good lipophilicity, SIH can be administered orally. It readily enters the cells, effectively chelates the intracellular Fe ions, and is therefore able to very efficiently inhibit the Fe-dependent processes,

such as production of ROS, but also the synthesis of some proteins and enzymes and the processes they regulate (e.g., cellular growth and proliferation).

In this work we focused on the design, synthesis and *in vitro* evaluation of novel SIH analogues with modified ligands, in particular the thio-analogue of SIH, analogues derived from (di)hydroxybenzophenone (**1**) and 2,6-dihydroxybenzaldehyde (**2**, Fig. 1).

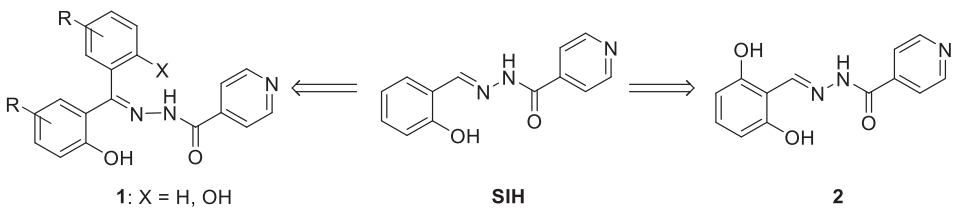


*Fig. 1.* Structures of SIH and its analogues studied in this work.

We prepared 17 analogues of SIH so far and assessed their ability to protect H9c2 cardiomyoblast cells against hydrogen peroxide-induced injury, studied their toxicity in the same cell line and their antiproliferative effects in HeLa and MCF-7 cell lines. Among the studied compounds, 2,6-dihydroxybenzaldehyde 4-chlorobenzohydrazone showed the most promising results.

*The study was supported by the Czech Science Foundation (1315008S) and Charles University (SVV 260 401).*

## TOTAL SYNTHESIS OF HUMAN 6-HYDROXYSPHINGOSINE

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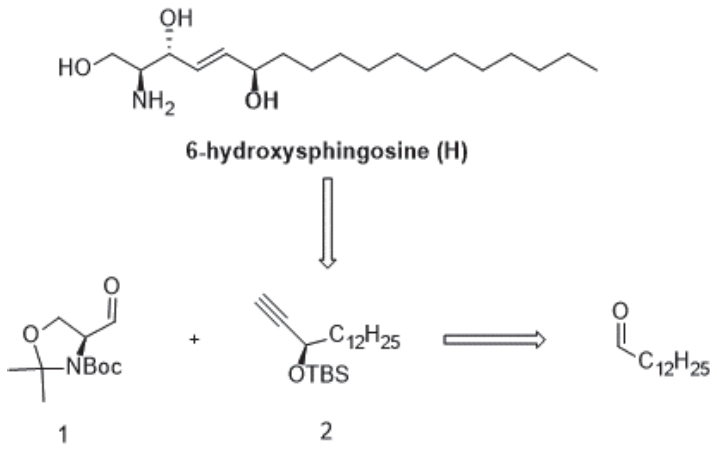
Ceramides (Cer) as members of sphingolipid family, play an important role in cell signalling. On the other hand, Cer occur in the human skin, where play another role in barrier function. Cer together with free fatty acids and cholesterol (in equimolar ratio) form intercellular multi-lamellar lipid matrix of the uppermost skin layer (stratum corneum). The primary function of this lipid matrix is to ensure a permeability barrier, thus, to provide water and electrolyte homeostasis, and event entry of harmful substances.[1]

Cer are composed of amino alcohol, e.g., sphingosine, and fatty acid acyl part. Cer derived from 6-hydroxysphingosine (H),3 i.e., (2S,3R,4E,6R)-2-aminooctadec-4-ene-1,3,6-triol (4E-t18:1), are the most unusual sphingolipids. 6-Hydroxyceramides (H-Cer) are not typical for all mammals, i.e., none were detected in pig skin but were found in some dog breeds.[2] Moreover, their function and biosynthesis are still unclear. However, various studies showed a relationship between lower concentrations H-Cer classes (relative to the

healthy skin) and atopic dermatitis.[3] The major limitation of understanding the nature of H-Cer is that these species are not commercially available.

Therefore, the aim of this work was to explore of synthetic route towards H as a precursor of all known H-Cer subclasses.




*Scheme 1.* Structure and retrosynthesis of 6-hydroxysphingosine.

The total syntheis of H was based on the reaction of commercially available tridecanal with trimethylsilyl acetylene. Strategy for synthesis of H involved an alkynylation of (S)-Garner's aldehyde (protected L-serinal) (1) with protected (R)-pentadec-1-yn-3-ol (derived from tridecanal), followed by selective two-step trans-reduction of triple bond(2). The reduction was performed by mild and selective [Cp*Ru(CH3CN)3]PF6-catalyzed Trost's hydrosilylation followed by protodesilylation.

In conclusion, physiological sphingoid base H was prepared in 7 reaction steps. Moreover, Cer NH (N-lignoceroyl-6-hydroxysphingosine) was also prepared by the acylation of free H with lignoceric acid. In the future, the free sphingoid base (H) will serve as precursor for the synthesis of all known H-Cer subclasses, i.e., alfa-hydroxylated Cer AH, omega-hydroxylated Cer OH and Cer EOH (with ester-linked linolenic acid).

*This work was supported by the Czech Science Foundation (16-25687J) and Charles University (SVV 260 401).*

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## PYRAZINE DERIVATIVES AS POTENTIAL ANTITUBERCULOTICS, SYNTHESIS AND BIOLOGICAL EVALUATION

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Over the past few years, the treatment of tuberculosis proved to be more and more challenging due to appearance of many resistant strains of *Mycobacterium spp*. Therefore, once considered not so important systematic research of new potential antituberculotics is becoming to be one of the priorities in research among developed countries.[1]

The importance of pyrazinamide in treatment of tuberculosis is undeniable. However, because of commonly and rapidly growing resistance to this potent antituberculotic drug, modern research is trying to find other new drugs or at least other derivatives of known ones as potent as pyrazinamide to overcome the resistance problem. In 1978, Foks and Janowiec[2] described antimycobacterial activity of 1-phenyl-3-(pyrazin-2-yl)urea but did not continue with the research in this area.

Our research group decided to further investigate this finding in hope to find another potent antituberculotic by preparing and testing derivatives of this promising molecule.

We synthetized a series of 1-phenyl-3-(pyrazin-2-yl)urea derivatives by substituting phenyl group with other aromatic compounds, and had them tested so far on 3 strains of Mycobacteria: *M. aurum, M. phlegmatis and M. tuberculosis*. None of the synthetized proved to be sufficiently active against *M. aurum* and *M. phlegmatis*, but some showed activity against *M. tuberculosis*. With more time, our research will continue and we hope to find other potential new antituberculotic drugs.

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## COMPOUNDS COMBINING PYRAZINAMIDE AND *PARA*-AMINOBENZOIC ACID AS POTENTIAL ANTITUBERCULARS

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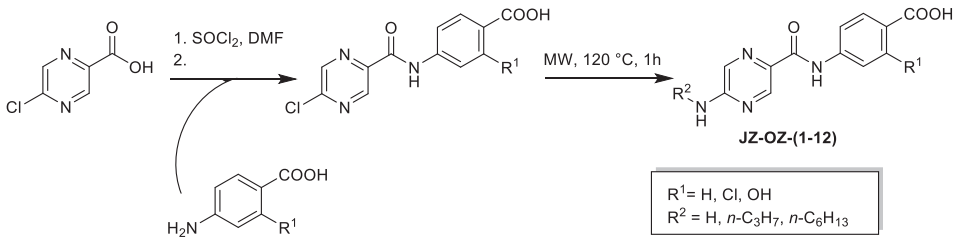
A series of new compounds combining pyrazinamide and *p*-aminobenzoic acid was prepared and *in vitro* tested for antimycobacterial activity against *M. tuberculosis* H37Rv, *M. avium*, *M. kansasii*, *M. aurum* and *M. smegmatis*. Previously prepared 4-(5-chloro-

pyrazine-2-carboxamido)-2-hydroxybenzoic acid ($R^1$ = OH) exerted micromolar activity against *M. tbc* H37Rv and low *in vitro* cytotoxicity in HepG2 cells.[1] *Para*-Aminosalicylic acid (PAS) has significant antitubercular properties based on its resemblance to *p*-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 12), 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 Czech Science Foundation project No. 17-27514Y and by SVV 260 401.*

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## DERIVATIVES OF 5-ALKYLPYRAZINE-2-CARBOXYLIC ACID AS POTENTIAL ANTI-INFECTIVES

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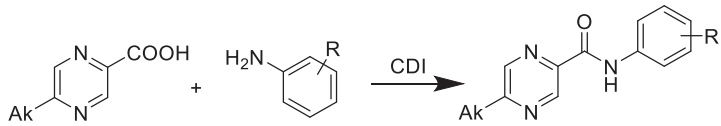
In our previous study, we have demonstrated that 5-alkylamino-*N*-phenylpyarazine-2-carboxamides with longer alkyl chain ($C_5$–$C_8$) exerted micromolar growth inhibition activity against *M. tuberculosis* H37Rv.[1] We speculated that the long alkylamino chain could facilitate the penetration of lipophilic mycobacterial cell envelope. To test this hypothesis, we performed the amino to methylene isosteric exchange and designed a series of 5-alkylamino-*N*-phenylpyarazine-2-carboxamides. 5-Alkylpyrazine-2-carboxylic acids (5-Ak-POA) were prepared by homolytic alkylation of commercially available pyrazine-2-carbonitrile by respective alkanoic acid, followed by hydrolysis of the carbonitrile group.

Final derivatives were prepared by CDI mediated coupling of 5-Ak-POA with corresponding aniline at RT.

Final compounds were described by melting point, elementary analysis, IR spectroscopy and $^{1}H$, $^{13}C$ NMR. Then they were tested *in vitro* for antimycobacterial activity against *M. tuberculosis* H37Rv and several non-tuberculous mycobacterial strains. Several compounds exerted MIC of 3.13–6.25 μg $mL^{-1}$. Compounds with R = 3-$CF_3$ had a broad spectrum of activity covering the non-tuberculous mycobacteria. Detailed structure-activity relationships will be discussed.




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

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## PYRAZINECARBOXYLIC ACID DERIVATIVES AS POTENTIAL ANTIMYCOBACTERIAL COMPOUNDS

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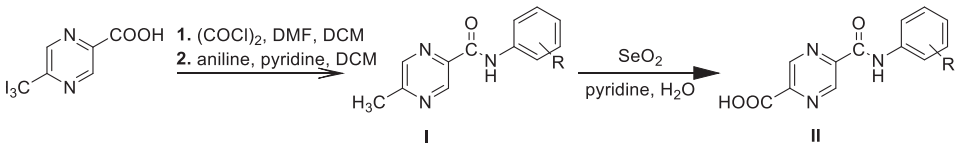
A series of new compounds related to pyrazinoic acid (POA) was synthesized, characterized by analytical data and *in vitro* tested for antimycobacterial activity against *M. tuberculosis* H37Rv and several non-tuberculous mycobacteria.

Anilides of 5-methylpyrazine-2-carboxylic acid (**I**) were prepared to broaden the previously published series of POA anilides with different substitution on the pyrazine core.[1] One of the proposed mechanisms of action of POA is the inhibition of mycobacterial trans-translation by binding to ribosomal protein RpsA. Lipophilic substituents in C-5 or C-6 of POA should be compatible with the binding mode of POA to RpsA[2] and could enhance the permeation of mycobacterial cell wall due to the increased lipophilicity. With this intention, we designed and prepared lipophilic derivatives of POA of general structure **II** by oxidation of **I** by selene dioxide. Among the tested compounds (incomplete results), **I** with R = 4-Cl proved to be the most potent compound against *M. tbc* H37Rv (MIC = 1.65 μg $mL^{-1}$). Generally, oxidation of the methyl to carboxylic moiety decreased the activity. Structure-activity relationships will be discussed.

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## STRUCTURAL STUDIES OF SELECTED PROTEIN-LIGAND INTERACTIONS

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Molecular docking is a computational method that predicts interaction between two molecules (receptor and ligand). This method studies the binding mode and the position of the ligand in a protein's binding site.

The goal of the work was to predict interactions of novel compounds designed on Department Toxicology and Military Pharmacy before their synthesis. Based on the predicted data, compounds for chemical synthesis and biochemical testing were chosen.

Our targets were human BACE-1 (beta-secretase 1) that plays a role in Alzheimer's disease, GluN2B that is a part of glutamate (*N*-methyl D-aspartate) receptor and its antagonists have neuroprotective and anti-Parkinsonian effects, and COX-2 (cyclooxygenase-2) as a target for non-steroidal anti-inflammatory drugs.

We chose appropriate complexes from RCSB Protein Data Bank and we prepared proteins and ligands for molecular docking using software Avogadro, Chimera and AutoDock Tools. The computational part was realized through AutoDock. Suitability of the approach was verified by re-docking of ligands co-crystalized with the targets.

# EVALUATION OF CLINICAL PHARMACIST´S INTERVENTIONS IN THE HOSPITAL

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The Drug-Related Problem (DRP) is an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes.[1] DRP may disrupt patient's safety. Pharmaceutical care allows solution of DRPs and contributes to optimization of pharmacotherapy.

The aim of the research was to describe and evaluate the most common DRPs occurring in the selected department in the hospital.

The research was provided in the hospital in Šumperk, at the department of the internal medicine, the nursing section. Data was collected from medical records of hospitalized patients. Subjects for analysis were patient's characteristics, diagnosis, using drugs, selected laboratory markers and drug-related problems. DRPs have been identified during reviews of the clinical pharmacist. Description and evaluation of DRPs include management, acceptation of the pharmacist's intervention and economic aspect of intervention. The classification of DRPs was according to the Pharmaceutical Care Network Europe classification version 5.01. Results were evaluated by statistical method. 53 patients were involved in the research. DRPs were detected at 85 percent of analyzed patients. On average one patient suffered from 1.9 DRPs. The most frequent DRPs were "Drug choice problem" (58%), followed by "Dosing problem" (26%), "Others" (9%), "Drug use problem" (4%), "Interaction" (3%). "Adverse reaction" were not identified even once.

The most related drugs to DRPs were omeprazole, cholecalciferol and atorvastatin. Recommended interventions of the clinical pharmacist were discussed with doctor and mostly accepted. Interventions did not mean cost reduction on drugs. Occurrence of DRPs in hospital is high. Clinical pharmacist helps to prevent and identify DRPs and minimize their occurrence among hospitalized patients.

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## ANALYSIS OF DRUG-RELATED PROBLEMS IN HEALTHCARE FACILITY

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Drug-Related Problems (DRPs) are defined as pharmacotherapy related events, which actually or prospectively interfere with a therapeutic purpose.[1]

The goal of the work is focused on obtaining and evaluating of DRPs in *Hamzova odborná léčebna pro děti a dospělé* (Luže-Košumberk), which is the important center of rehabilitation in the Czech Republic. Patient´s data were collected from their medical documentation, uploaded to special web database, and statistically evaluated by descriptive statistic. The data of 94 patients from the three departments of the rehabilitation center were analyzed. Together patients used 672 drugs (i.e. 7.15 drugs per patient). Overall, 272 DRPs were registered and 84 (91%) patients had at least one DRP. Most of them were related to drugs from the anatomical group C – Cardiovascular system in accordance with ATC classification of drugs. According to PCNE (Pharmaceutical Care Network Europe) classification the most problems were recorded in groups P3 (Dosing problem, 47%) and P2 (Drug choice problem, 33%).

The most frequent DRPs were regarding to unclear drug indication, drug absence despite the clear indication, bad dosing scheme, too high drug dose and the unclear drug signature. DRPs had a prospective character. Because of the methodology, this study contains no information about undesirable side effects. Most of the DRPs were considered of little or medium importance.

The conclusion of the work is that influence of pharmacotherapeutic audits is very important because they help to detect and solve DRPs frequently occurring in healthcare facilities. The most occurred DRPs were discussed with physicians.

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## PRESCRIBING OF POTENTIALLY INAPPROPRIATE MEDICATIONS TO ELDERLY PEOPLE NEGATIVE OUTCOMES

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Older persons represent a quickly growing segment of the population worldwide and more vulnerable population to various adverse drug reactions and adverse outcomes. Many explicit criteria of potentially inappropriate medications (PIMs) have been developed in different countries in order to improve the quality and safety of geriatric prescribing and to reduce the risk of drugs in older adults.

The aim of this diploma thesis was to summarize (using narrative literature review) the outcomes of potentially inappropriate medications documented in pharmacoepidemological studies published by 2016 year.

Based on the systematic literature review using PubMed, Web of Science datasets during the period 10/2015–1/2017, literature review was conducted. 421 studies were identified during the primary literature search and after thorough consideration of abstracts 69 (16%) of studies were selected for works on summary tables. In the literature review only outcomes studies published during the period 2003–2016 were included. 36 (52%) of prospective, 27 (39%) of retrospective and 6 (9%) of cross-sectional studies were identified. The majority of studies included patients aged ≥ 65 years living in community, nursing homes or hospitalized patients. 57 (83%) of studies were made on representative sample of population (> 300 patients). The most of studies found positive association with hospitalization, impairments in physical functioning, higher health care cost and higher health care utilization. No negative impact on mortality, HRQOL, occurrence of ADE/ADR or drug-drug interactions was observed.

Further outcome studies using improved methodology (particularly in the part of the number of older people, length of data collection period, study design) are needed to better understand relevant outcomes of PIM use in older patients in Europe.

## POLYPHARMACY IN THE ELDERLY – NEGATIVE OUTCOMES

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Older persons usually suffer from multiple chronic disorders and consequently use more medications than younger adults and often polypharmacy. The aim of this diploma thesis was to summarize by narrative literature review the negative outcomes of polypharmacy in older adults documented in pharmacoepidemological studies published between 2005 and 2015 years. The outputs of this diploma thesis create part of results of the research subgroup "Aging and Changes in the Therapeutic Value of Medications in the Aged" and the EU COST Action initiative IS1402 (2015–2018).

Using narrative literature review of Web of science, Medline, PubMed, EMBASE datasets during the period (2014–2015), we summarized outcome studies on polypharmacy in older patients published between 2005–2015 years. 563 studies were identified during the primary literature search and after reading of studies' abstracts, 70 (13%) of studies were

selected for summary tables. 496 (87%) of studies were excluded because they did not focus on outcomes of polypharmacy.

We identified 23 (33%) prospective, 22 (32% retrospective and 25 (35%) cross-sectional studies. Seniors mainly aged 65 years from ambulatory care, nursing homes, acute care or living in their homes were included. The main outcomes positively associated with polypharmacy were mortality, falls, hospitalizations, non-adherence, poorer nutritional status and GIT symptoms and poor quality of life. Cognitive decline was not significantly associated in most of the studies.

We confirmed that polypharmacy in older adults have mostly negative impact on health status in the elderly, especially on mortality, falls, higher rate of hospitalizations, adherence and other factors. Further outcome studies are needed to better understand relevant negative outcomes of polypharmacy in older patients in Europe.

## PARALLEL RE-EXPORT OF MEDICINES FROM CZECH REPUBLIC

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Re-export of medicines is one of the most discussed issues, both in professional and public scene of pharmacy and therefore, we decided to focus on this topic and try to help keeping balance between shortages of particular drugs and income of distributors.

There are still severe shortages of vital human medicines on the Czech market as a result of their mass parallel re-export to other countries. This is clearly a long-term problem that has not been dealt with effectively. The parallel re-export of medicines as a result of low prices on the Czech market has led to national shortages.

Under EC law, Member States can impose limits on the percentage volume of medicines that can be exported from the country if those medicines cannot be substituted for another substance with the same medicinal properties. Under EC law, Member States can take action within the framework of their own laws to limit exports of medicinal products (maximum re-export limits) for the purpose of protecting human health and human lives and on the basis of the proportionality of such action.

Aim of the study was to get complex knowledge from distributors, pharmacies with approval for distribution and producers on recent situation including legislative, common praxis with essential data and also their own experiences and opinions how to solve the problem, in order to maximize results of the research. Personalised questionnaires were made and the answers were analyzed, amplified with further up-to-date information from verified sources.

Conclusions are greatly interesting and promising, so continuing long-term research is going on already.

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## ANALYSIS OF COMMUNITY PHARMACY MARKETING

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Community pharmacy works as any other economical subject so it needs to generate profit from its customers. Therefore, it is important to analyze customers' needs and decide for the right marketing communication.[1] According to growing number of patients that use internet and social media for health related reasons it is good to use these technologies as the part of marketing communication also in the community pharmacy.[2]

This research focuses on the issues of patients' choice of pharmacy and investigates their opinion on pharmacy marketing practice and importance of internet communication. Data were collected via the questionnaire survey among the users of internet and customers in one independent community pharmacy in Beroun. Link on the electronic version of questionnaire was placed on Facebook sites dealing with the medicines and healthy life style and on the internet forums for mothers and pregnant women. Customers of community pharmacy in Beroun were asked to complete the questionnaire right in the store.

More than one-half of respondents prefer the nearest pharmacy. The third of them (33.7%) appreciate the quality of provided services. Almost one-half of addressed people assess marketing activities of pharmacies as positive and 30.9% of respondents admitted that these activities influence them. For twenty-four percent of respondents aged between 25 and 64 years is important communication of pharmacy on the internet.

According to outcomes of this research, there is significant part of patients seeking for high-quality marketing communication and internet presentation of the community pharmacy.

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# MEDICATION ADHERENCE AND SELF-MANAGEMENT IN KIDNEY TRANSPLANT RECIPIENTS

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Kidney transplantation (KT) requires a lifelong medical regimen of immunosuppressive treatment (IS). Our study is focused on medication adherence (MA) in KT outpatients, as the medical non-adherence is one of the leading (yet preventable) cause of graft rejection.

Furthermore, we focus on analysis of the main self-management tasks. The prospective cross-sectional study was undertaken in one year period from March 2016 to March 2017 at the Haemodialysis Centre in the Teaching Hospital Hradec Králové. Patients ≥ 18 years old and at least 3 weeks after KT were addressed within their regularly scheduled visit to the nephrologist. Structured interview was performed by pharmacist to determine patients' self-reported MA to IS using validated Czech version of Medication Adherence Report Scale (MARS-CZ). In addition, patients were interviewed about other self-management issues. Simultaneously, medication records were reviewed. Data analysis was performed by means of descriptive statistics. A total of 211 patients with the mean age of 55.8 ± 12.41 years completed the interview. Patients were 7.4 ± 5.75 years after KT and used in average 11.3 ± 2.96 drugs. The mean score of MARS-CZ was 24.7 ± 0.74 (MIN 16; MAX 25). Using the cutpoint of < 23, signs of non-adherence to IS were observed in 6 patients. In terms of medication taking, tacrolimus on empty stomach was taken by 44 (35.2%) patients and prednisone after the breakfast by 160 (79.6%) patients. A number of 174 (82.5%) patients followed their dietary recommendations. Out of 211, 153 (72.5%) patients used some kind of sun protection, 172 (81.5%) measured blood pressure at home and 24 (11.4%) were regular smokers.

According to current findings, the level of self-reported MA seems to be satisfying. Nevertheless, lower acceptance of other self-management tasks may also cause serious problems. Interventions on multiple levels including education and psychosocial support should be implemented to daily routine to minimize the risks of therapy failure. The engagement of the pharmacists should be beneficial in this point. This is the first complex study in patients after KT conducted in the Czech Republic.

*This study was supported by Charles University (Project SVV 260 417).*

# ATTITUDES TOWARDS TREATMENT AND KNOWLEDGE OF HORMONAL CONTRACEPTIVES AMONG FINAL YEAR PHARMACY STUDENTS

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Hormonal contraception (HC) belong among worldwide birth control methods used in a broad age groups of women. Based on the health status, lifestyle, user's preferences or non-contraceptive benefits it is possible to choose from the wide spectrum of HC which differs in dosage and composition. The aim of this thesis was to determine the attitudes toward the treatment and knowledge about the HC among the final-year pharmacy students upon the completion of mandatory 6-months long practical training in pharmacy and knowledge acquired during the studies. The data were collected using online questionnaire. The questionnaire consisted of 33 questions, which were divided into several sections focused on obtaining for instance social-demographic information, information about dispensation of HC during practical training, knowledge about HC risks, information about correct and safe dosage of HC etc. The questionnaire was sent during one month to the students' school e-mail addresses at 2 faculties of pharmacy (Faculty of pharmacy in Hradec Králové (HK) and Faculty of Pharmacy in Bratislava (BA)). A total of 382 students were requested. The collected data were processed using the descriptive statistics. In the questionnaire, 109 students from HK (57.1%) and 74 students from BA (38.7%) have responded. Most of the respondents were women, in HK it was 95 (87.2%) and in BA 64 (84.2%). The mean age of the respondents was 23.6 years (SD = 0.9) in HK and 23.6 years (SD = 1.1) in BA. If students provided advice on the supply of HC (HK 43.1%, BA 32.8%), they focused mainly on the new users and counseling was mostly in the range of dispensing minimum (HK 61.5%, BA 47.3%). As absolute contraindications of combined HC students from both faculties reported correctly mostly venous thromboembolism history, breast carcinoma and smoking more than 15 cigarettes by women over 35 years, however in case of progestin-only HC, majority of students did not know the correct answers. Only 17.6% of students in BA and 21.1% students in HK would properly advise the patient how to solve the problem with unstuck contraceptive patch.

In conclusion, knowledge and attitudes of pharmacy students at both faculties were comparable, but there are still some limits of knowledge, which should be improved to enhance rational pharmaceutical care.

## ATTITUDES TOWARDS TREATMENT AND KNOWLEDGE OF HORMONAL CONTRACEPTIVE USERS

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Hormonal contraception (HC) is very popular and reliable birth control method, although, as any other pharmacological therapy it is not entirely risk-free. The main aim of this work was to determine the attitudes towards treatment and awareness of HC risks of its users.

The research was carried out as questionnaire survey. Anonymous questionnaire consisted of 35 questions to obtain sociodemographic data, information concerning HC use as well as form of HC, information about the extent of knowledge concerning HC risks and HC misconcepcion awareness. Last part of questionnaire was focused on smoking while using HC. Questionnaires were distributed among women older than 15 years of age who attended a small private pharmacy in the centre of Prague with prescriptions to get their HC. The respondents could fill out the written questionnaire by themselves directly in pharmacy but mostly they chose to fill it out at home and send it to the Faculty in the prepaid envelope. The survey lasted from October 2016 to January 2017 and totally 51 (respond rate 63.8%) questionnaires were gathered. Collected data were then evaluated by descriptive statistics.

The mean age of respondents was 28 ± 8.2 years while more than 50% of women reached higher education. Survey results show that respondents were mostly aware of side effects that do not pose a direct threat to their health and most of them are not even proven to be caused by HC. One quarter of respondents was also smokers but only 23.1% of them were afraid of thromboembolism while using this form of birth control method and smoking. Respondents took mostly affirmative attitude (agreed or strongly agreed) for a claim that HC users are able to enjoy sexual relationship to the greater extent.

It was discovered that women's knowledge and awareness is quite limited, especially when it comes to the most serious risks of HC. However, these outcomes cannot be globalized because of small number of respondents, yet, they can serve as a background for further research needed for obtaining more accurate results in this topic.

## PHARMACY STUDENTS' QUALITY OF LIFE

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The quality of life of pharmacy students may change throughout their university life and differ from the quality of life of the general population. In the Czech Republic, quality of life of pharmacy students was not yet examined.

The aim was to determine the quality of life of second year students at the Faculty of Pharmacy at Charles University in Hradec Králové. Secondary aim was to compare the results of the Bern Subjective Well-Being Questionnaire with the short version of World Health Organization Quality of Life questionnaire and the Subjective quality of life analysis questionnaire.

The data were collected during 2011. Questionnaires were filled in by second-year students of Faculty of Pharmacy in Hradec Králové. The Bern Subjective Well-Being Questionnaire, the short version of World Health Organization Quality of Life questionnaire and the Subjective quality of life analysis questionnaire were used. The data were analyzed using the MS Excel computer program.

The results of the questionnaires showed that most of the students rated their quality of life as good or very good (82%). Fifty three percent and seventeen percent of the students were satisfied and very satisfied with their health, respectively. Students were mostly satisfied with the environment they lived in (housing, health care, safety, money). They were least happy with the lack of free time. Students lacked time for relaxation, hobbies and friends. A statistically significant difference between the quality of life of men and women was confirmed in third domain of the short version of World Health Organization Quality of Life questionnaire, at the scale of somatic disorders, at the scale of self-respect and at the scale of depressed mood of the Bern Subjective Well-Being Questionnaire ($p < 0.05$). At the other scales, a statistically significant difference was not confirmed ($p > 0.05$). Worse results in quality of life of our respondents, compared with Prague's population have not been confirmed. We found relationships between second domain of the short version of World Health Organization Quality of Life questionnaire and the scale of a positive attitude towards life of the Bern Subjective Well-Being Questionnaire, between third domain of the short version of World Health Organization Quality of Life questionnaire and the scale of joy of life of the Bern Subjective Well-Being Questionnaire and between second dimension of the Subjective quality of life analysis questionnaire and the scales of somatic disorders of the Bern Subjective Well-Being Questionnaire.

We can indicate that quality of life of students from second year was good although our students were not satisfied with their free time. The same conclusion was found in students from the Czech Technical University in Prague.[1] Further investigation into quality of life of pharmacy students across all years is needed.

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## UP-SCALING AND FURTHER DEVELOPMENT OF MATRIX LIPOSOMES

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Liposomes have been investigated as promising drug delivery systems since their first discovery back in the 1960s. Up to date, scientists are facing many challenges in order to increase their bioavailability after oral administration, improve and simplify manufacturing processes and avoid microbial contamination with the aim to extend their shelf-life.[1] Matrix liposomes are small spheroid vesicles whose lipid bilayer is composed of egg-phosphatidylcholine and cholesterol. Entire particles of liposomes are embedded in a gelatin matrix while the final formulation is solid at room temperature. In addition, the presence of gelatin suggested to increase the stability of the formulation under gastric and intestinal conditions in the GI-tract. Therefore, it is considered to become a prospective formulation for oral protein drug delivery.[2]

The aim of this study is to up-scale and optimize the matrix liposomes formulation by dual asymmetric centrifugation method. This method is based on a combination of two *contra* rotating movements which generate shear forces and thus lead to efficient homogenization of lipid blend.[3]

Due to the development of a new speed mixing device, liposomes were formulated in higher batch sizes while the step of forming dried lipid film completely omitted. The new technology is not only less time-consuming but also enables to decrease consumption of organic solvents and make the entire process more environmentally friendly.

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## STUDY OF SKIN DISEASES USING MONOLAYER LIPID MODELS

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Skin is an organ that protects the body against external influences and excessive water loss. The proper skin barrier is localized in the outermost layer of the epidermis, the stratum corneum (SC). The function of this barrier depends on the composition and or-

ganization of the extracellular space of SC. It consists mainly of ceramides (CERs), free fatty acids and cholesterol in approximately equimolar ratio. An imbalance in the lipid composition leads to disruption of the skin barrier function.[1]

Ceramidase is an enzyme which cleaves CERs or glucosylated ceramides (precursor of CERs) to lysosphingolipid (lysoSph) and fatty acid. Abundance or deficit of this enzyme causes dysfunction of skin barrier and the manifestation of skin diseases, e.g. Farber's disease. This systemic disease causes the development of subcutaneous nodules, which appear a few months after birth and lead to death during the first few years of life.[2,3]

The aim of this work was to study the monolayer lipid models whose compositions reflect the abundance of ceramidase in the skin. Lipid models were studied by Langmuir monolayers at the gas-liquid interface and at the solid surface, including Brewster angle microscopy and atomic force microscopy.

With increasing addition of lysoSph (0–75%) – including increasing addition of free fatty acids, the theoretical area per molecule of the lipid is decreasing. The modus of compressibility of the lipid mixtures is increasing to the 10% addition of lysoSph to the mixture containing only CERs and then it is decreasing again.

Results brought finding that the addition of lysoSph to the lipid mixture affects the lipid organization and therefore it affects the function of the skin barrier.

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## ARTICLES

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ŠOLÍNOVÁ, J., MALÝ, J.: Individuální konzultace poskytované starší pacientce ve veřejné lékárně (Individual counseling in community pharmacy – case report of geriatric patient). Prakt. Lékárenství, 11 (2), 2015, 64–67.

## MONOGRAPHIES

DOHNAL, F.: Některé významné postavy zdravotnické služby v československém legionářském vojsku a jejich další osudy (On Some Important Persons of the Health Service in the Czechoslovak Legion Army and Their Further Fate). In: Kapitoly z dějin medicíny, farmacie a veterinárního lékařství. Brno: Technické muzeum v Brně, 2015, 14–17. ISBN 978-80-87896-17-4.

KVĚTINA, J., RUSEK, V.: Pražská farmaceutická škola a její osobnosti v proměnách farmaceutického vysokoškolského vzdělávání v meziválečném období (Prague school of pharmacy and its personalities in the changing pharmaceutical higher education in the interwar period). In: Kapitoly z dějin medicíny, farmacie a veterinárního lékařství. Brno: Technické muzeum v Brně, 2015, 115–118. ISBN 978-80-87896-17-4.

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ŠUSTR, P., LAMKA, J., RĄPAŁA, R., ZENDULKOVÁ, D., TESA, K., ERNST, M., ROBOVSKÝ, J., SVOBODOVÁ, K., POHLOVÁ, L., ŠIROKÝ, Z., BLAŽEK, P., TUREČEK, J.: Jeleni v Krkonoších (Deers in Krkonoše). Vrchlabí / Jelenia Góra: Správa KRNAP / Dyrekcja KPN, 2015, pp. 200. ISBN 978-80-87706-91-6.

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## TEXTBOOKS

Nová, A., Pávek, P.: Základy ADME a toxického hodnocení léčiv v preklinickém vývoji. Olomouc: Univerzita Palackého, 2015, pp. 62. ISBN 978-80-244-4539-7.

## DEGREES

*Lecture for the Professorship Appointments, Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2015*

Doc. Pharm. Dr. MILAN NOBILIS, CSc.: Associate Professor, Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Hradec Králové.
*Discipline:* Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
*Inaugration:* 11. 3. 2014
*Continuation:* 10. 6. 2014
*Title of Lecture:* Studium osudu léčiv v organismu z pohledu bioanalytika (Studies of drugs fate in organism from bioanalytics view) 10. 6. 2014
*Appointment:* 1. 5. 2015

Doc. RNDr, PETER MIKUŠ, Ph.D.: Associate Professor, Head of Department of Pharmaceutical Analysis and Nuclear Pharmacy.
*Discipline:* Analytical Chemistry, MŠMT 24 295/2007- 30/1
*Inauguration:* 6. 11. 2015
*Continuation:* 8. 12. 2015
*Title of Lecture:* Pokročilé metody kapilárnej elektroforézy vo farmaceutickej a biomedicinskej analýze (Advanced methods of capillary electrophoresis in pharmaceutical and biomedical analysis) 8. 3. 2016.

*Habilitation Thesis (Associate Professor), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2015*

Pharm.Dr. RADIM KUČERA, Ph.D.: Senior Lecturer, Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Hradec Králové.
*Discipline:* Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
*Inauguration:*15. 9. 2014
*Continuation:*14. 10. 2014

*Title of Lecture:* Alternativní stacionární fáze využívané v analýze biologicky aktivních látek (Alternative stationary phase used in analysis of biological active substances) 9. 12. 2014.
*Appointment:*1. 3. 2015

Pharm.Dr. VERONIKA NOVÁKOVÁ, Ph.D.: Senior Lecturer, Department of Biophysic and Physical Chemistry, Faculty of Pharmacy, Hradec Králové.
*Discipline:* Pharmaceutical Chemistry, MŠMT 24 295/2007-30/1
*Inauguration:* 9. 9. 2014
*Continuation:* 14. 10. 2014
*Title of Lecturer:* Fluorescenční senzory pro biologicky relevantní analyty (Fluorescense sensors for biologically relevant analytics) 12. 2014.
*Appointment:* 1. 3. 2015

Pharm.Dr. LUDMILA MATYSOVÁ, Ph.D.: Senior Lecturer, Department of Analytical Chemistry, Faculty of Pharmacy, Hradec Králové.
*Discipline:* Analytical Chemistry, MŠMT 24 295/2007-30/1
*Inauguration:* 18. 11. 2014
*Continuation:* 9. 12. 2014
*Title of Lecture:* Validace chromatografických metod – legislativa a provedení (Validity of chromatographic methods – legislature and practise) 3. 3. 2015.
*Appointment:* 1. 5. 2015

*Doctoral Dissertation Thesis (Ph.D.), Faculty of Pharmacy in Hradec Králové (CZ), Charles University (CZ), 2015*

PharmDr. IVANA ŠRÁMKOVÁ, Ph.D.: Využití neseparačních průtokových metod ve farmaceutické analýze (Application of non-separation flow methods in pharmaceutical analysis). 29.09.2015.
PharmDr. ZDENĚK NOVÁK, Ph.D.: Analýza struktury alkaloidů metodami multidimensionální NMR spektroskopie (Analysis of the structure of alkaloids using multidimensional NMR spectroscopy). 24.09.2015.
Mgr. JIŘÍ KRATOCHVÍL, Ph.D.: Syntéza specificky substituovaných heterocyklů katalytickými reakcemi (Synthesis of Specifically Substituted Heterocycles via Catalytic Reactions). 24.09.2015.
RNDr. LUCIE RAISOVÁ-STUCHLÍKOVÁ, Ph.D.: Metabolismus a účinky nových anthelmintik u helmintů a jejich hostitelů (Metabolism and effects of new anthelmintic drug in helminths and its hosts). 24.09.2015.
Mgr. BARBORA ŠKOLOVÁ, Ph.D.: Syntéza a studium analogů ceramidů (Synthesis and study of ceramide analogues). 24.09.2015.
PharmDr. JIŘÍ BINDER, Ph.D.: *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). 17.06.2015.
PharmDr. PETR VRBATA, Ph.D.: Nanovlákenné membrány jako nosiče léčiv (Nanofibrous Membranes as Drug Delivery Systems). 01.07.2015.
PharmDr. KATEŘINA LÁDOVÁ, Ph.D.: Význam hodnocení adherence k léčbě pomocí výpovědi pacienta v posouzení účinnosti farmakoterapie (The Importance of Self-Reported Medication Adherence in the Evaluation of the Effectiveness of Pharmacotherapy). 02.07.2015.
PharmDr. MICHAL ŘÍHA, Ph.D.: Screening nových látek chelatujících železo/měď – *in vivo* a *in vitro* studie). 04.09.2015.
Mgr. HELENA HENDRYCHOVÁ, Ph.D.: Fytochemická studie jednotlivých taxonů rostlin rodu *Bergenia* (Phytochemical study of individual plant species of *Bergenia* genus). 21.09.2015.
Mgr. KUMAR UDAY KILLI, Ph.D.: Interakce muskarinových receptorů a cholinesterasy: Studie funkcí inhibitorů esterasy u potkana (Interactions of muscarinic receptors and choline esterases: Functional examinations of esterase inhibitors in the rat). 24.09.2015.
Mgr. VĚRA DAŇKOVÁ, Ph.D.: Identifikace nových faktorů virulence intracelulárního patogena *Francisella tularensis* (Identification of new virulence factors in intracellular pathogen *Francisella tularensis*). 20.05.2015.
PharmDr. ABOBAKR ABASAEED ELHAG, Ph.D.: Analysis of the Use of Antibiotics in the United Arab Emirates. 19.03.2015.

PharmDr. ANNA HOŠŤÁLKOVÁ, Ph.D.: 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). 01.07.2015.

*Rigorous Thesis (Pharm.Dr.) Faculty of Pharmacy in Hradec Králové (CZ) Charles University (CZ), 2015*

Mgr. BRLICOVÁ, MONIKA PharmDr.: Imunohistochemická detekce PECAM-1, endoglinu a VCAM-1 v aortě transgenních myší se zvýšenými hladinami solubilního endoglinu (Immunohistochemical detection of PECAM-1, endoglin and VCAM-1 in aorta of transgenic mice with high levels of soluble endoglin). 23.09.2015.

Mgr. BROŽOVÁ, IRENA PharmDr.: Vliv stearanu vápenatého na lisovatelnost mikrokrystalické celulosy (Effect of calcium stearate on the compressibility of microcrystalline cellulose). 04.12.2015.

Mgr. BRYCHTOVÁ, JANA PharmDr.: Využití květu z pěstovaných odrůd bezu černého ve farmacii (Pharmaceutical utilization of flowers from cultivated varieties of *elderberry*). 23.04.2015.

Mgr. ČÁŇOVÁ, KRISTÝNA PharmDr.: Vliv flubendazolu na proliferaci buněk kolorektálního karcinomu *in vitro* (Effect of flubendazole on proliferation of colorectal carcinoma cell lines *in vitro*). 16.06.2015.

Mgr. ČERNOHLÁVEK, MILAN PharmDr.: Výsledky kontroly parazitóz ve dvou oborních chovech přežvýkavé spárkaté zvěře (Parasite Control Results in Two Preserve Ruminant of Hoofed Game Breedings). 23.09.2015.

Mgr. DOSTÁLOVÁ, ALŽBĚTA DOROTA Pharmdr.: Vliv přídavku viskozifiantů na vlastnosti nanoemulzí II. (The effect of addition of thickening agents on nanoemulsion properties II). 03.09.2015.

Mgr. DRTINOVÁ, LUCIE PharmDr.: Testování nových potenciálních látek pro léčbu Alzheimerovy nemoci (Testing of new potencial substances for the treatment of Alzheimer's Disease). 23.09.2015.

Mgr. DUDOVÁ, ADRIANA PharmDr.: Vliv *spiruliny platensis* na experimentální aterogenezi (*Spirulina platensis* effects in experimental atherogenesis). 23.09.2015.

Mgr. ENDLICHER, RENÉ RNDr.: Studium změn energetického metabolismu hepatocytů: působení oxidačního stresu a trijodtyroninu (The study of the changes of hepatocyte energy metabolism: the effect of oxidative stress and triiodthyronine). 10.02.2015.

Mgr. HÁJEK, JAN PharmDr.: Biological activity of antioxidant compounds in monocytes THP-1. 10.02.2015.

Mgr. HROUDOVÁ, JANA PharmDr.: Effects of antidepressants and depressive disorders on mitochondrial functions. 19.02.2015.

Mgr. HRSTKA, VÁCLAV PharmDr.: Alkaloidy rodu *Narcissus* (Amalyllidaceae) a jejich biologická aktivita (The alkaloids of the genus *Narcissus* (Amaryllidaceae) and its biological aktivity). 29.10.2015.

Mgr. HUDSKÁ, KLÁRA PharmDr.: Syntéza a studium ceramidů s deuterovaným acylem (Synthesis and study of ceramides with deuterated acyl chain). 30.06.2015.

Mgr. IBRMAJEROVÁ, LUCIE PharmDr.: Přírodní látky a jejich biologická aktivita X. Screening vybraných alkaloidů na inhibici prolyloligopeptidasy (Natural compounds and their biological activity X. Screening of selected alkaloids on inhibition of prolyloligopeptidase). 04.06.2015.

Mgr. JANDOVSKÁ, KATEŘINA PharmDr.: Příprava analogů ceramidů a dihydroceramidů a hodnocení jejich vlivu na bariérovou funkci kůže (Synthesis of ceramide and dihydroceramide analogues and evaluation of their effects on the skin barrier properties). 06.02.2015,

Mgr. JAROŠOVÁ, PATRÍCIA PharmDr.: Separace vybraných anorganických iontů pomocí sekvenční injekční chromatografie (Separation of selected inorganic ions using the sequential injection chromatography). 18.06.2015.

Mgr. JEŘÁBKOVÁ, MARKÉTA PharmDr.: Měď redukující účinky flavonů (Copper reducing effects of flavones). 25.03.2015.

Mgr. JIRKOVSKÝ, EDUARD PharmDr.: Kardiotoxicita antracyklinových antineoplastických látek a možnosti farmakologické kardioprotekce (Kardiotoxicita antracyklinových antineoplastických látek a možnosti farmakologické kardioprotekce). 23.09.2015

Mgr. JOHN, KLÁRA PharmDr.: Dávkování očních kapek – charakterizace očních kapátek 2. 27.04.2015.

Mgr. KAŠKOVÁ, JANA PharmDr.: Validace HPLC hodnocení amlodipinu a atorvastatinu v kombinovaném léčivém přípravku (Validation of HPLC evaluation of amlodipine and atorvastatin in combinated dosage forms). 18.03.2015.

Mgr. KITTLEROVÁ, ZDEŇKA PharmDr.: Biologicky aktivní metabolity rostlin VI. Alkaloidy *Eschscholtzia californica cham*. a jejich biologická aktivita (Biologically activ metabolites of plants VI . Alkaloids from *Eschscholtzia Californica Cham*. and their inhibiting activity to). 10.09.2015.

Mgr. KOBLÁSOVÁ, ANNA PharmDr.: Měď redukující vlastnosti flavanonů (Copper reducing properties of flavanones). 25.03.2015.

Mgr. KOLOUCH, JAN PharmDr.: Stanovení chelatačních vlastností 6,7-dihydroxykumarinu (Assessment of chelating properties of 6,7-dihydroxycoumarin). 23.09.2015.

Mgr. KOZLOVÁ, LUCIE PharmDr.: Ekotoxikologická studie směsi fluorochinolonových antibiotik na řasách (Ecotoxicological study of fluoroquinolone antibiotics mixture on algae). 23.04.2015.

Mgr. KRÁL, JAN PharmDr.: Syntéza a biologické hodnocení takrin-amantadinových derivátů (Synthesis and biological evaluation of tacrine-amantadine derivatives). 13.11.2015.

Mgr. KRÁLOVÁ, ANNA PharmDr.: Výskyt a analýza lékových problémů u pacientů v léčebně dlouhodobě nemocných (Incidence and Analysis of Drug-Related Problems in a Long-Term Care Facility). 25.06.2015.

Mgr. KRESOVÁ, JANA PharmDr.: Studium možností využití alternativních sorbentů pro úpravu vzorku (Study on possibility of utilization of alternative sorbents for sample preparation). 18.03.2015.

Mgr. KŘÍŽOVÁ, ŠÁRKA PharmDr.: Stanovení katechinů a fenylpropanových kyselin ve vybraných doplňcích stravy (The determination of catechins and phenylpropanoid acids in selected). 23.04.2015.

Mgr. KUBEŠOVÁ, ZUZANA PharmDr.: Optimalizace kultivačních podmínek pro explantátové kultury *catalpa bignonioides* pěstovaných v bioreaktoru (Optimization of cultivation conditions for plant tissue culture of *Catalpa bignonioides* grown in bioreactor ). 23.04.2015.

Mgr. KUŽELOVÁ, KRISTÝNA PharmDr.: Validace HPLC metody stanovení piroxikamu v plasmě s využitím SPME a deproteinace .26.03.2015.

Mgr. KVITA, VOJTĚCH PharmDr.: Účinek polyfenolů ze zeleného čaje na antioxidační enzymy u myší *in vivo* (The effect of green tea polyphenols on antioxidant enzymes in mice *in vivo*). 10.02.2015.

Mgr. LABAŠTOVÁ, PETRA PharmDr.: Fluorescenční detekce metsulfuron methylu v SIA systému (Fluorescence detection of metsulfuron methyl in the SIA systém). 26.03.2015.

Mgr. LÁDOVÁ, KATEŘINA PharmDr.: Význam hodnocení adherence k léčbě pomocí výpovědi pacienta v posouzení účinnosti farmakoterapie (The Importance of Self-Reported Medication Adherence in the Evaluation of the Effectiveness of Pharmacotherapy). 04.11.2015.

Mgr. LÁSKOVÁ, MIROSLAVA PharmDr.: Syntéza azaftalocyaninů nesoucích jeden 2,6-di(terc-butyl)fenolický substituent (Synthesis of azaphthalocyanines bearing one 2,6-di(tert-butyl)phenol substituent). 13.11.2015.

Mgr. LEBEDOVÁ, VÁCLAVA PharmDr.: Studium smykového chování velikostních frakcí sorbitolu (Study of shear behaviour of the sorbitol size fractions). 04.12.2015.

Mgr. LEHAROVÁ, EVA PharmDr.: Obsah anthokyanů v plodech bezu černého a jejich biologická aktivita (The content of anthocyanins in elderberry fruits and their biological activity). 23.04.2015.

Mgr. LEVOROVÁ, LUCIE PharmDr.: Účinek brusinkového extraktu na vybrané biotransformační enzymy u normálních a obézních myší (Effect of cranberries extract on selected biotransformation enzymes in normal and obese mice). 10.02.2015.

Mgr. LINHARTOVÁ, ANNA PharmDr.: Oxidační stres v modelu katecholaminové kardiotoxicity (Oxidative Stress in the Model of Catecholamine Cardiotocitiy). 21.04.2015.

Mgr. MAJEROVÁ, JITKA PharmDr.*: Hypericum perforatum in vitro* – abiotická elicitace. 23.04.2015.

Mgr. MALINOVÁ, BARBORA PharmDr.: Asociace parametrů složení těla stanovených pomocí bioimpedanční spektroskopie a prediktorů morbidity a mortality u pacientů s CHOPN (Association of body composition parameters established using bioimpedance spectroscopy and predictors of morbidity and mortality in patients with COPD). 23.09.2015.

Mgr. MÁROVÁ, MARIE PhamDr.: Vyhodnocení aktivity potenciálně antimikrobních látek pomocí mikrodiluční bujónové metody (Evaluation of the activity of potentional antimicrobial substances through the use of microdilution broth method). 19.02.2015.

Mgr. MAŘÍKOVÁ, MARTINA PharmDr.: Sledování potenciálních lékových interakcí na vybraných pracovištích FN HK (Assessment of potential drug interactions in two inpatient wards in university hospital in Hradec Králové). 15.01.2015.

Mgr. MLADĚNKOVÁ, MARKÉTA PharmDr.: Stanovení parazitostatu a účinnosti vybraných léčiv ověřené ve dvou chovech jelení a daňčí zvěře (Assessment of the parasites' status and the effectiveness of selected drugs verified in two herds of deer and fallow deer). 19.02.2015.

Mgr. MORKUSOVÁ, MICHAELA PharmDr.: Protidestičkové účinky 5.7-dihydroxy-4-metylkumarinů (Anti-platelet effects of 5,7-dihydroxy-4-methylcoumarins). 19.02.2015.

Mgr. MUDROVÁ, MICHAELA PharmDr.: Analýza lékových problémů v lůžkovém zdravotnickém zařízení II. (Analysis of drug-related problems in ward health care facility II.). 19.03.2015.

Mgr. NEHYBOVÁ, MONIKA PharmDr.: Anthokyany v plodech kulturních odrůd *Sambucus nigra* L. – Izolace a stanovení antioxidační aktivity (Anthocyanins in the fruit of *Sambucus nigra* L. cultivars – isolation and determination of antioxidant aktivity). 23.04.2015.

Mgr. NĚMEČKOVÁ, ANETA PharmDr.: Biotransformace vybraných anthelmintik u tasemnice ovčí (*Moniezia expansa*) (Biotransformation of selected anthelmintics in sheep tapeworm (*Moniezia expansa*). 16.06.2015.

Mgr. NEPRAŠOVÁ, MARIE PharmDr.: Studium matricových tablet s dvěma typy Carbopolu (The study of matrix tablets with two types of the Carbopol®). 27.04.2015.

Mgr. NEZVEDOVÁ, MARKÉTA PhamDr.: Využití hydrofóbních materiálů na bázi oxidu zirkoničitého pro úpravu vzorku (Application of hydrophobic materials based on zirconium dioxide for sample pre-treatment). 26.03.2015.

Mgr. NOVÁKOVÁ, IVETA PharmDr.: Transport of Nsaids across a blood-brain barrier *in vitro* model based on cell line pbmec/c1-2. 23.09.2015.

Mgr. PALAN, MATYÁŠ PharmDr.: Effect of the subchronic and early-life permethrin exposures on rat liver: oxidative stress and endogenous antioxidant responses. 10.02.2015.

Mgr. PAUŠÍMOVÁ, ZUZANA PharmDr.: Vliv objemnosti neperiferně vázaných substituentů ftalocyaninů na jejich absorpční a fotofyzikální vlastnosti (The Effect of Bulkiness of Non-peripheral Substituents of Phthalocyanines on their Absorption and Photophysical properties). 13.11.2015.

Mgr. PAVLÍČKOVÁ, STELA PharmDr.: Srovnání účinnosti inhibitorů acetylcholinesterasy takrinu, rivastigminu a donepezilu při léčbě kognitivní poruchy vyvovalné 3-Quinuclidinyl benzilátem (Comparison of efficacy of acetylcholinesterase inhibitors tacrine, rivastigmine and donepezil for the treatment of cognitive disorders induced by 3-Quinuclidinyl benzilate). 23.09.2015.

Mgr. PEŠKOVÁ, PAVLA PharmDr.: Susceptibility profile of biofilms of non-albicans spp. to *echinocandins* . 19.02.2015.

Mgr. PODLIPSKÁ, KATEŘINA PharmDr.: Hodnocení toxicity směsi léčiv (Evaluation of drugs mixture toxicity). 23.04.2015.

Mgr. POSPÍCHALOVÁ, NAĎA RNDr.: Úprava biologického materiálu před HPLC analýzou – stanovení alfa tokoferolu v erytrocytární membráně (Treatment of biological samples before HPLC analysis – determination of alfa-tocoferol in erythrocyte membrane). 10.11.2015.

Mgr. PRAVDÍKOVÁ, KATEŘINA PharmDr.: Studium protektivních vlastností série nových aroylhydrazonových chelátorů iontů železa před poškozením srdečních buněk oxidačním stresem (Study of the protective properties of the series of novel aroylhydrazone iron chelators against the oxidative stress-induced cardiomyocyte injury). 10.02.2015.

Mgr. SAIFROVÁ, ZUZANA PharmDr.: Vývoj HPLC metody pro sledování stability roztoků kofeinu (Developement of HPLC method for monitoring stability of caffeine solutions). 26.03.2015.

Mgr. SEDLÁŘOVÁ, LUCIE PharmDr.: Analýza oxidačního stresu u modelu isoprenalinové kardiotoxicity po intravenozním podání rutinu (Analysis of oxidative stress in the model of isoprenaline carditoxicity after intravenous administration of rutin Cardiovascular). 19.02.2015.

Mgr. SKORKOVSKÁ, JANA PharmDr.: Vliv polyfenonu na vybrané biotransformační enzymy u myší (Effect of polyphenon on selected biotransformation enzymes in mice). 10.02.2015.

Mgr. STONAWSKÁ, MICHAELA PharmDr.: Porovnávání ferrozinové a přímé spektrofotometrie při stanovení chelatace železa (Comparison of ferrozine and direct spectrophotometry by assessment of iron chelation). 23.09.2015.

Mgr. STRÁŽNICKÁ, JULIE PharmDr.: Formulace a hodnocení stability perorální suspenze s nitrofurantoinem pro pediatrii (Formulation and stability evaluation of the oral nitrofurantoin suspension in paediatrics). 27.04.2015.

Mgr. SUPRUNOVÁ,VLASTA PharmDr.: Vývoj bioanalytické metody pro stanovení nových potenciálních léčiv odvozených od thiosemikarbazonu v biologickém materiálu (Development of a bioanalytical method for the determination of novel drug candidates derived from thiosemicarbazone in a biological materiál). 18.03.2015.

Mgr. ŠANDEROVÁ, PETRA PharmDr.: Genová výbava producentů biologicky aktivních látek v půdních bakteriálních společenstvech (Genetic apparatus of biologically active compounds producers in soil bacterial communities). 16.06.2015.

Mgr. ŠILHAVÁ, TEREZA PharmDr.: Ekotoxikologické hodnocení léčiva Diazepamu (Ecotoxicological screening of diazepam drug). 23.04.2015.

Mgr. ŠMIDRKALOVÁ, TEREZA PharmDr.: Využití HPLC pro stanovení barviv ilegálně používaných v potravinách (Using of HPLC for illegal colorants determination in food). 18.03.201.

Mgr. ŠRÁMKOVÁ, IVANA PharmDr.: Application of non-separation flow methods in pharmaceutical analysis. 03.12.2015.

Mgr. ŠTĚTKOVÁ, KATEŘINA PharmDr.: Studium vlivu složení mobilní fáze na selektivitu a retenci analytů na HILIC stacionárních fázích (Study of influence of mobile phase composition on selectivity and retention of the analytes on HILIC stationary phases). 18.06.2015.

Mgr. TAMBOR, VOJTĚCH PharmDr.: Identification of potential intraamniotic infection and inflammation biomarkers in amniotic fluid from preterm birth patients. 10.02.2015.

Mgr. VALÁT, MARTIN PharmDr.: Metabolismus monepantelu u parazitů a jejich hostitelů (Metabolism of monepantel in parazites and their hosts). 10.02.2015.

Mgr. VAŇKÁTOVÁ, HELENA PharmDr.: The analysis of eating habits of diabetics in the USA. 25.06.2015.

Mgr. VAVŘIČKOVÁ, HANA PharmDr.: Syntéza symetrických derivátů azaftalocyaninů substituovaných objemnými aromatickými substituenty (Synthesis of symmetrical azaphthalocyanines substituted with bulky aromatic substituents). 13.11.2015.

Mgr. VÍTOVÁ, LUCIE PharmDr.: Studium antiproliferačních vlastností série nových aroylhydrazonových chelátorů iontů železa (Study of the antipoliferative properties of the series of novel aroylhydrazone iron chelators). 10.02.2015.

Mgr. VRBATOVÁ, LUCIE PharmDr.: Stanovení derivátů estrogenu metodou sekvenční injekční chromatografie (Determination of estrogen derivatives by sequential injection chromatography). 18.06.2015.

Mgr. ŽELAZKOVÁ, JANA RNDr.: *In vitro* and *in silico* evaluation of biological activity of new compounds. 16.06.2015.

## PATENTS

Hrabálek, A., Roh, J., Karabanovich, G., Klimešová, V., Němeček, J., Pávek, P.: Substituovaný diazol, jeho použití a farmaceutický přípravek ho obsahující (Substituted diazoles, their use and pharmaceutical composition containing thereof). Praha: Úřad průmyslového vlastnictví, 2015. Patent No. CZ 305680 B6.

Hrabálek, A., Roh, J., Karabanovich, G., Klimešová, V., Němeček, J., Pávek, P.: Substituovaný tetrazol, jeho použití a farmaceutický přípravek ho obsahující (Substituted tetrazole, its use and pharmaceutical composition containing thereof). Praha: Úřad průmyslového vlastnictví, 2015. Patent No. CZ 305622 B6.

Miletín, M., Kopecký, K., Nováková, V., Zimčík, P., Cidlina, A., Švec, J.: Použití derivátů pyrazinu a jejich isosterů jako sloučenin vážících se do malého žlábku DNA (Use of the pyrazine derivatives and their isosters as DNA minor groove binders). Praha: Úřad průmyslového vlastnictví, 2015. Patent No. CZ 305332 B6.

# SOCIAL HAPPENINGS

## REMEMBRANCE OF Assoc. Prof. PhMr. RNDr. VĚRA SPURNÁ, DrSc. (* 26. 8. 1925 – † 30. 4. 2017)

On Sunday, the last day of April this year, a significant woman, assoc. prof. V. Spurná, née Košová, one of the first lectures of the newly founded Pharmaceutical faculty of the Masaryk University in Brno (1952). Although she has reached a respectable age of 92, and we all know that passing away is untenable, we have accepted this message with deep sorrow. When I recalling her life, I reveal Špát's perfect film essay Respice Finem; she filled the story of her life with Matthew's Gospel: Petite et dabitur vobis, quaerite et invenietis, pulsate et aperietur vobis.

Věra Košová was born in Olomouc where she also became a figure skater under the tutelage of her father. Her greatest achievements included the title of Junior Champion of Bohemia in Pardubice in 1946. Later she performed successfully in the Prague Revues. After ending her sport career she started to work in Brno and intensively devoted her activities to the training-methodical field and especially arbitration. She had become a reputable international referee, the only one in our country had received the prestigious Honorary ISU Referee title from the International Skating Union. She had also been for many years a very active worker within the national ice skating union; within the Bureau of the Union she held the position of the chairwoman of the Training and methodological section.

She graduated at the Faculty of Science of Masaryk University in Brno, where she obtained PhMr. degree in 1948, degree of RNDr in 1950 and in 1963 the degree of Associate Professor. She to the great extend contributed establishment of the Center of Medicinal Plants at Kraví Hora. She had been forced to leave the faculty in 1959 due to the political purg. She got a new job as a scientist at the Biophysical Institute of the Czechoslovak Academy of Sciences in Brno (under the supervision of Prof. Herčík) in the Department of Cytogenetics. In 1982, she earned a scientific rank of Doctor of Science. At the end of her career she returned to teaching at the Pharmaceutical Faculty at the newly-established Veterinary and Pharmaceutical University in Brno. Around a year 2000, she lectured for several years' Pharmaceutical botany to foreign students at the Faculty of Pharmacy of Charles University in Hradec Králové, where she had become a very popular pedagogue, always smiling, with excellent relations with students. In addition to this teaching activity, I had been with assoc. prof. Spurná cooperating in field of science much earlier: from 1974 to 1985, the Department of Pharmaceutical Botany cooperated with the Biophysical Institute of the Czechoslovak Academy of Sciences in the area of cytogenetics, more precisely

on the effect of ionizing radiation on some pharmaceutically significant plants. Assoc. prof. Spurná headed this research.

In 1960 she got married to the assistant professor of Department of Natural Sciences at Masaryk University RNDr. Miloš Spurný. After 19 years the sharing of their life in common, full of activities and workload, ended with the death of her husband. In memories of Miloš, a botanist and landscape ecologist, she dedicated (as a co-author) a book "Farewell, Old River", in which she very sensitively approached her husband's photographic work.

In the last 30 years of her life Mrs. Věra spend along with her friend, artist Miloš Kuda and his children, so in the late age she became aware of the family's concerns and moods from the perspective of mother (although unfortunately stepmother) and grandmother.

Great woman had left: selfless, hardworking, tenacious, giving joy, a person capable of encouraging, pointing the way and strengthening the situations of her own life, a person we no longer have a chance to ask. The ancient sentence quidquid agis, prudenter agas et respice finem, written at the head of the sundial in the Middle Ages, Mrs. Věra filled perfectly.

Tribute to her memory!

*Prof. RNDr. L. Opletal, CSc.*

# Prof. KAREL WAISSER, PhD., DSc., Deceased

Slightly more than one year has passed since I wrote about the 80th anniversary of Professor Karel Waisser's birthday. Today's message is far from cheerless, since, following a short and serious illness, he passed away on 6 June 2017.

On this sad occasion, I would like to have a few recollections of Karel, who was an enthuasiastic chemist, and, above all, a very good friend.

Karel joined the teaching staff at the Faculty of Pharmacy in Hradec Králové as a young and enthusiastic Lecturer following his return from a postdoctoral in Canada.

He got involved in somewhat difficult teaching of chemistry at the Faculty, which was literally being built at that time, both in terms of physical construction and curriculum. In fact, teaching chemistry existed just "on paper". Karel also immediately started participating in faculty research, namely in the synthesis of potential antituberculotics. Introduction of mathematical methods to new drug development, known under the abbreviation of QSAR, can be regarded as his noticeable personal contribution to the faculty research. Having been a pioneer of this approach in then Czechoslovakia in late 1970s and early 1980s, his activities in the field soon became famous all over the country. As a result, Prof. Waisser is registered as an author or co-author of several hundreds of papers, 140 out of which are in impacted journals, and the rest in local ones without an IF. Publishing in local journals used to be a standard in the 1970s and 80s, since access to "noble" journals (as he used to call them) was not entirely straightforward.

Due to his political opinions, to which he proudly and openly adhered regardless of the difficult times following the Soviet invasion in 1968, Karel's promotion to a Reader and then a full Professor was out of question for a long time. Mainly for this reason, he was promoted to the former rank in 1987, and became a full Professor and a Doctor of Science (DSc.) as late as in the beginning of the 1990s.

Having been a very good teacher, Prof. Waisser was popular with his students. He tried to bring organic chemistry closer to them through using elements of fun in teaching. His group dances demonstrating vibrations of organic molecules irradiated by IR light, educational songs explaining the need for using boiling chips (Betulin blues) and a Song of Meerwein-Ponndorf rearrangement in the molecule of betulin testify to this. He loved the character of the fictitious Czech genius Cimrman by Zdeněk Svěrák, and the performances

of actors Vodňanský and Skoumal. He embraced their humour, understood its principle, and was able, in his own specific way, to develop it further.

Karel Waisser, however, was above all a very nice person, and a good friend and fellow. He liked fun, and hated foul tricks, falseness and lies, something we currently encounter almost daily. Me and him spent numerous moments over coffee up to his last days here, when he just now and then stopped by at the Department. We used to have lots of fun during these, without exception pleasant, visits.

Karel, we will miss you. Your presence always left positive feelings in those of us who knew you and had the honour and the pleasure to collaborate with you. You knew that even at the times when something was going wrong in our lives (and yours was no exception), there was always a reason to go on. From your way of life, we got to understand that developing of things and events in contrast to our wishes should serve as an incentive to us to go on, and to do our best to reverse this or at least make things go a bit more favourably.

Karel, we hope you feel better now, and maybe you will meet your big friend George up there. You will know, whom I have in mind.

*Alexandr Hrabálek*
Faculty of Pharmacy, Charles University, Hradec Králové

# INSTRUCTIONS FOR AUTHORS FOR FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

Manuscripts should be submitted on the A4 paper, in English, typed in editor Microsoft Word, format Times New Roman 12 normal.

Manuscript should be divided into sections:

Title – (14, left alignment, (SPECTROPHOTOMETRIC DETERMINATION OF …). Write the title in lowercase letters and then format it using Font – All caps (Písmo – Všechna velká).

Names of Authors – first name and surname with the reference to institution's name (Times New Roman 12, center alignment) (JIŘÍ GASPARIČ[1], MILENA ČERMÁKOVÁ[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, centre alignment) (1 Department of …, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic).

Email address – (Times New Roman 10, centre alignment) (e-mail: …).

The text should be written continuously in Times New Roman 12, normal, left alignment with line space 1.5, without indent, only using left alignment, and starting a new paragraph by "enter". Bold and Italic may be used. Manuscript should be divided into sections:

Headings of individual sections for original Papers:
Abstract – 12, bold, left alignment (ABSTRACT)
Keywords – maximum 5 keywords – 12, bold, left alignment (KEYWORDS: extraction – spectrophotometry)
Introduction – 12, bold, left alignment (INTRODUCTION)
Experimental – 12, bold, left alignment – (EXPERIMENTAL)

Text can be further divided:
Main Chapter (12 bold, left alignment), Subchapter (Italics, 12 bold, left alignment) and Further (Italics, 12, left alignment).
If it is not absolutely necessary, do not use more than three levels of headlines.
Results and discussion – 12, bold, left alignment (RESULTS AND DISCUSSION).

Figures must be submitted (colourless)< in the best quality and 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, centre alignment).

<Koukal_Fig2.jpg>
Fig. 1. Structures of the studied compounds

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

Chemical structures should be drawn with a suitable drawing program.

Acknowledgements – 12 italic, left alignment (Acknowledgements)
Text (12, italic, left alignment)

References – 12, left alignment (References)
References must be numbered continuously and indicated as an upper index in the text.
References from journals:
1. Agrawal, Y. K., Patel, D. R.: Spectrophotometric Determination of Clioquinol. Indian J. Pharm. Sci., 47, 1985, 207–209.
References from books:
1. Němcová, I., Čermáková, L., Gasparič, J.: Spectrophotometric Reactions. New York, Marcel Dekker Inc., 1996.

Manuscripts should be submitted in one copy (on a paper and on CD) to the editor (Assoc. Prof. RNDr. Pavla Žáčková, CSc., Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové, Heyrovského 1203, 500 05, Czech Republic; e-mail: folia.@faf.cuni.cz).

UNIVERSITAS CAROLINA PRAGENSIS

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