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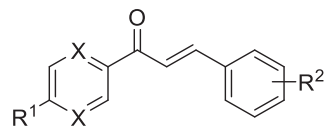
Long-term complications of diabetes have been associated with the distinct activation of polyol pathway enzyme – aldose reductase (AKR1B1) that is responsible for sorbitol production and its accumulation under hyperglycemic conditions which contributes to oxidative stress.[1]

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].

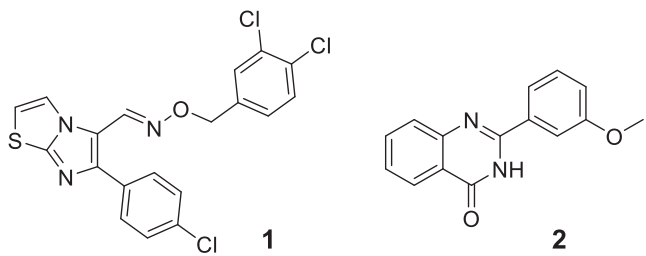
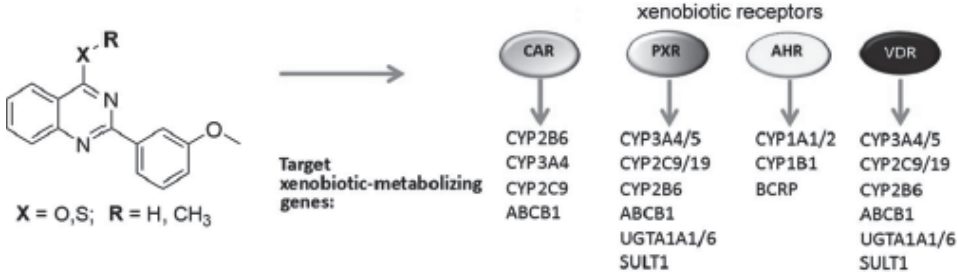


Fig. 1.

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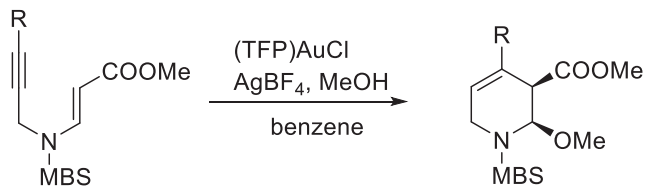
In order to expand our research[1,2] on chemoselective gold(I)-catalyzed cyclization of β-propargylamino acrylic esters, we developed the nucleophile-assisted diastereomeric cyclizations to furnish substituted aminoacetals (Scheme 1).



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).

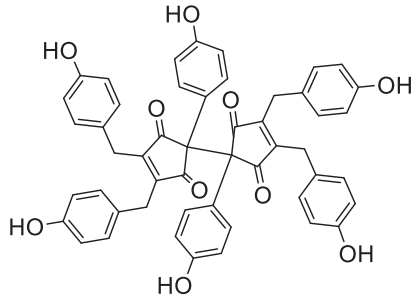


Fig. 1. Nostotrebin 6.

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RED-EMITTING AZAPHTHALOCYANINE SENSORS HIGHLY SELECTIVE TOWARD POTASSIUM CATIONS

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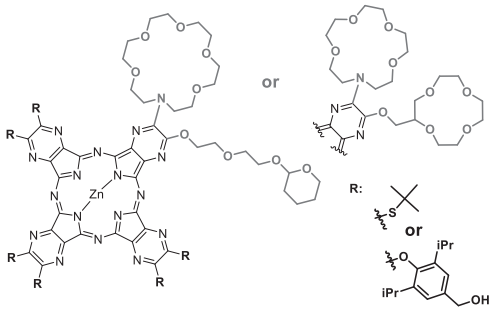
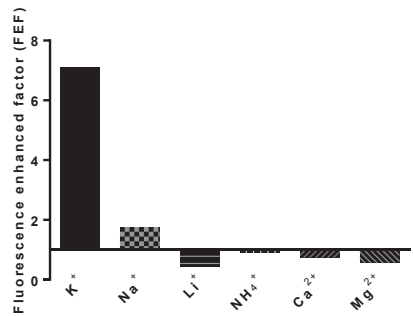
Azaphthalocyanines (AzaPcs) are planar macrocyclic compounds with advantageous fluorescence properties like absorption and emission over 650 nm, high extinction coefficient (approx. 200 000 L mol^{-1} cm^{-1}) and high fluorescence quantum yields. Recently, AzaPcs bearing aza-crown on periphery have 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

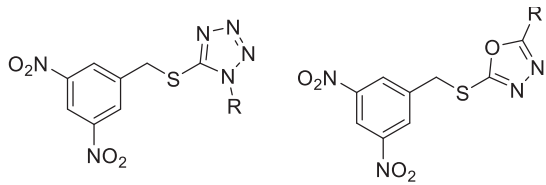

Fig. 1.

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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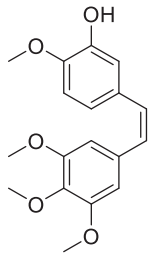
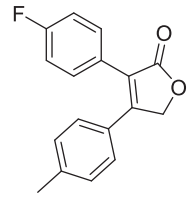
The aim of this project was to synthesize a novel library of α,β-diphenyl furanones as potential antitumor and antimicrobial agents. Model structures were designed as analogues of the stilbenoid combretastatine (**1a**), without oxygenation of the phenyl rings. Our previous results have shown that derivatives 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



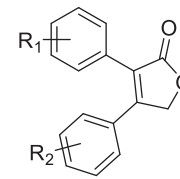
1a

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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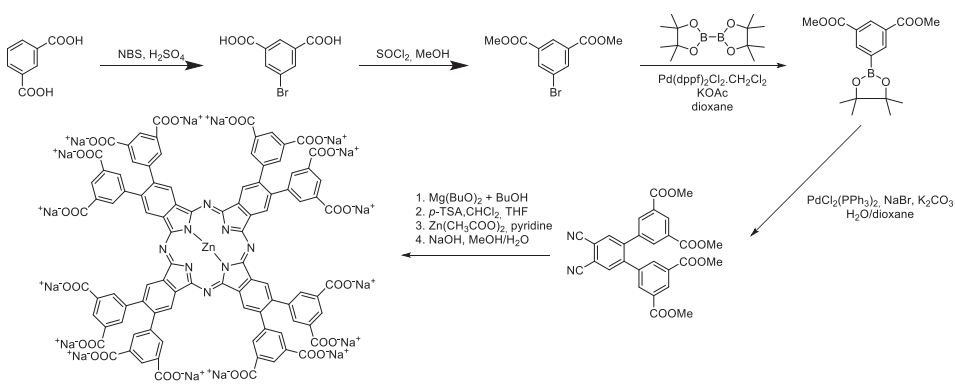
Phthalocyanines (Pc) represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. This work closely follows the previous project[1] that 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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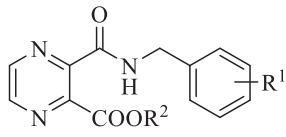
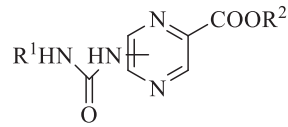
Tuberculosis (TB) is a common infection that had been successfully treated with proper first line anti-TB drugs. Yet this curable infection has become a frequently deadly illness due to the increasing antimicrobial resistance (AMR).[1] This serious issue has driven efforts to find new therapeutic drugs 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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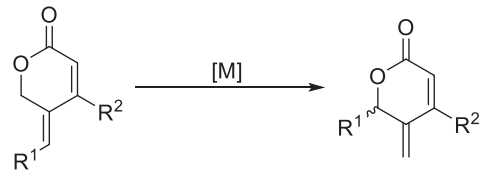
Our group has previously reported an interesting rearrangement of lactones (**1**) prepared by Migita-Stille coupling.[1] The original coupling products rearranged to the isomeric 5,6-dihydro-5-methylene-2*H*-pyran-2-ones (**2**).

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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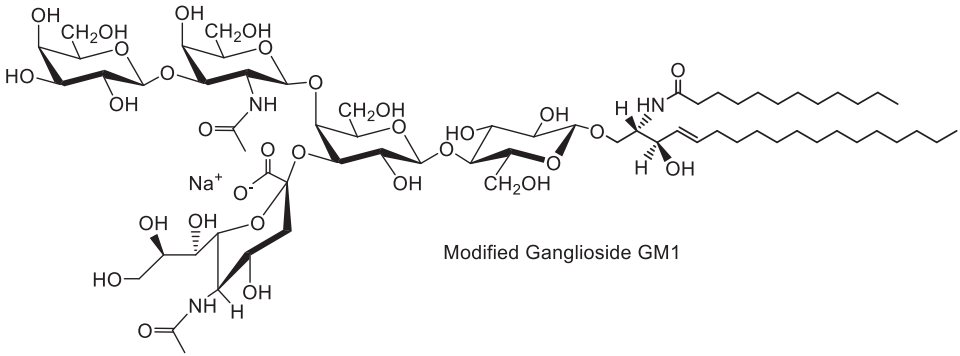
Gangliosides are acidic glycosphingolipids that contain one or more sialic acid residues and are particularly prevalent on neuronal cells. They seem to be involved in cell-to-cell interactions and regulations of activities of receptor tyrosine kinases in the plasma membrane. The regulation of the receptor 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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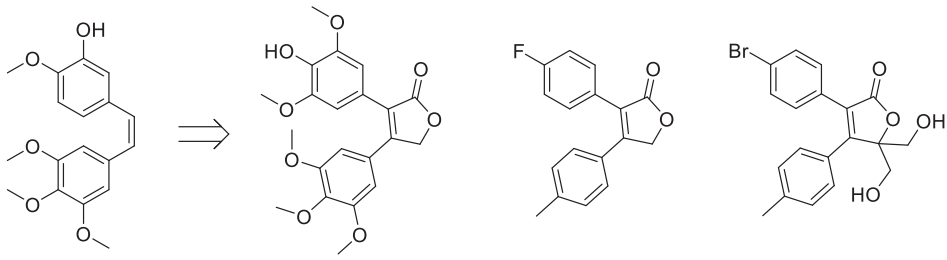
Synthesis, derivatization and biological activity evaluation of 3 libraries of α,β-diaryl furanones have been executed.

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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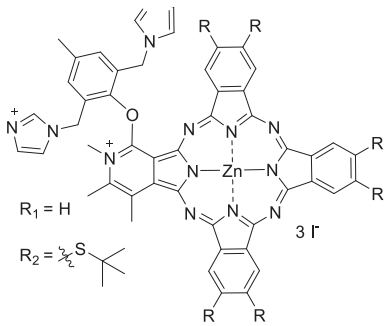
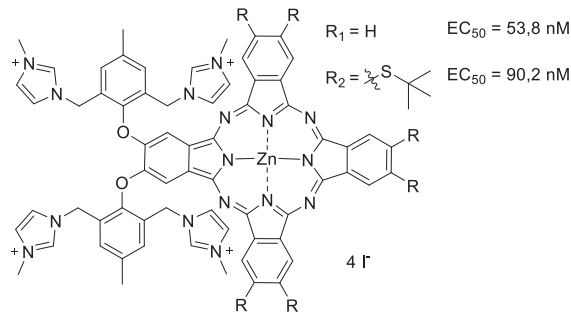
Phthalocyanines (Pcs) are planar macrocyclic compounds, which have ability to produce efficiently singlet oxygen. Singlet oxygen is the cytotoxic species in photodynamic therapy of cancer that besides this agent involves also light and photosensitizer (*i.e.* Pc). Recent results of research of our group 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.

The study was supported by Charles University (project GA UK No. 772216), the Faculty of Pharmacy (SVV/2016/260295), MH CZ – DRO (UHHK, 00179906) and PRVOUK P40.

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.

The study was supported by GAČR 13-15008S, PRVOUK P37/05 and UNCE 33/2012.

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.

The study was supported by GAUK 338315 (170/50/55006).

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.

The study was supported by SVV/260292/2016.

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.

The study was supported by SVV 260292/2016.

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.

The study was supported by Charles University (projects GAUK 344 615 and SVV 260 291).

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.

The study was supported by grants SVV 260291, SVV 260292 and Charles University grant Nr. 17/2012/UNCE.

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.

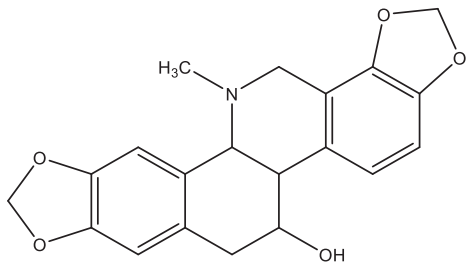

Fig. 1.

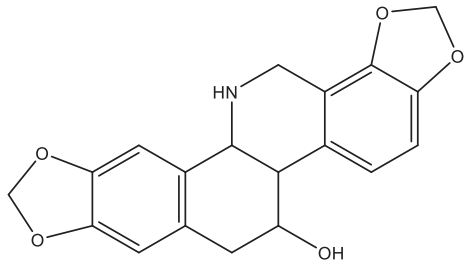

Fig. 2.

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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.

The study was supported by PRVOUK P40.

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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.

The study was supported by the Czech Science Foundation (13-15008S).

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.

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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.

The study was supported by Charles University (GAUK 1404213 and 88615, SVV 260 291), and by the Czech Science Foundation (13-23811S).

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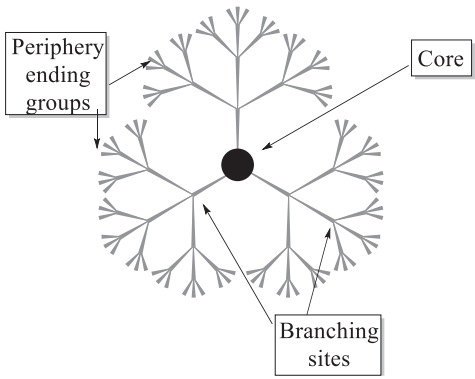
Highly branched, multivalent and monodisperse polymeric molecules, generally known as dendrimers (Scheme 1), have received continuous interest in recent years because of the global demand for new nanomolecules that are useful in advanced technology and medicine.[1]



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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