ABSTRACTS

5th POSTGRADUAL AND 3rd POSTDOCTORAL SCIENTIFIC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ (CZ), CHARLES UNIVERSITY (CZ)

HRADEC KRÁLOVÉ, 3–4 FEBRUARY 2015

PLENARY LECTURE

CONTINUING THE EXPLORATION OF IN-SYRINGE STIRRING: APPLICATIONS BEYOND DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION

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The "Lab-On-Valve" technique[1] can be considered as an ideal and versatile approach to automate SPE procedures in a Sequential Injection Analysis system as it presents an optimized manifold format to handle sorbent beads and to create renewable SPE packings.

"In-Syringe Analysis" or "Lab-In-Syringe" can be then considered as a complement tool to automate liquid-liquid microextraction (LLME) protocols. Using a magnetic stirring bar inside the syringe[2], it further allows mixing of numerous solutions homogenously and widely independent from volumes and viscosities.

Mostly, dispersive LLME but also head-space single-drop micro-extraction have been automated with this approach[3]. In this presentation, we demonstrate the potential of in-syringe analysis with magnetic stirring on two further approaches of sample treatment.

Firstly, automated in-syringe fixation of dissolved oxygen determination according Winkler standard method with less than two minutes compared to 24 h recommended incubation time.

Secondly, salting out homogenous LLME is presented as a proof-of-concept, which allows extraction of moderately hydrophobic compounds into chromatography compatible solvents.

Financial support by Charles University by Project PRVOUK P40/1105 and Charles University Research Centre (UNCE 204026/2012).

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BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

STUDIES TOWARDS THE SYNTHESIS OF MORPHINE PRECURSOR

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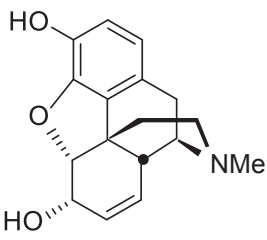
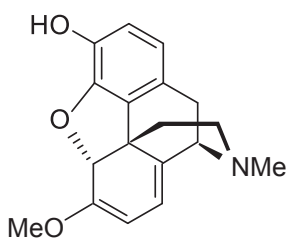
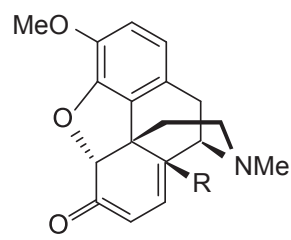
Morphinans are known to be excellent analgesics since centuries. Especially, morphine is referred to as a gold standard in the management of severe pain. These opiate structures have continued to attract the organic chemists for their challenging syntheses. A plethora of publications have been reported since the groundbreaking work of Gates in 1952.[1] More than twenty syntheses are reported for morphine alone, however, none of them offer practicality on large scale. Also, the unnatural derivatives of opiates, whether agonists or antagonists, are all derived by semisynthesis from the naturally occurring alkaloids.[2] Thus, the situation demands efficient, cost effective and practical method for the synthesis of this class of compounds. In the presented work, we have designed a concise route for the synthesis of hydromorphone, a precursor to morphine and related compounds. The chirality is achieved by enzymatic oxidation and key steps of the synthesis involves Diels-Alder reaction, Mitsunobu reaction, Wittig reaction, Corey-Chaykovsky Reaction, and Oppenaur oxidation.



morphine (1), R = H
codeine (2), R = Me



thebaine (3), R = Me
oripavine (4), R = H



oxycodone (5), R = OH
hydrocodone (6), R = H

The study was supported by Ministry of Education, Youth and Sport through the operational programme ECOP (Grant No. CZ.1.07/2.3.00/30.0061).

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COMPUTATIONAL APPROACH TO SEARCH FOR NOVEL ANTITUBERCULOTICS

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Tuberculosis remains one of the world's leading infectious diseases not only in developing countries. *Mycobacterium tuberculosis* multidrug resistant strains, HIV co-infection and the prospect of nosocomial transition are currently highly problematic issues[1].

Mycobacterial enoyl-ACP-reductase is an enzyme contributing in mycolic acids biosynthesis. It has been established within FAS II system as a promising target for the novel inhibitors. The development of inhibitors effective without previous activation by catalase/peroxidase system, seems to be a rational approach[2].

We hereby present the results of virtual screening study. MOE 2013 software package was used throughout the experiments. ZINC Natural Derivatives – chemically modified natural products database (almost 38,000 entries) was selected to be screened for substances with potential affinity towards the mycobacterial enoyl-ACP-reductase. Three enoyl-reductase crystallographic protein structures (pdb no: 2X23, 1P44, 4TZK) were used as a receptor. Almost 300 compounds surpassed affinity of the known inhibitors. Successful compounds were subsequently subjected to the molecular docking for further selection. Suitable candidates were consequently docked using the induced fit protocol and binding mechanism of most successful potential inhibitors was proposed.

The study was supported by project no. CZ.1.07/2.3.00/20.0235.

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SYNTHESIS OF NOVEL A,B-DIPHENYL FURANONES AS POTENCIAL ANTITUMOROUS AND ANTIMICROBIAL AGENTS

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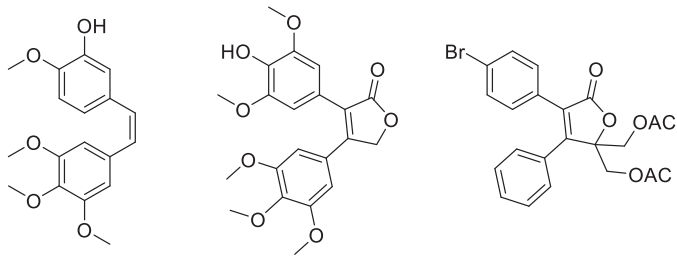
The project deals with the synthesis, derivatization and biological activity evaluation of a libary of α,β-diphenyl furanones related to natural combretastatins. The lead structure is derived from *cis*-stilbene with high degree of oxygenation of both phenyl rings (Fig. 1). Two combretastatine analogues are currently under clinical trials as potential antineoplastic drugs. Our derivatives also show potent anticancer activities and relatively low toxicity to normal cells.

As shown by our results, molecules with halogen or methyl group on both cores and molecules with functionalized γ-position of the furanone skeleton also possess an interesting antimicrobial activity.



Combretastatine A-4

This work was supported by Charles University (SVV 260 062 and GAUK 19062 14) and Czech Science Foundation (P207/10/2048).

NOVEL ABAD MODULATORS FOR MODIFYING TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is one of the most frequent neurodegenerative disorders in elderly where extracellular amyloid-beta (Aβ) presents to be one of the hallmarks in AD pathogenesis. However, it is well known that Aβ is also located intracellularly interacting with many proteins and altering their proper functions. One of the affected proteins is mitochondrial amyloid-binding alcohol dehydrogenase (ABAD), enzyme directly interacting with Aβ. Among other, altered function of ABAD leads to disruption of cell homeostasis consequently resulting in cell death. Thus, ABAD and ABAD-Aβ interaction represent potential drug target for AD treatment[1–3].

A series of novel frentizole analogues was prepared with different substitution of phenyl and benzothiazolyl moieties (Fig. 1). Consequently, their potency to affect ABAD enzymatic activity was evaluated. Two compounds were found to decreased ABAD activity by 80%, where reference compound K691 with IC_{50} of 2.16 μM[4] exhibited only 60% decrease in ABAD activity. Furthermore, one compound showed ~20% increase in ABAD activity.

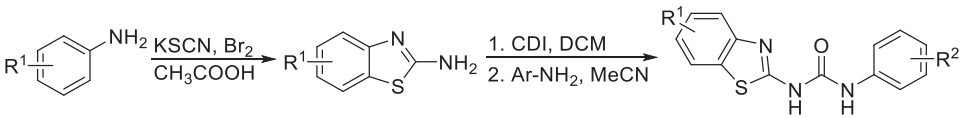

Fig. 1. Preparation of substituted benzothiazolylureas.

The work was supported by the European Social Fund, the state budget of the Czech Republic (Project no. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB), GAUK B-CH/992214 and SVV 260 062.

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PREPARATION OF QUINAZOLINE COMPOUNDS WITH BRONCHODILATORY ACTIVITY

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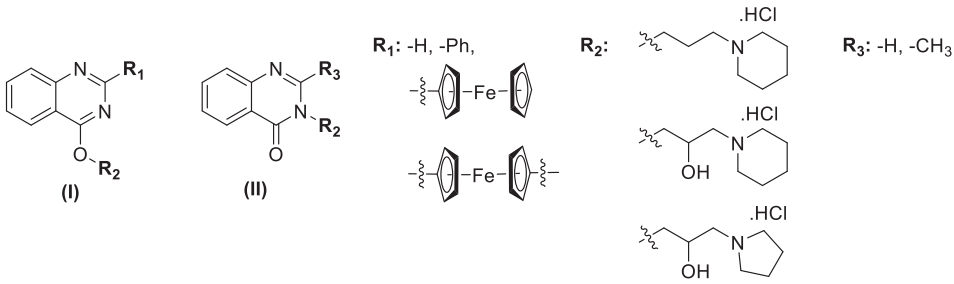
Two most active compounds from previous screening were selected as model structures, substituted *O*-alkylquinazolinone (**I**) and *N*-alkylquinazoline (**II**).[1,2] The "active"



(piperidine-1-yl)propyl and its variations were attached to quinazoline ring to examine relationship between the bronchodilatory effect and the heterocycle.

Preliminary results of bronchodilatory screening showed very promising effect of the compounds in this series. Even though the activity of the most successful derivatives are not yet comparable to ipratropium bromide, due to the fact that their mode of action is still not explored, it renders them possible target for further development.

This work was supported by the Czech Science Foundation (project No. P207/10/2048) and by Charles University (SVV-260-062).

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PREPARATION AND BIOLOGICAL EVALUATION OF NOVEL PYRAZINAMIDE DERIVATIVES SYNTHESIZED UNDER MICROWAVE CONDITIONS

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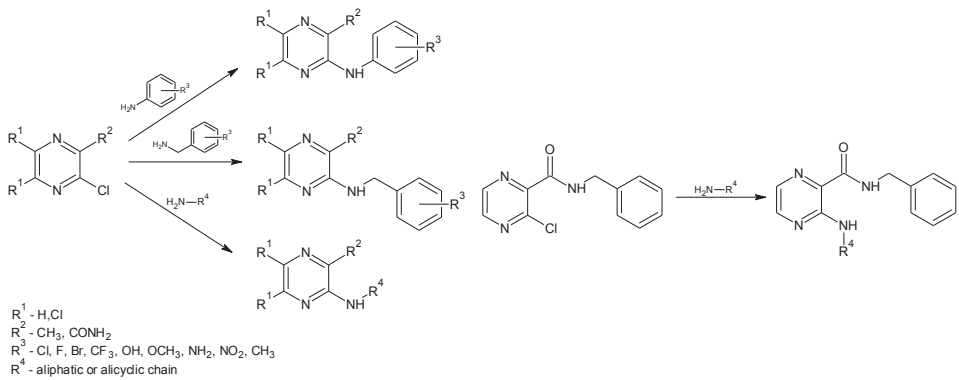
The latest epidemiological reports are contradictory. The incidence of new tuberculosis (TB) cases is diminishing since 2006 according to WHO. Problems have arisen with drug resistant strains of *Mycobacterium tuberculosis* that are appearing more frequently than ever before despite the fact that global efforts are oriented to better public awareness and intensive research.[1] HIV co-infection is also problem connected to TB treatment. These consequences lead to urgent need of finding the effective, safer and innovative drugs.

One of possibilities is to modify known molecules. One of them is pyrazinamide (PZA), which is counted among the first-line antituberculotic agents. Its' advantages are the activity against dormant forms of *M. tuberculosis* and its' mode of action that was found to

be multiple (acidification of inner compartment of mycobacterial cell, inhibition of FAS I enzyme, inhibition of *trans*-translation).[2,3,4]

This research project is focused on synthesis of derivatives of pyrazinamide because this small molecule is very suitable for structure modifications due to its unique chemical properties.



Three starting substances (5-chloro-6-methylpyrazin-2,3-dicarbonitrile, 3-chloropyrazine-2-carboxamide, 3-chloropyrazine-2-carbonylchloride) were treated with compounds containing amino group (anilines, benzylamines, aliphatic amines) under microwave conditions that were determined experimentally. Products were characterized by analytical data (NMR, IR, melting point, elemental analysis). Lipophilicity was calculated as log *P* and experimentally determined as log *k*.

Biological screening was focused on antimycobacterial, antibacterial, antifungal and herbicidal evaluation. Antimycobacterial assays were carried out against *M. tuberculosis* and two non-tuberculosis strains using PZA and isoniazide as standards. Antibacterial (8 strains) and antifungal (8 strains) tests were completed using five antibiotic and four antimycotic standards. Herbicidal activity was measured as the inhibition of photosynthetic electron transport in spinach chloroplasts using industrial herbicide DCMU as standard.

A group of substances has shown antimycobacterial and herbicidal activities and some of these values were the same or better than the activity of standards. Structure-activity relationships were predicted either for active compounds or whole group with the same basic structure traits based on the influence of lipophilicity parameters.

The study was supported by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB. This study was also co-financed by the Grant Agency of Charles University (B-CH/710312, B-CH/1594214), by Ministry of Health of Czech Republic (IGA NZ 13346) and by Ministry of Education, Youth and Sports of Czech Republic (SVV 260-062).

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DRUG DESIGN AND DEVELOPMENT OF NOVEL DRUGS FOR ALZHEIMER'S DISEASE TREATMENT

KORABEČNÝ, J.,[1,2] NEPOVIMOVÁ, E.,[1,2] SPILOVSKÁ, K.,[1,2] ANDRS, M.,[1,2] BABKOVÁ, K.,[1,2] MUSÍLEK, K.,[1,2,3] SOUKUP, O.,[1,2] KUČA, K.[1,2]

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Alzheimer's disease (AD) is a multifactorial disorder and apparently involves several different etiopathogenetic mechanisms. Up-to-date, there are no curative treatments or effective disease modifying therapies for AD. On the other hand many aspects of AD are currently debated or even unknown. Current efforts in the development of novel drugs aimed against AD are represented by the so-called Multi-Target-Directed Ligands (MTDLs), the therapeutic strategy followed not only in the AD research but also in other diseases. MTDLs combine drugs action at different levels of the neurotoxic cascade. MTDLs represent challenging approach giving people suffering from AD a new hope to slow down or even cure this insidious disease. Within our contribution, novel trends in designing and development of novel MTDLs as potential anti-AD drugs will be presented.

The work was supported by the Grant Agency of the Czech Republic (No. P303/11/1907), by Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011) and by MH CZ – DRO (University Hospital Hradec Králové, No. 00179906).

HALOGENATED PYRAZINE-BASED CHALCONES AS POTENTIAL ANTIMICROBIAL AGENTS

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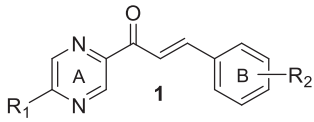
Chalcones are naturally occurring precursors of flavonoids that are biologically active plant components. However, chalcones exert a wide range of bio-activities themselves. Chalcones are chemically 1,3-diphenylprop-2-en-1-ones.[1] Our research group focused on their pyrazine analogues (**1**), several series were synthesized, and fungal susceptibility to these compounds was tested. Some compounds showed activity comparable to that of fluconazole against *Trichophyton mentagrophytes*. It was found that alkyl substitution on the pyrazine ring has no decisive and unequivocal influence on the *in vitro* antifungal activity against this strain. The highest potency was exhibited by derivatives with electron withdrawing groups (EWG) in positions 2 and 4 of the ring B.[2,3]



1

R^1 = H, alkyl
R^2 = electron-donating or -withdrawing group

As halogens also have EWG properties, halogenated derivatives were prepared and submitted for antifungal and antibacterial tests. Some structure-activity relationships have been deduced.

The study was supported by PRVOUK P40 (Charles University).

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THE ROLE OF SIZE OF AZA-CROWN RECOGNITION MOIETY IN CATION-SENSING AZAPHTHALOCYANINE FLUORESCENCE INDICATORS

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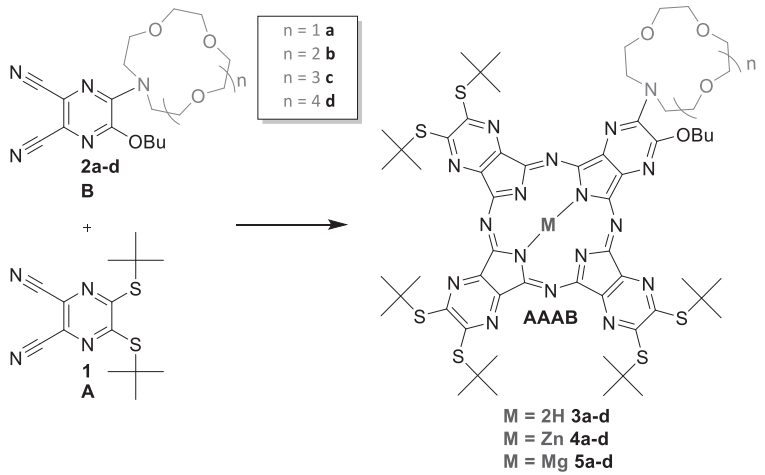
Azaphthalocyanines (AzaPcs) are macrocyclic planar compounds with unique absorption (over 650 nm) and interesting photophysical properties (emission over 650 nm, high Φ_F and Φ_Δ). Their sensoric properties are based on intramolecular charge transfer (ICT). ICT occurs between donor (peripheral alkylamine) and acceptor (macrocyclic core) moiety

of AzaPc and is responsible for quenching of fluorescence. After coordination of metal cation ICT is switched off leading to increase of fluorescence[1].

This work is focused on the study of AzaPcs as potential fluorescence indicators sensitive to metal cations. The series of unsymmetrical AzaPc indicators with different size of aza-crown recognition moieties was synthesized according to the Scheme 1 below. The precursors 1 (A) and **2a–d** (B) were prepared by nucleophilic substitution. Their statistical condensation initiated by $Mg(BuO)_2$ led to a mixture of six different AzaPc congeners. Magnesium cation was removed from the center by *p*-toluensulfonic acid. Asymmetric congener AAAB (**3a–d**) was isolated and purified as a metal-free derivative. Then zinc or magnesium was coordinated into the center leading to the final AzaPcs **4a–d** or **5a–d**.



Scheme 1. Statistical condensation.

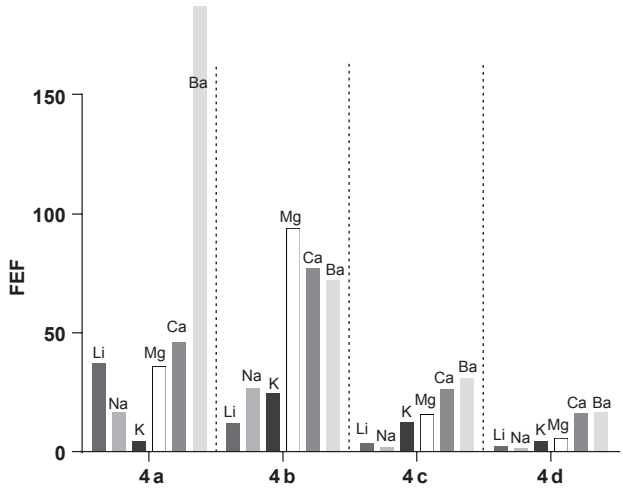


Fig. 1. Fluorescence titration experiment.

Desired AzaPc indicators were studied by the mean of fluorescence titration experiments. Selectivity to different cations (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}) was observed as increase of fluorescence after addition of metal cation (Figure 1). The results showed that the cavity size of aza-crown recognition moiety affected selectivity to the certain metal cation, especially in the group of alkali earth metals. Emission at longer wavelengths and insensitivity to the pH of the medium[2] are great advantage. These properties are very promising for the next developing fluorescence indicators.

The study was supported by GA UK 494214 and SVV 267 001.

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SYNTHESIS OF SUBSTITUTED HETEROCYCLES USING TRIS(2-FURYL)PHOSPHINE GOLD (I)

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Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as a catalyst. In order to expand our research[1] on cyclisation of propargyl vinyl ethers to dihydropyrans using tris(2-furyl)phosphine gold(I) chloride and silver tetrafluoroborate, we employed the same catalytic system on enynes with other heteroatoms. The synthetic protocol was optimized and a series of substituted nitrogen heterocycles synthesized.

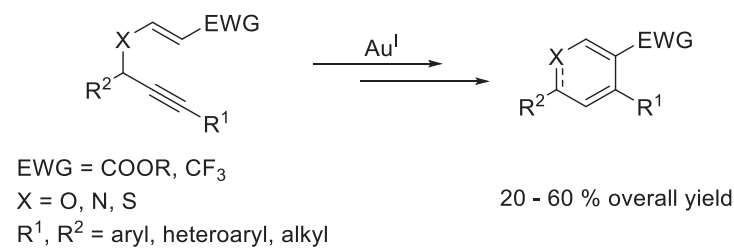

Fig. 1.

The study was supported by Charles University (SVV 260 062 and GAUK 5671/2012) and Czech Science Foundation (P207/10/2048)

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COMPLETE SYNTHESIS OF ULTRA LONG HUMAN SKIN CERAMIDES

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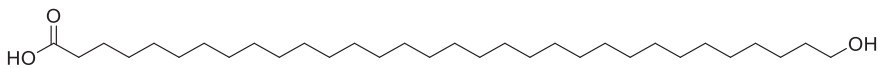
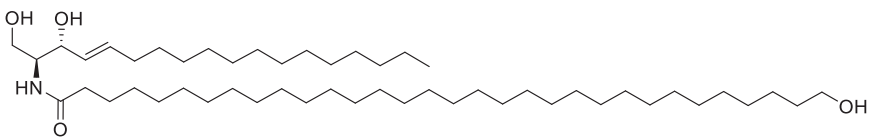
The main skin barrier is situated into stratum corneum, the top layer of the skin. Corneocytes (flat cells) in stratum corneum are surrounded by lipidic matrix, which is composed of equimolar mixture of cholesterol, free fatty acids and ceramides.

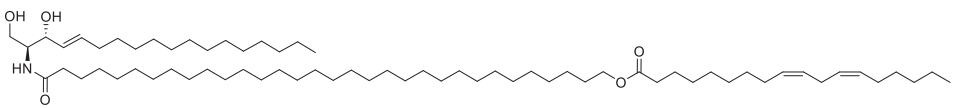
The aim of our work was to prepare ceramides with ultra long chain (also known as acylceramides or ceramides of the O and EO classes), because these molecules are not commercially available, but they are essential for the proper skin barrier function.



Scheme 1. 32-hydroxydotriacontanoic acid.



Scheme 2. Ceramide OS.



Scheme 3. Ceramide EOS.

Synthesis of O- and EO-type ceramides started from 16-bromohexadecanoic acid. The long chain was obtained in several steps, including Wittig reaction. Product was converted to succinimidylester, hydrogenated and deprotected to obtain succinimidylester of 32-hydroxydotriacontanoic acid, which was directly used in synthesis of ceramides of the O class. Linoleic acid was connected to the omega-hydroxy group using Yamaguchi reaction and after the reaction with sphingoid base, ceramides of the EO class were obtained.

This work was supported by the Czech Science Foundation (13-23891S) and by Charles University (SVV 260 062).

RECENT OUTCOMES OF NOVEL MODIFICATIONS ON THE STRUCTURE OF SALICYLANILIDES

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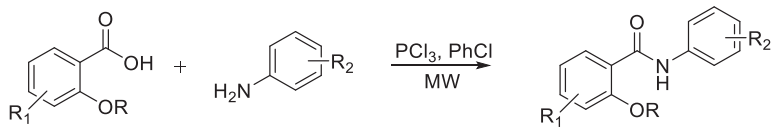
Salicylanilides and their derivatives have shown great promise as antimicrobial agents over the past years.[1,2]



The present work is highlighting our recent work on the field, including new modifications on the basic structure of salicylanilides and commenting the effect of these modifications to the activity against different pathogens as well as the impact to the toxicity of the final molecules.

The study was supported by by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW PREPARED *N*-ALKYL-3-(ALKYLAMINO)PYRAZINE-2-CARBOXAMIDES AND THEIR PRECURSORS

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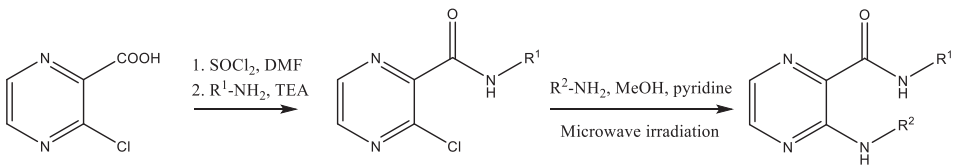
Tuberculosis (TB) still remains a major global problem. There were 9.0 million of new TB cases in 2013 and 1.5 million of TB deaths.[1] Multidrug-resistant (MDR) and exten-

sively drug-resistant (XDR) TB along with HIV co-infection are important reasons for developing new potential antitubercular drugs. Pyrazinamide (PZA) belongs to the most important first-line antituberculotic agents used in TB therapy and offers many possibilities to develop new highly effective compounds.

Series of *N*-alkyl-3-chloropyrazine-2-carboxamides **(1)** and *N*-alkyl-3-(alkylamino) pyrazine-2-carboxamides **(2)** were prepared according to the results of antimycobacterial evaluation of 5- and 6-(alkylamino)pyrazine-2-carboxamides reported previously (6-octylaminopyrazine-2-carboxamide, MIC = 1.56 μg/mL; 5-octylaminopyrazine-2-carboxamide, MIC = 6.25 μg/mL; *M. tuberculosis* H37Rv).[2]



R[1], R[2] - methyl-octyl

1 **2**

All compounds were characterized with analytical data and tested *in vitro* for their antimycobacterial (*M. tuberculosis* H37Rv, *M. avium* and *M. kansasii*), antibacterial and antifungal activity. 3-Octyl-, 3-heptyl- and 3-hexylamino-*N*-methylpyrazine-2-carboxamide were the most effective against *M. tuberculosis* (MIC = 25 μg/mL). Other compounds exerted lower or none activity.

The study was financially supported by the Grant Agency of Charles University, project B–CH/1594214, and SVV 260 062. This work is co-financed by the European Social Fund and the state budget of the Czech Republic. Project TEAB no. CZ.1.07/2.3.00/20.0235.

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THE STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF 3,5-DINITROBENZYLSULFANYL HETEROAROMATES, NEW HIGHLY EFFICIENT ANTIMYCOBACTERIAL AGENTS

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Tuberculosis (TB) is becoming worldwide health and economic problem. Bacterial strains causing this disease have developed resistance against approved antiTB drugs which have been presented several decades ago and since then there was no compound introduced to clinical practice.

Recently our group have developed heteroaromatic compounds containing dinitrobenzyl fragment with high and selective antimycobacterial activity. Heteroaromatic fragment is represented by variously substituted tetrazole,[1,2] oxadiazole and thiadiazole.[3] Moreover it was discovered that the presence of nitro groups is essential for high antiTB effect. In this work we aimed on study of the impact of nitro groups' position and further substitution on benzyl fragment on antimycobacterial activity, selectivity and toxicity of these compounds.

This work was supported by Czech Science Foundation (project GAČR 14-08423S) and Charles University (project SVV 260 062).

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ALKYLAMINO DERIVATIVES OF *N*-PHENYLPYRAZINE-2-CARBOXAMIDE: SYNTHESIS AND ANTIMYCOBACTERIAL EVALUATION

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According to the WHO Global Tuberculosis Report 2014, there were an estimated 9.0 million new cases of tuberculosis (TB) and 1.5 million deaths associated with TB (2nd leading cause of death from an infectious disease worldwide) in 2013.[1] The situation is worsening due to the co-infection with HIV and increasing number of resistant TB-forms.[1] Pyrazinamide (PZA),[2] an essential component of short-course anti-TB chemotherapy, is used as a model compound for substances referred in this research project.

Based on the results of previously published active alkylamino derivatives[3], series of 5- (**I**) and 6-alkylamino-*N*-phenylpyrazine-2-carboxamides (**II**) was synthesized, characterized by analytical data and screened for *in vitro* antimycobacterial activity (against *Mycobacterium tuberculosis* H37Rv, *M. kansasii* and two different strains of *M. avium*). To study influence of simple aliphatic chain on activity, derivatives with modified alkyl chain (containing terminal methoxy or hydroxy group) as well as phenylalkylamino derivatives were prepared.

5-alkylamino (**I**) and 6-alkylamino (**II**) isomers exhibited similar antimycobacterial activity against *M. tuberculosis* H37Rv (activity in the range MIC = 0.78–12.5 μg/mL). On the other hand, modification of alkylamino chain with terminal methoxy or hydroxy group led to compounds with decreased or none activity, the decrease was proportional to the decrease of lipophilicity.

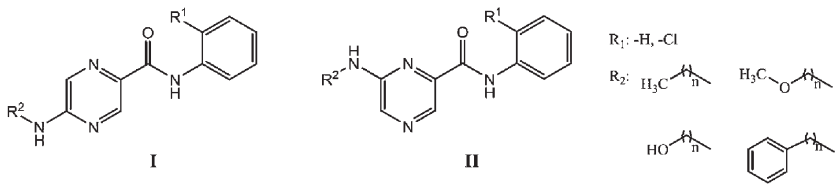

Fig. 1. Final structures **I** and **II**.

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COUPLING APPROACH TO PYRAZINE-2,3-DICARBONITRILES WITH Π-EXTENDED LINKERS BETWEEN DONOR (*N*,*N*-DIMETHYLAMINO) AND ACCEPTOR MOIETIES

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Azaphthalocyanines (AzaPc) are well-known synthetic dyes structurally close to porhyrins with outstanding photophysical and photochemical properties. Intramolecular charge transfer (ICT) is responsible for quenching of triplet state and can occur at aminosubstituted AzaPc[1]. Peripheral amine serves as a donor and the AzaPc core as an acceptor of the

electrons. This phenomenon can be used for sensoric applications[2]. The aim of this study is to evaluate the effect of the distance between donor and acceptor moiety on ICT efficiency.

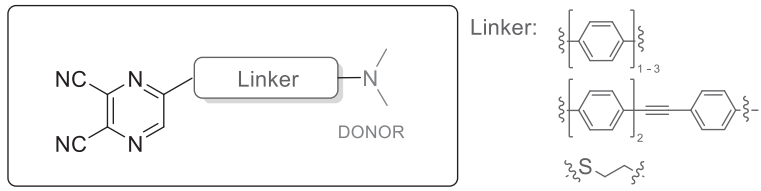

Fig. 1. Pyrazines with π-extended linkers.

The synthesis of AzaPc usually starts from the suitably substituted precursors – pyrazine-2,3-dicarbonitriles. *N*,*N*-Dimethylamino substituted pyrazine-2,3-dicarbonitriles with π-extended linkers between the donor and pyrazine presented by one, two or three 1,4-phenylene units and with three 1,4-phenylene units with inserted triple bond were prepared (Fig. 1). The π-extended linkers bearing donor group and arylboronic acid pinacol ester were prepared from 1-bromo-4-iodobenzene and 4-bromo-*N*,*N*-dimethylaniline by palladium catalyzed multiple steps reactions[3]. 5-Chloropyrazine-2,3-dicarbonitrile and 5-(4-iodophenyl)pyrazine-2,3-dicarbonitrile were prepared by condensation of diaminomaleonitrile with corresponding diketones. Afterwards, they were allowed to react with prepared linkers under Suzuki-Miyaura reaction conditions to form desired precursors. Precursor where the donor is not in conjugation with pyrazine was prepared by nucleophilic substitution of 5-chloropyrazine-2,3-dicarbonitrile by 2-(dimethylamino)ethanethiolate (Fig. 2)

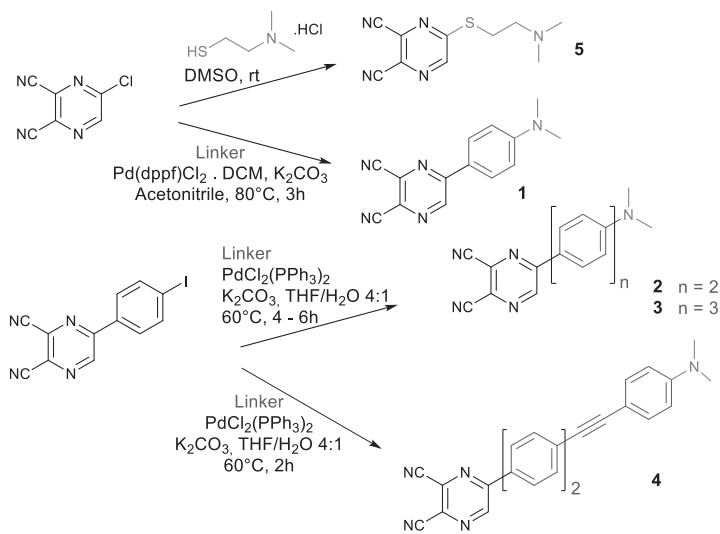

Fig. 2. The synthetic pathway for prepared precursors.

The study was supported by GA UK 1182313/2013 and SVV 267001.

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EVALUATION OF TRANSDERMAL PERMEATION ENHANCERS BASED ON TERPENES

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Transdermal drug delivery has many advantages compared to other conventional routes. However, skin acts as a very effective barrier preventing most environmental substances from entering our body. One of the methods, how to overcome this barrier, is the use of skin permeation enhancers, substances which reversibly decrease skin barrier properties, so the drug can get over it.

In this study we prepared esters of 6-(dimethylamino)hexanoic acid with selected alcoholic terpenes or their analogues (menthol, citronellol, linalool, farnesol, borneol, geraniol, nerol, carveol, perillyl alcohol and cinnamyl alcohol) by a two-step reaction.

The *in vitro* enhancing experiments on human skin with two model drugs (theophylline-TH and hydrocortisone-HC) showed, that the best activity possess esters of borneol, citronellol and cinnamyl alcohol (BBN, BCN and DMC). BCN enhanced flux of TH 15times, flux of more lipophilic HC was enhanced by DMC, BBN and BCN 92, 67 and 63times, respectively, compared to control.

Infrared studies using isolated human stratum corneum suggested that the mechanism of action of these substances involves interaction with barrier lipids.

Reversibility of action of selected substances (BBN, BCN and DMC) after 24 h application on human skin was proved using electrical impedance and transepidermal water loss. Afterwards, we studied toxicity of three most potent substances on two cell lines. On 3T3 fibroblasts, BCN showed lowest toxicity with IC_{50} values 177μM, IC_{50} values for DMC and BBN are 121μM and 73μM respectively. Best IC_{50} values on HaCaT keratinocytes has DMC (742 μM), followed by BCN and BBN (IC_{50} values 167 μM and 44μ M, respectively).

The study was supported by Charles University (GAUK 1404213 and SVV260 062) and the Czech Science Foundation (13-23891S).

THE EFFECT OF STEREOCHEMISTRY AT C-3 OF CERAMIDES NS AND NdS ON THE PERMEABILITY AND MICROSTRUCTURE OF MODEL STRATUM CORNEUM LIPID MEMBRANES

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The uppermost layer of the skin called the *stratum corneum* (SC), contains ceramides (Cer), cholesterol (Chol), free fatty acids (FFA) in equimolar ratio and small amount of cholesteryl sulphate (CholS). These main barrier lipids form a lamellar structure in the SC. Typical human sphingoid bases include sphingosine (S) and dihydrosphingosine (dS), phytosphingosine or 6-hydroxysphingosine, which can be *N*-acylated by non-hydroxylated (N), α- (A) or ω-hydroxylated (O) fatty acid. Considering their stereochemistry, all sphingoid bases share the common D-*erythro* configuration, which is (2*S*,3*R*) in S and dS.[1] This work focused on the effect of stereochemistry at C-3 on the barrier function and microstructure of model SC lipid membranes containing Cer NS or Cer NdS. We prepared Cer (2*S*,3*S*,4*E*)-NS and (2*S*,3*S*)-NdS with C_{24} acyl chain and compared them with their natural diastereomers with (*3R*) configuration. We prepared model membranes composed of Cer/FFA($C_{16–24}$)/Chol/CholS. Their permeability was assessed in Franz-type diffusion cells using four permeability markers: electrical impedance, water loss through the membrane and flux of two different model drugs. To elucidate the mechanisms of Cer effects on skin permeability, their biophysical properties were investigated by infrared spectroscopy and X-ray powder diffraction. The change of configuration at C-3 led to higher permeability of the model membranes; the highest values being found in Cer (2*S*,3*S*)-NdS membrane. The results confirmed that the correct stereochemistry is highly important for the skin barrier lipids.

The study was supported by The Czech Science Foundation (13-23891S) and Charles University (GAUK 1868214 and SVV 260062) and was co-financed by the European Social Fund and the state budget of the Czech Republic, project no. CZ.1.07/2.3.00/30.0061.

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1,2,5-CHALCOGENADIAZOLE-ANNULATED TRIPYRAZINOPORPHYRAZINES

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Tetrapyrazinoporphyrazines (TPyzPAs) are known for their promising photophysical properties usable in photodynamic therapy. Their ability to produce singlet oxygen and emit fluorescence after their excitation are important characteristics for this application. The preferred way of relaxation is driven mostly by process which is called heavy atom effect (HAE). HAE was described for the first time by McClure.[1] It was shown mostly on Pc that the presence of heavy atoms in the structure enhances the probability of compounds to undergo the intersystem crossing leading to the increase of singlet oxygen production.[2]

The aim of this work was to synthesize precursors and series of low-symmetrical TPyzPAs containing chalcogenadiazole ring (oxygen, sulfur, selenium or tellurium) in their structure and to disclose their effect on TPyzPAs' photophysical properties (i.e. HAE). Appropriate precursors A and B underwent statistical condensation leading to a mixture of TPyzPAs congeners from which required ABBB congener was isolated by the mean of column chromatography. Different cyclotetramerization methods were tried and MgII, ZnII complexes and metal free derivatives were synthesized. The influence of the chalcogen atom on the electronic absorption, emission spectra, and singlet oxygen production will be discussed as well as the stability of prepared macrocycles.

Acknowledgements. The Project is co-financed by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061

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SALICYLANILIDE DERIVATIVES AND THEIR CONJUGATION WITH PEPTIDE CARRIERS

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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 TB and drug-resistant TB forms (multidrug-resistant TB, extensively drug-resistant TB and recently reported totally drug-resistant TB). According to the WHO report, about one third of the world population is infected by the latent TB.[1,2]

Salicylanilide derivatives belong to the potentially promising groups of such compounds. It has been reported that the prodrug design of phenolic drugs, e.g., by their esterification can provide compounds with improved properties – increased activity, reduced toxicity, improved physicochemical properties and thus enhanced bioavailability or absorption, which are often the limiting factors for their activity.[3] Several salicylanilide-based molecules have shown a high *in vitro* activity against both drug-sensitive and resistant TB strains as well as nontuberculous mycobacteria with minimum inhibitory concentrations ≥ 0.125 μmol/L.

Another possibility for improving both pharmacokinetics and pharmacodynamics are drug delivery systems (DDSs). In cooperation with Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Budapest, we are working on the drug delivery systems (DDSs) based on oligotuftsine derivatives. We have synthesised several oligotuftsine peptides consisting of tandem pentapeptide repeated unit [TKPKG]*n* (*n* = 2, 4, 6, 8) based on the canine tuftsin sequence TKPK which is conjugated with our most active modified salicylanilide derivatives. These derivatives will be assayed against TB infected macrophages and determined *in vitro* activity, cytotoxicity and cytostasis.

The work was financially supported by the Research project IGA NT 13346 (2012).

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PHARMACEUTICAL ANALYSIS SECTION

HOW DILUENTS USED IN THE SENSING PHASE OF AN ELECTROCHEMICAL GENOSENSOR AFFECT TO THE GENOSENSOR PERFORMANCE

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Gold electrode surfaces are advantageous due to its easy immobilization protocol and high compatibility with mass fabrication of micro/nano electronic arrays, gold (Au)–thiol chemistry has been extensively studied in DNA hybridization sensing technology[1,2]. To detect a specific DNA sequence the complementary sequence containing an –SH group (SHCP) is immobilized to gold surface of the electrode. This immobilized sequence tends to adsorb on the electrode surface which making difficult a hybridization reaction. Therefore the co-immobilization of complementary probe, called capture probe and a blocking agent, usually mercaptohexanol (MCH) is proceeds. However, recent studies have indicated that such two-component SHCP + MCH monolayers still display nonspecific background contributions due to incomplete backfilling and related surface defects that results in an irreproducible absorption of the SHCP resulting in erroneous readings[3]. Because of this, we optimized sensing phase using different diluents.

Between the blocking agents tested, hexanedithiol, (HDT) and dithiothreitol (DTT) provide improvements primarily by the remarkably higher resistance to nonspecific adsorption leading to a decrease in the background current in comparison with the common binary layer[4]. These dithiol agents are arranged parallel to the surface and plugged holes remaining on the electrode surface. Blocking agents with the shorter chain lengths, mercaptopropionic acid (MPA), n = 3, and MCH, n = 6, are perpendicular arranged and they keep the negatively charged head groups OH and COOH closer to the surface by hydrogen bonding[4]. Differences in the discrimination effects are influenced also by the chain length[2]. Due this reason 11-mercaptoundecanol (MUD) and 11-mercaptoundecanoic acid (MUDA) were tested.

In our study, the influence of different diluents (HDT, DTT, MPA, MUD and MUDA) at tree or more concentration levels for detection of the DNA encoding gliadin and ARA h2 were investigated. The best results were achieved using HDT for both analytes. Also DTT and MUD provide very good results for detection of gliadin.

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LC-MS/MS METHOD FOR ANALYSIS OF AROYLHYDRAZONE PRO-CHELATOR AND ITS ACTIVE FORM IN PLASMA; APPLICATION TO A PILOT PHARMACOKINETIC STUDY

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Aroylhydrazone iron chelators possess interesting antioxidative, cytoprotective and antiproliferative properties. They are biocompatible, well tolerated compounds, which can be potentially used to treat several pathological conditions (e.g. Parkinson's and Alzheimer's diseases, cancer, malaria, tuberculosis). Unfortunately, they suffer from a short biological half-life associated with splitting of hydrazone bond.[1] Boronyl salicylaldehyde isonicotinoyl hydrazone – BSIH was prepared as a pro-drug of salicylaldehyde isonicotinoyl hydrazone – SIH in order to improve its stability in biological materials. BSIH contains protective mask which can be selectively remove only in the presence of oxidative stress that allow to focus its effect solely on spot of disease.[2]

The aims of this study were: (1) to develop and validate LC-MS/MS conditions for the simultaneous analysis of SIH and BSIH in rat plasma, (2) to compare the plasma stability of both analytes *in vitro*, (3) to perform a pilot pharmacokinetic study in rats.

Separation of SIH and BSIH was achieved on Zorbax Bonus-RP column (150 × 3 mm, 3.5 μm), using mobile phase composed of 2 mM ammonium formate and a mixture of methanol and acetonitrile (40:60, *v/v*), in a ratio of 60:40 ($v_{water}/v_{organic}$). All plasma samples were treated with methanol, centrifuged (16,800 g, 10 min) and filtered (0.22 μm) prior the analysis. The LC-MS/MS method was validated according to FDA guidelines. The linearity of the method was proven within the ranges of 0.05–23 μM and 0.24–23 μM for determination of BSIH and SIH, respectively. Both analytes were incubated in rat plasma (100 μM, 37 °C) to evaluate its stability. BSIH was administered to rats (n = 5; 10 mg/kg; *i.v.*) in a pilot pharmacokinetic study.

It was found, that BSIH is significantly more stable in plasma compared to its active chelator – SIH. Moreover, the estimated PK parameters showed an improvement in elimination halflife of BSIH over SIH. To conclude, this study showed that the concept of boronic ester based pro-chelators seems to be a promising strategy for future aroylhydrazone development and targeted structure modifications.[3]

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UHPLC METHOD DEVELOPMENT FOR DETERMINATION OF IMPORTANT ANTIOXIDANTS

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Term vitamin E includes two groups – tocopherols and tocotrienols, each of them consists of four isoforms – α, β, γ, δ. In last decades mainly α-tocopherol was studied and its anti-inflammatory and anti-proliferative effect was described. Recently anti-cancer effect of β-, γ- and δ-tocopherols was discovered and the association between their levels and cancer risk has been demonstrated[1].

In this study, the novel Ultra High Performance Liquid Chromatography (UHPLC) method coupled with fluorescent detection for determination of α-, β-, γ-, δ-tocopherols and retinol in human serum was developed. During method development various types of chromatographic columns (Aquity UPLC Beh Amide, Aquity UPLC PFP and Kinetex PFP) and different working conditions were tested. Best results were achieved using Kinetex Pentafluorophenyl column (4.6 × 100 mm, 2.6 μm) as stationary phase and mixture of methanol and ammonium acetate buffer in the ratio 84:16 (v/v) as mobile phase, temperature was set at 50 °C. Liquid–liquid extraction as a sample preparation procedure was optimized and miniaturized using Eppendorf tubes. For detection of vitamin E forms and retinol the fluorescence detector was set at excitation wavelength 295, 325 and emission wavelength 325, 480, respectively.

Development of this new chromatographic method might help to extend the knowledge of the role of each isomer in various metabolic pathways and their relevance for serious diseases.

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DEVELOPMENT OF UHPSFC-PDA METHOD FOR DETERMINATION OF TOCOPHEROLS AND TOCOTRIENOLS

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Recently, supercritical fluid chromatography has become very popular in modern chromatographic laboratories. Due to the properties of supercritical fluid and high flow-rates, the analysis time can be substantially reduced in comparison with LC procedures, while maintaining or increasing the separation efficiency, especially when using sub-2-µm particles, known as ultra-high performance supercritical fluid chromatography (UHPSFC). The analysis of tocopherols and tocotrienols is very challenging due to many isomeric forms, which are not easy to resolve even using highly efficient liquid chromatographic approaches. Similarly to LC, stationary phase is the key factor for successful separation in UHPSFC. Four stationary phases including BEH, BEH 2-EP, HSS C18 and CSH PFP using isocratic elution with CO_2/MeOH were tested in the method development. Among them, BEH and BEH 2-EP provided complete baseline resolution of all tested compounds. Finally BEH 2-EP was chosen for analysis due to best separation of tocopherols. The columns were kept at 50 °C and the BPR pressure was set-up at 1885 psi. The sample volume and solvent type were optimized in order to increase sensitivity and maintain symmetric peak shape. PDA was used for the detection with the selected wavelength of 290 nm for data processing. Method repeatability, linearity and sensitivity were determined before its application to real samples, which were in the first instance hop and barley extracts. Due to the fact that developed method provides sufficient separation of all analyzed substances with time of analysis up to 3.5 minutes, UHPSFC-PDA seems to be a suitable technique for analysis of isomeric forms of vitamin A.

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DEVELOPMENT OF UHPLC-MS/MS METHOD FOR DETERMINATION OF VANCOMYCIN IN SURGICAL PATIENTS WITH IMPORTANT SEQUESTRATION OF LIQUIDS

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Vancomycin (VCM) is a tricyclic glycopeptide antibiotic, which is indicated especially in severe staphylococcal and streptococcal infections resistant to penicillin and oxacillin[1]. The aim of the project was development of rapid and sensitive UHPLC-MS/MS method for determination of VCM in human biological fluids with simple sample preparation. The best results for chromatographic separation were achieved with core-shell YMC Meteoric Core C18 BIO column, 2.7 µm particle size C18, 100 × 4.6 mm (YMC Europe GmbH, Germany) and acetonitril with 0.2% (v/v) FA in mixture with water (pH 2.58) as the mobile phase. VCM and teicoplanin (IS) were determined by a triple quadrupole mass spectometer with electrosprey ionization source (LCMS 8030, Japan). Total time of analysis was 1.7 min. This new UHPLC-MS/MS method will be used for determination of VCM for surgical patients with systemic inflammatory response syndrome (SIRS) caused by multi-trauma or serious bacterial infection and accompanied with a large fluid sequestration.

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MONITORING OF SEPTIC CONDITIONS BY BIOLOGICAL MARKERS

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According to WHO sepsis is the most common and least recognized state in urgent medicine, with long-term complications. Highest mortality rate is observed in patients with

decreased function of immune system, like geriatric, polymorbid and immuno-suppressed patients, regardless of age and gender[1]. Crucial step in monitoring of sepsis is early diagnosis by biomarkers[2]. In 2011 a novel highly specific marker called presepsin was discovered. Presepsin may help to stratify and reveal septic conditions before they manifest[3]. So far in our region no clinical study has been made and only few departments analyze it.

During 10 months, 697 patients with development of sepsis were observed and biomarkers: procalcitonin, C – reactive protein, interleukin – 6, lactate, D – dimer, fibrinogen, white blood cell count and presepsin were analyzed and statistically compared to determine their diagnostic value. Presepsin is fast and reliable predictor of possible complications and should be used in monitoring of sepsis. Unfortunately, the need for expensive and one purpose appliance used only for its determination with specialized kits calls for new accurate analytical methods for its determination.

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METHODS FOR ANALYSIS OF VITAMIN D IN MILK

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Vitamin D is a hormone precursor with a steroid structure that is present in either of 2 different forms, vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol). A lack of vitamin D in children can lead to soft, thin, and brittle bones, a disease known as rickets. Due to the very low concentrations of vitamin D and its metabolites in human breast milk, its precise measurement in these samples is quite challenging and includes sample pre-treatment before analysis. Traditional sample preparation involves saponification, liquid–liquid extraction, solvent evaporation, manual solid phase extraction, and pre-concentration[1].

Techniques for the determination of vitamin D in milk can be categorized into immunological techniques (CPBA, ELISA, RIA) and non-immunological techniques (HPLC, LC-MS). Various types of detection have been used with LC, including MS^2, MS and UV. LC-MS/LC-MS^2 are the methods of choice in vitamin D analysis since this methodology is sensitive, accurate and provides high specifity and offers quantify multiple analytes in a single assay[2]. The separation is usually performed on a reversed-phase analytical column packed with C18 particles. The best results are achieved by using isotope-labeled internal standards and MS detection[1].

Goal of this study is summarizing current chromatographic methods with sample preparation of vitamin D in bovine and human breast milk.

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ZIRCONIA BASED SORBENTS FOR SOLID PHASE EXTRACTION OF METHOTREXATE FROM BIOLOGICAL MATRICES

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Today, zirconia-based chromatography columns are easily commercially available and they are used in many described methods for their stability and unique chromatographic properties. Our group is trying to transfer knowledge of zirconia stationary phases from liquid chromatography to the field of sample preparation, specifically to the solid phase extraction (SPE) of polar compounds.

Zirconium coated with carbon (Zr-CARB) combines Lewis acid/base properties of zirconia and hydrophilic/hydrophobic retention mechanisms of carbon, what might be very advantageous for many pharmaceutical compounds.

Methotrexate (MTX) is a widely used anticancer and antirheumatic drug. It suffers from high toxicity, therefore monitoring of its levels and metabolism is very important. Prior analysis, it is usually necessary to extract MTX from biological fluids such as plasma or urine. Our work brings a new possibility for extraction of MTX on solid phase.

An SPE method using 10% MeOH for column conditioning and washing and 0.2% ammonia in MeOH as elution agent was developed. Mean recoveries of MTX from plasma and urine were 93.8% and 98.5%, respectively. Employing UV detector, the calibration graph of MTX in plasma was linear within the range of 8–150 μg/ml. Relative standard deviations and accuracies in concentrations 8, 50 and 100 μg/ml were less than 7.3%.

The used concentrations of MTX were 100–1000 times higher than real levels in biological fluids. Therefore the next step of our research will be to use fluorescence or MS detectors for final determination, what will allow us to lower MTX concentrations for extraction.

Results of our work conclude, that sorbent based on carbon coated zirconia is applicable for solid phase extraction of methotrexate from both plasma and urine with relatively high recoveries and good linearity for higher concentrations.

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STUDY OF OCHRATOXIN A AND CITRININ CONTENT IN CZECH LAGER BEERS BY FAST METHOD USING DIRECT SAMPLE INJECTION COMBINED WITH FUSED CORE COLUMN ON-LINE SPE-HPLC WITH FLUORESCENCE DETECTION

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A new fast and sensitive method of high performance liquid chromatography for simultaneous determination of mycotoxins ochratoxin A (OTA) and citrinin (CIT) using column-switching system for on-line sample pretreatment was developed. OTA and CIT are produced by some species *Aspergillus* and *Penicillium,* which can contaminate cereals and their products such as beer. Some other mycotoxins have already been tested in beer.

The analytes were on-line preconcentrated and separated in time less than 6 min, after direct injection of 100 μL of filtrated beer. Preconcentration of OTA and CIT from beer samples was performed on Ascentis Express RP-C-18 guard column (5 × 4.6 mm), particle size 2.7 μm, with mobile phase methanol/water solution of 0.5% acetic acid pH 3.0 (30:70, v/v) at flow rate 2.0 mL min^{-1} and at temperature 50 °C. The time of flow switch from extraction column to analytical column in back-flush mode was set at 2.0 min and the separation was performed on the fused-core column Ascentis Express Phenyl-Hexyl (100 × 4.6 mm), particle size 2.7 μm, with mobile phase acetonitrile/water solution of 0.5% acetic acid pH 3.0 in gradient elution at a flow rate of 1.0 mL min^{-1} and temperature 50 °C. Fluorescence excitation/emission detection wavelengths were set at 335/497 nm.

The optimized and validated method showed high sensitivity with limit of detection 10 and 20 ng L^{-1} for OTA and CIT, respectively, and accuracy as the mean recoveries of OTA and CIT both in light and dark beer samples were in the range 98.3–102.1%.

The mycotoxins were analyzed in 49 Czech beer samples, the content in light, dark and wheat lagers was studied. Low concentration levels of OTA and CIT below the maximum tolerable limit were found.

The study was supported by the Charles University – project 17/2012/UNCE and project of specific research, no. SVV 260 063

PHARMACOKINETIC STUDY OF THE TRANSPORT OF A FLUORESCENT MARKER THROUGH CELL MONOLAYER USING AN SIA SYSTEM

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In this present work, the main goal is the monitoring of the transport of a fluorescent marker (Rhodamine) through the cell monolayer. Usually, this type of experiments is evaluated using until 3 points for 2–4 hours testing which leads to an incomplete information. Using the fully automated system with automated sampling small volumes, it is possible to obtain detailed profile concerning the interaction of Rho with cell membrane transporters.

Using the same system, monitoring different substances (drugs) which can act as activators or inhibitors of membrane transporters and thus can affect the transport of the marker which corresponds to a change of the fluorescent signal was carried out.

Another aim is the connection of more Franz cells (permeation units) to the same flow system and to optimize flow conditions such as volume and aspiration flow rate, integration time and procedure to get repeatable sampling, filling up the volume and sensitive detection. For the present system with three Franz cells, the procedure consists in two samplings for each Franz cell in each seven minutes, when 60 µL of marker solution is sent to the detector with a flowrate of 30 µL s^{-1} and an integration time of 60 ms.

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A MODEL OF NATURAL DEGRADATION OF 17-A -ETHINYLESTRADIOL IN SURFACE WATER AND IDENTIFICATION OF DEGRADATION PRODUCTS BY GC-MS

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In the last decade the environmental analysis is one of the most progressive part of analytical research and over time daily analytical practice as well. Environment is polluted by a huge spectrum of exogenous chemicals from human living and their influence on every part of any ecosystem can be devastating. The great attention focuses on the group of steroidal endocrine disruptors and especially estrogen hormones, comes from human living. The

aim of our work is to determine the kinetics of natural degradation made by physicochemical factors and to identify the degradation products of 17-α-ethinylestradiol, the massively used estrogenic drug. The photodegradation, oxidation and termostability conditions were chosen according to ICH requirements for pharmaceuticals stability testing. The simple 72 hours photodegradation study in purified water exhibits a significant 1st order kinetic decrease with kinetic constant $k = 0.0303\ h^{-1}$ and degradation halftime 22.8 h. GC-MS analysis showed five major degradation products with preserved steroidal structure with changes on ring A and B. Two of them were tri-hydroxy derivatives (m/z = 528), the other two were dehydrogenated tri-hydroxy derivatives (m/z = 526), and one was tetra-hydroxy derivative (m/z = 616). Derivatization by phenylboronic acid excluded hydroxylation to position 2- and 4- so degradation products were identified as 1-hydroxy EED, 6-hydroxy EED, 1,6-dihydroxy EED. However all MS data did not show an exact position for dehydrogenation. Potentiation of photodegradation by oxidative processes using hydrogen peroxide accelerated degradation rate to halftime 2.3 hours and $k = 0.3014\ h^{-1}$. GC-MS data showed the degradation of steroidal structure to low mass compounds and TOC analysis results determined the decrease of organic carbon in solution by 8% in 72 hours. The influence of sea salt and temperature exhibited no significant changes in degradation kinetics.

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SOLID-PHASE MICROEXTRACTION – ISOLATION OF THIOSEMICARBAZONE ANTI-CANCER AGENTS AND EVALUATION OF NOVEL SORBENTS

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Solid-phase microextraction (SPME) invented by Pawliszyn in early 90s is currently under intensive development for the isolation of analytes from complex matrices. The main advantage of the technique stems from incorporation of sampling, extraction and preconcentration into one step as well as low consumption of organic solvents needed. SPME is considered as a non-exhaustive sample preparation technique where extraction is based on equilibrium of the analyte between sample matrix and the extraction phase. The technique is applicable as a sample preparation step before analysis using a gas or liquid chromatography[1]. Moreover, recent studies proposed direct connection of SPME with mass spectrometry (direct analysis in real time) resulting into quick, solventless and sensitive analysis[2]. SPME should be considered as an alternative sample preparation technique for problematic analytes due to high configuration flexibility as well as the possibility of utilization of wide spectrum of extraction phases[1].

Analytical evaluation of thiosemicarbazone anti-cancer agents is complicated by their ability to chelate several metal ions, extensive plasma protein binding and adsorption to various materials. Hence the first part of this lecture will be aimed at testing of possible

utility of SPME in 96-well format for the extraction of these agents from PBS buffer and human plasma.

The second part of the lecture will be focused on novel graphene based sorbents as a possible alternative material for SPME. The sorbents were prepared by binding of graphene oxide to polyethyleneimine-coated zirconia particles. SPME fibers were prepared by a novel technique based on attachment of the sorbent on stainless steel wire covered with a polydimethylsiloxane glue.

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SYNTHESIS OF MOLECULARLY IMPRINTED MATERIAL FOR SOLID-PHASE EXTRACTION OF β-*N*-METHYLAMINO-L-ALANINE FROM CYANOBACTERIAL EXTRACT

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Analysis of neurotoxic, non-proteinogenic amino acid β-*N*-methylamino-L-alanine (BMAA) that is hypothesized to be linked to amyotrophic lateral sclerosis was performed using HPLC-MS/MS. This method was developed and validated for environmental samples by Combes et al.[1] whose sample preparation method, based on mixed mode solid-phase extraction, was not specific enough because considerable matrix effects were observed. Molecularly imprinted solidphase extraction (MISPE) can be used for a more selective and efficient clean-up of a complex matrix. Molecularly imprinted polymers are synthetic materials possessing specific recognition sites (cavities) that are tailor-made for a target analyte. Altogether 12 different MISPE sorbents were synthesized by solgel approach in polar media and subsequently tested. Extraction procedure for BMAA was developed in pure medium and then applied to cyanobacterial extracts. MISPE procedure used as an additional step of sample preparation completely eliminated the matrix effects that affected quantification of BMAA in cyanobacterial samples.

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European Social Fund and the state budget of the Czech Republic, TEAB project no. CZ.1.07/2.3.00/20.0235.

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THEORETICAL AND PRACTICAL ASPECTS OF A GENERALIZED CALIBRATION STRATEGY IMPLEMENTED TO FLOW TECHNIQUES

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Generalized calibration strategy (GCS) is a novel approach in analytical chemistry. This method enables to obtain six estimations of an analytical result in a single calibration procedure and to verify analytical results in terms of systematic errors. This innovative strategy is based on several elements[1]: instrumental (development of systems based on flow techniques); methodological (integration of different calibration methods) and laboratory (addition of a standard to a sample and gradual dilution of the sample and standard solutions).

The presentation shows the complex theoretical studies involving above issues. For this purpose a dynamic mathematical model was developed defining the impact of various interferences in estimations of the analyte concentration received.

Moreover, the GCS was tested in terms of verification and elimination of systematic errors in case of determination of calcium by FAAS in synthetic and natural samples[2] and in case of determination of selenium by HG-AFS for natural samples[3].

Another part of the presentation is focused on adaption of GCS strategy to two-component analysis on example of the determination of paracetamol and caffeine in pharmaceuticals samples.

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UHPLC-MS/MS ANALYSIS OF THIOSEMICARBAZONE IRON CHELATORS – A PHARMACOKINETIC STUDY

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The thiosemicarbazone iron chelator di-(2-pyridyl)ketone 4-cyclohexyl-4methyl-3-thiosemicarbazone (Dp44mT) exhibits potent anti-neoplastic and anti-metastatic effect[1]. Nonetheless, at high sub-optimal doses Dp44mT induces cardiac fibrosis in rats. The new lead compound among thiosemicarbazone anti-cancer agents – di-(2-pyridyl)ketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) – retains the anti-cancer efficacy of its predecessor but lacks its toxicity. It is known that pharmacokinetics (PK) and metabolism can vastly influence toxicity of a drug[2]. As no such data are available for these novel thiosemicarbazones, it is not clear whether their metabolism and disposition may have any connection to the toxicity or efficacy of these agents. In our pilot *in vivo* study we discovered that Dp44mT is demethylated to Dp4mT while DpC seems to resist metabolic decomposition. However further studies are needed to discover PK of both compounds in detail.

Therefore the aim of this work was to develop and validate an UHPLC-MS/MS assay for determination of Dp44mT, Dp4mT and DpC in rat plasma and to use it to estimate the PK parameters of these compounds.

For the analyses, a Shimadzu Nexera UHPLC system coupled with LCMS-8030 QqQ mass detector was used. All separations were achieved on Acquity BEH C18 stationary phase with aqueous ammonium formate (with 5 μM EDTA) and acetonitrile as mobile phase in gradient mode. Plasma samples were treated with a combination of protein precipitation and liquid-liquid extraction. The analyses were complicated by the chelation ability of the analytes, thus another chelator (preferably EDTA) had to be added in nearly all steps of analysis. This method was fully validated according to FDA guidelines with respect to selectivity, linearity, accuracy, precision, stability, extraction recovery and matrix effects. Subsequently, rats were administered DpC or Dp44mT, respectively ($n \geq 6$, 2 mg/kg, *i.v.*). Their plasma was taken in predefined intervals and analysed. The PK parameters were described using population analysis with both non- and 2-compartmental modelling.

In spite of the very close chemical structures of Dp44mT and DpC, major dissimilarities were found in metabolism, terminal half-lives, total AUC, clearance as well as other PK

characteristics of these compounds, which might be the reason for the distinct toxicities. However, further studies are necessary to confirm this proposal.

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APPLICATION OF THE SEQUENTIAL INJECTION TECHNIQUE FOR AUTOMATION OF SAMPLE PRETREATMENT IN PHARMACEUTICAL ANALYSIS

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Sequential injection analysis (SIA) is a flow technique based on programmable, bidirectional discontinuous flow. The feasibility of SIA makes it an advantageous tool for sample pre-treatment since this step is often tedious, time consuming, and prone to errors when performed manually.

Different microextraction methods, both liquid and solid phase-based, can be performed using a SIA system, as demonstrated here by the following novel applications: A. Dispersive liquid-liquid microextraction (DLLME) e.g. in analysis of thiocyanates in human saliva samples[1]; B. Head-space single-drop microextraction (HS-SDME) for analysis of ethanol in wine[2]; C. Microextraction by packed sorbent (MEPS) coupled with chromatographic separation in an SIA system for determination of betaxolol in human urine.

In this presentation, the benefits and potential of employing SIA in sample pre-treatment, especially regarding the analysis time, reagents, sample and solvents consumption, waste production, automation and simplification, are shown and discussed.

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COMPARISON OF 3 MULTISTATIN MEPS-UHPLC-MS/MS METHODS FOR DETERMINATION OF 17 STATINS AND RELATED COMPOUNDS IN HUMAN SERUM

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Statins are used for the treatment of hypercholesterolemia. Some recent studies revealed the extralipid effects of these cholesterol-lowering drugs. In recent years, especially the number of studies dealing with anticancer statin activity has grown. Therefore a fast and sensitive method for the determination of 7 commercially available statins, their interconversion products and metabolites (17 analytes in total) in serum using UHPLC-MS/MS was developed and validated. Deuterium labeled standards for most analytes were used for reliable quantification. The method was developed on three different MS instruments: an old platform (I), a newer one (II) and the State-of-the art (III) triple quadrupole system. The results were compared in terms of the sensitivity, selectivity and other validation parameters. Protein precipitation (PP) followed by microextraction by packed sorbent (MEPS) were selected as the sample preparation techniques. Both LC conditions and sample preparation procedure were the same for all the three instruments. Separation of analytes was performed on BEH C18 analytical column (50 mm × 2.1 mm, 1.7 µm), using gradient elution by mobile phase consisting of ACN and 0.5 mM ammonium acetate at pH 4.0. Higher flow rate of mobile phase was possible to use for instrument II and III. MS conditions were optimized individually for each mass spectrometers. Instrument II and III enabled fast, selective and sensitive analysis due to the higher scan speed and different construction of the ion source. All the three methods were validated. However, due to the sensitivity, only the methods II and III were applicable for the real sample analysis of patients treated by atorvastatin and rosuvastatin.

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GRADIENT CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SOTALOL AND SORBATE IN PEDIATRIC ORAL LIQUID PREPARATIONS

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A gradient HPLC-UV method for the determination of sotalol hydrochloride and potassium sorbate in five types of oral liquid preparations was developed and validated. The separation of an active substance sotalol hydrochloride, an antimicrobial agent potassium sorbate and other substances (for taste and smell correction etc.) was performed using an Ascentis® Express C18 (100 × 4.6 mm, particles 2.7 μm) solid core HPLC column. Linear gradient elution mode with a flow rate of 1.3 mL min^{-1} was used, and the injection volume was 5 μL. The UV/Vis absorbance detector was set to a wavelength of 237 nm, and the column oven was conditioned at 25 °C. A sodium dihydrogen phosphate dihydrate solution (pH 2.5; 17.7 mM) was used as the mobile phase buffer, organic solvent was acetonitrile. The total analysis time was 4.5 min (+ 2.5 min for re-equilibration). The method was successfully employed in a stability evaluation of the developed formulations, which are now already being used in the therapy of arrhythmias in pediatric patients. The method is also suitable for general quality control, i.e. not only just for extemporaneous preparations containing the mentioned substances.

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SAMPLE PRETREATMENT IN DETERMINATION OF EFAVIRENZ IN OPTIMEM® REDUCED SERUM MEDIA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

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Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor used in a combination with other antiretroviral drugs to treat HIV-positive patients. Its interactions with membrane located drug transporters could lead to drug-drug interactions during antiretroviral therapy and has been therefore intensively studied using transport experiments on cellular monolayers. The aim of this work was to find a simple sample pre-treatment before EFV determination by high-performance liquid chromatography (HPLC) method with UV detection and to use this method for analysis of samples obtained in the transport studies.

The EFV had to be analyzed in OptiMEM® Reduced Serum Media that contains essential nutrient components. Because of quite complex matrix and low sample volumes (50 µl), the optimization of sample pre-treatment step was necessary. The low sample volume prevented an application of commonly used techniques, such as protein precipitation, solid-phase extraction or liquid-liquid extraction. Sample filtration in a combination with centrifugation using syringe filters (pore size of 0.2 µm) of different membrane materials were tested, but loss of EFV was observed. Finally, sample dilution with an internal standard solution and direct sample injection onto the column with higher particle size (5 µm) was chosen.

HPLC method was optimized and real samples were measured. HPLC system Nexera X2 (Shimadzu Corp., Japan), analytical column Discovery HS C18 (150 × 4.6 mm, 5 µm, Supelco, USA) and mobile phase consisted of acetonitrile and ultra-pure water (65:35, v/v) were used. Analyses took 5 min and were performed at the flow rate of 1.6 ml/min at the temperature of 25 °C. Injected sample volume was 10 µL. UV detector was set up at 245 nm. Method was linear in a concentration range of 0.5–10 µmol L^{-1} ($R^2 = 0.9985$).

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PHARMACOLOGY AND TOXICOLOGY SECTION

CD44V6 RECEPTOR MAY INTERACT WITH TUMOR ASSOCIATED CELL SURFACE PROTEINS

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CD44 glycoproteins belong to the large family of cell adhesion molecules included in cell-cell and cell-matrix interactions. Among its variants, CD44v6 is the isoform, which is implicated in tumorigenesis, tumor cell invasion and metastasis. CD44v6 may interact with the other tumor associated receptors in tumorigenesis process like epidermal growth factor (EGF) receptor (EGFR), tyrosine kinase receptor Met and vascular endothelial growth factor (VEGF) receptor (VEGFR). In this study, we searched for the possible interactions

among CD44v6 and either EGFR or Met or VEGFR. For the study, human squamous carcinoma cells were incubated with either EGF or hepatocyte growth factor (HGF) or VEGF or none of the natural ligands (the control) for 48 hours at 37 °C and 5% CO_2. Moreover, cells were also incubated in the presence of EGFR activity inhibitors either gefitinib or lapatinib. The CD44v6 expression was estimated using fluorescence-activated cell sorting (FACS) machine, when fitc-labelled anti-CD44v6 was used. UT45 cells showed small decrease of the CD44v6 expression, but A431 and LK0412 cells both demonstrated high increase in receptor expression upon EGF and HGF incubation (no effect of VEGF). The co-incubation of natural ligands with either gefitinib or lapatinib changed the CD44v6 expression. For example LK0412 cells, either gefitinib or lapatinib alone increased the CD44v6 amount almost twice. When combined with EGF, only gefitinib showed positive effect on the CD44v6 expression. HGF combined with lapatinib increased the CD44v6 amount on cell surface five times and VEGF combined with either gefitinib or lapatinib twice. These preliminary tests demonstrated the mutual cooperation between CD44v6 and EGFR and between CD44v6 and Met. No cooperation was found for VEGFR unless incubated with either gefitinib or lapatinib. Nevertheless, more investigation is needed to decipher the exact inter-receptor communication.

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CHRYSIN, BALCALEIN AND GALANGIN ARE INDIRECT ACTIVATORS OF THE HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR)

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The constitutive androstane receptor (CAR) is a crucial transcriptional regulator of key xenobiotic-metabolizing enzymes such as cytochrome P450 CYP3A4, CYP2C9 and CYP2B6. The flavonoids chrysin, balcalein and galangin have been reported to activate CAR. Nevertheless, it is not known if these flavonoids are direct CAR ligands or indirect phenobarbital-like CAR activators *via* the inhibition of epidermal growth factor receptor (EGFR) signaling.

We analyze the interactions of chrysin, galangin and balcalein and its glycoside baicalin with human CAR. We have employed methods that can demonstrate direct interaction with the CAR ligand binding pocket. Secondly, we determined if the compounds affect EGFR signaling and interact with EGFR.

Employing a TR-FRET coactivator assay with recombinant CAR or CAR assembly assay, a consistent activation of CAR with flavonoids and phenobarbital was not observed. It was determined, however, that galangin, chrysin, and baicalin may repress EGFR-Tyr1068

autophosphorylation after EGF treatment. Only chrysin significantly inhibited the downstream ELK1 transcription factor of EGFR signaling.

These data suggest that flavonoids chrysin, galangin and balcalein are not direct human CAR agonists and that they may interfere with EGFR signaling. This study also demonstrates the need for the testing of the direct CAR interaction of both natural and synthetic ligands.

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THE ROLE OF NUCLEOSIDE TRANSPORTERS IN A TRANSFER OF THYMIDINE AND ABACAVIR ACROSS THE HUMAN TROPHOBLAST MICROVILLOUS MEMBRANE

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Trophoblast is the key structure controlling passage of exogenous as well as endogenous compounds across the human placenta. Its microvillous membrane (MVM) directly faces maternal blood and is well equipped with a variety of efflux transporters that can play protective role to the trophoblast, as well as influx transporters, providing a mechanism that enable active materno-fetal transfer of various compounds. The aim of this study was to evaluate the activity of nucleoside transporters in the MVM isolated from human term placentas. Particularly we aimed to address the following issues: (i) To evaluate the transport of endogenous nucleoside thymidine across the MVM and distinguish between the activity of Na^+ dependent concentrative uptake transporters (CNTs) and equilibrative nucleoside transporters (ENTs). (ii) To reveal whether abacavir, an antiretroviral drug being a nucleoside analogue, can utilize nucleoside transporters for its own uptake into the trophoblast.

Our data show active time-dependent uptake of thymidine into MVM vesicles, which could be inhibited by ENTs inhibitors uridine and NBMPR. Contrary, no Na^+ dependent component of the uptake could be detected. These results confirm contribution of ENTs in the uptake of thymidine across MVM into the trophoblast layer and absent role of CNTs in the thymidine uptake. Uptake of abacavir into the MVM vesicles has revealed large variability, nevertheless the same inhibitory pattern as thymidine. To conclude, our results confirm the functional activity of ENTs in the human trophoblast MVM. These transporters can be utilized by abacavir for its passage across the placenta, while CNTs do not seem to mediate abacavir uptake.

The study was supported by the Czech Science Foundation (GACR P303/ 13-31118P).

RADIOLABELING OF ANTIBODY IGG M75 FOR EPITOPE OF HUMAN CARBONIC ANHYDRASE IX BY ^{61}CU AND ^{64}CU

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The subject of the work was conjugating antibody IgG M75 for epitope human carbonic anhydrase IX with noncommercial new chelator denoted as "phospinate" specific for copper isotopes. Human carbonic anhydrase IX is a membrane enzyme that is significantly expressed in cancer cells. The antibody IgG M75 was successfully conjugated with phosphinate, and the conjugation was optimized and is well-reproducible. The conjugate was then labeled with two positron emitters, namely copper radionuclides ^{61}Cu (3,333 h) and ^{64}Cu (12,701 h). The labelling resulted in the product of high specific activity (1.0 and 7.4 MBq/mg, respectively) and of high radiochemical purity (> 95%). The labelled molecule has considerable potential as a radioimmunopharmaceutical suitable for imaging of tumors expressing carbonic anhydrase IX by positron emission tomography (PET).

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LONG TERM ADMINISTRATION OF TENOFOVIR OR EMTRICITABINE TO PREGNANT RATS DOES NOT AFFECT *ABCB1A*, *ABCB1B*, AND *ABCG2* EXPRESSION IN MATERNAL AND FETAL CELLS

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Nucleotide/nucleoside reverse transcriptase inhibitors tenofovir and emtricitabine belong to current backbone of the antiretroviral combination regimens for prevention of perinatal HIV transmission. To date many studies showed that absorption, distribution

and elimination of both drugs is not affected by activity of two widely expressed drug efflux transporters P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). However, knowledge whether tenofovir/emtricitabine administered in long-term fashion might alter ABCB1 and/or ABCG2 expression in biological barriers of pregnant women and their fetuses is lacking. To investigate this issue we treated pregnant Wistar rats with i.m. injection of tenofovir (2.25 mg/kg) or emtricitabine (3.5 mg/kg) for ten days (from 12th to 21st gestation day). On 22nd gestation day organs were collected and relative expression of *Abcb1a*, *Abcb1b*, and *Abcg2* mRNA in maternal/fetal organs (brain, kidney, intestine, and liver) and the placenta was evaluated. We found out that *Abcb1a*, *Abcb1b*, and *Abcg2* are expressed at term in all organs tested and *Abcb1a* and/or *Abcb1b* showed highly ontogenic expression in the brain, liver, and kidney. Subsequently it was demonstrated that neither tenofovir nor emtricitabine caused significant changes in expression of *Abcb1a*, *Abcb1b*, and *Abcg2* mRNA in all organs tested. In conclusion we confirmed ontogenic expression of *Abcb1a* and *Abcb1b* in rats and moreover, to our best knowledge, we bring the first evidence that long term treatment with tenofovir/emtricitabine might not alter expression of the tested transporters in both maternal and fetal organs. These data further extend the safety profile of tenofovir and emtricitabine.

The study was supported by GACR P303/120850.

PD0332991 REVERSES ABC TRANSPORTER-MEDIATED MULTIDRUG RESISTANCE AND SYNERGIZES WITH CANCER CHEMOTHERAPEUTICS IN HUMAN TRANSPORTER EXPRESSING MDCKII AND IN MCF-7 CELL LINES

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Multidrug resistance is one of the major causes of failure in cancer chemotherapy. This phenomenon is mainly associated with the overexpression of ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein) or ABCC1 (multidrug resistance associated protein 1) in tumor cells. Previously, we found that PD0332991, a cyclin-dependent kinase (CDK) inhibitor currently in phase 3 clinical trials for the treatment of breast cancer, is able to inhibit ABCB1 and ABCG2 transporters. In this project, we evaluated whether the inhibitory properties of PD0332991 can also reverse multidrug resistance conferred by the transporters. Moreover, we determined whether the combined treatment of PD0332991 with conventional chemotherapeutic drugs can increase the cytotoxic effects in transporter expressing MDCKII cell lines and in breast cancer MCF-7 cells.

XTT based cytotoxicity assay showed that PD0332991 significantly increased the sensitivity of resistant MDCKII-ABCB1 and MDCKII-ABCG2 cells to ABCB1 and ABCG2 substrate antitumor drugs, daunorubicin, topotecan, or mitoxantrone, whereas no such shift was observed in MDCKII-ABCC1 and parent cells. To further evaluate the cytotoxic ef-

fect of PD0332991 in combination with chemotherapeutic agents, we used combination index analysis. Significant synergy was observed between PD0332991 and daunorubicin (ABCB1 substrate) in MDCKII-ABCB1 cells and between PD0332991 and topotecan (ABCG2 substrate) in MDCKII-ABCG2 cells. These results confirm our hypothesis that the synergistic effects can be attributed to the ability of PD0332991 to inhibit the corresponding transporter and thus increase the intracellular accumulation of the cytotoxic anticancer drug. Furthermore, a considerable synergistic effect was observed when the combination of PD0332991 with daunorubicin or with raloxifene was applied in breast cancer cell line MCF-7, suggesting the suitability of these combinations for the treatment of breast cancer. To conclude, our data show synergistic effect of CDK inhibitor PD0332991 in combination with anticancer drugs that can be at least partly caused by overcoming ABC transporter-mediated multidrug resistance.

The study was supported by the Charles University (SVV 260 064).

SOLUBLE ENDOGLIN EFFECTS ON ENDOTHELIAL CELLS (HUVECS) – A PILOT STUDY

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Endoglin is an auxiliary receptor for ligands of TGF-β superfamily cytokines. It is an integral membrane protein highly expressed in the vascular endothelium. In addition to membrane bound endoglin, a soluble form of endoglin (sEng) present in plasma has been detected in various pathological conditions related to cardiovascular system. But the detailed relation between sEng and endothelial function or dysfuntion has not been uncovered yet. HUVEC (Human Umbilical Vein Endothelial) cells, which naturally express high amount of endoglin, were used in this study to asses possible effect of sEng treatment.

HUVEC cells (Lonza) were incubated for 16 h with 1nM recombinant human sEng (R&D Systems). Selected markers that characterize endothelial cells state and function (membrane endoglin (Santa Cruz Biotechnology), endothelial NO-synthase (eNOS, Santa Cruz Biotechnology), vascular endothelial growth factor (VEGF, Abcam), VE-cadherin (Cell Signaling), vascular cell adhesion molecule-1 (VCAM-1, Abcam) and heme-oxygenase (HO-1, Abcam)) were evaluated using Western blot analysis and immunofluorescence.

Western blot analysis and immunofluorescence so far demonstrated no significant differences between treated and control cells in the expression of selected markers in HUVECs. In conclusion, 1nM soluble endoglin probably do not directly affect chosen markers of TGF-β signaling and endothelial function in HUVEC cells. However, further studies (another concentrations, different incubation times, effect of inflammation, combination with cholesterol) are needed to evaluate whether soluble endoglin might affect endothelial cells.

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IN VITRO EFFECTS OF FLAVONOIDS ON THE ARACHIDONIC ACID PATHWAY OF PLATELET ACTIVATION

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Flavonoids are a large group of polyphenolic compounds, ubiquitously distributed in plants. Numerous studies have established that flavonoids and isoflavonoids exert a wide range of biological activities, such as anti-inflammatory, anti-ischemic, antiplatelet, anti-tumoral and immunomodulatory.[1]

In this study, effects of flavonoids on three major steps of the arachidonic acid pathway of platelet activation were analyzed using human platelets.

In particular, flavonoids possessing the isolated 7-hydroxyl group and/or the 4′-hydroxyl group acted as antagonists of thromboxane receptor TXA_2. Moreover, a blockade of the 7-hydroxyl group by glucose did not abolish the effect. Interestingly, isoflavonoids genistein and daidzein were more potent inhibitors of ovine cyclooxygenase-1 than acetylsalicylic acid (ASA). Although their effects were lower in comparison with ASA in human platelets, such activity may be clinically relevant. None of flavonoid had a relevant effect on TXA_2 synthase.

In conclusion, flavonoids inhibit at least two steps of the arachidonic acid pathway of platelet activation. Moreover, ongoing research has shown that some isoflavonoids are even more potent antagonists of TXA_2 receptor.

The study was supported by grants of The Czech Science Foundation No. P303/12/ G163 and Charles University No. SVV 260 064, PRVOUK P40.

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ORGANIC CATION TRANSPORTER 1 IS DOWN-REGULATED BY PREGNAN X RECEPTOR IN HEPARG CELL LINE AND PRIMARY HUMAN HEPATOCYTES

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Organic cation transporter 1 (OCT1, SLC22A1) is expressed mainly in the human liver. It is responsible for uptake of many endogenous substances and cationic drugs – e.g. antidiabetic drug metformin or some antivirotics. Regulation of its expression is controled mainly by hepatocyte nuclear factor 4α (HNF4α). Pregnan X receptor (PXR) plays an important role in regulation of xenibiotic and endobiotic metabolism. It affects gene expression of a wide variety of transporters and drug metabolizing enzymes. PXR is a ligand-activated transcription factor that has a wide variety of ligands, e.g. rifampicin.

We already described effect of PXR on down-regulation of the OCT1 reporter construct and on the level of OCT1 mRNA in HepG2 and HuH-7 cell lines. We observed the same effect also in non-hepatic cell line HeLa but only after co-transfection with both PXR and HNF4α.

The aim of this work was to confirm phenomenon of down-regulation OCT1 *via* PXR on well differentiated hepatoma cell line HepaRG and primary human hepatocytes. To suppress effect of rifampicin was used siRNA against PXR.

We identified statisticaly significant decrease of SLC22A1 in both HepaRG and all tested primary human hepatocytes after treatment with rifampicin. No significant effect was observed after silenging PXR both in HepaRG cells and primary hepatocytes.

We can conclude that PXR down-regulates OCT1 mRNA not only in hepatoma cell lines HepG2 and HuH-7 but also in well differentiated cells HepaRG and also primary human hepatocytes.

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CHARACTERIZATION OF ENDOTHELIAL FUNCTION IN AORTA OF TRANSGENIC MICE OVEREXPRESSING HUMAN SOLUBLE ENDOGLIN

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Soluble endoglin (sEng) is a plasma protein generated by the cleavage of the extracellular domain from membrane endoglin (CD105). Increased levels of sEng were found in patients with preeclampsia, type II diabetes, hypertension, hypercholesterolemia and related to induction of endothelial dysfunction. Therefore, this study was aimed to analyze whether high sEng levels induce endothelial dysfunction in aorta by using transgenic mice with high expression of human sEng (*Sol-Eng*$^+$).

Male and female *Sol-Eng*$^+$ transgenic mice on chow diet showed higher plasma levels of human sEng as well as increased blood arterial pressure, as compared transgenic littermates that do not develop high levels of human soluble endoglin (control animals in this study). Functional analysis in isolated aorta demonstrated that the endothelium-dependent vascular function was similar in *Sol-Eng*$^+$ and control mice. Western blot analysis of aorta showed no differences between *Sol-Eng*$^+$ and control mice in the protein expression levels of endoglin, endothelial NO-synthase (eNOS) and pro-inflammatory ICAM-1 and VCAM-1.

These results suggest that high level of human sEng alone do not induce endothelial dysfunction in aorta of *Sol-Eng*$^+$ mice. However, these data do not rule out the possibility that soluble endoglin might contribute to alteration of endothelial function in combination with other risk factors related to cardiovascular disorders.

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IL-1 RECEPTOR BLOCADE ALLEVIATES ENDOTOXIN-MEDIATED IMPAIRMENT OF RENAL DRUG EXCRETORY FUNCTIONS IN RATS

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Sepsis induced by gram-negative bacteria imposes acute kidney injury (AKI) by activation of severe immune response activated by their superficial lipopolysaccharides (LPS). In the present study we sought for possibility to prevent such impairment by two potent anti-inflammatory agents, dexamethasone and IL-1 receptor antagonist, anakinra. Biochemical and molecular hallmarks of AKI were evaluated in rats given endotoxin from *Salmonella typhimurium* after pretreatment by saline, dexamethasone or anakinra. Untreated endotoxemic rats developed within 10 h typical symptoms of AKI characterized by reduced GFR, microalbuminuria, increased fractional excretion of sodium, and decreased tubular secretion of azithromycin, the prototype substrate for multidrug transporters Mdr1 and Mrp2.

Pretreatment with either immunosuppressant alleviated all these symptoms and restored the azithromycin tubular secretory clearance to control values. This effect was related to up-regulation of basolateral organic anion transporters, but not to Mdr1 or Mrp2, which were paradoxically down-regulated by both agents. Moreover, dexamethasone also increased the urinary clearance of bile acids through reduction of their transporter for reabsorption, Asbt. Dexamethasone also showed more intensive effect on immune response – besides reduction of plasma cytokines seen after both agents, it also reduced plasma levels of nitric oxide as a result of reduced iNOS expression in the kidneys and liver. In conclusion, dexamethasone and anakinra were both able to mitigate AKI imposed by endotoxin and modulated impairment in the expression of major transporters for renal drug elimination. We demonstrated significant role of IL-1β for the development of AKI imposed by LPS.

The study was supported by the Grant Agency of Charles University, Prvouk P37/05 and SVV-2014-260058.

INTERACTIONS OF STEVIOL WITH SELECTED EFFLUX TRANSPORTERS AND THEIR IMPACT ON TRANSPORT OF TRIMETHOPRIM

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The natural sweetener stevioside belongs to the most abundant diterpenoid glycosides of the plant *Stevia Rebaudiana* Bertoli. Stevioside is converted in the gastrointestinal tract to steviol that permeates through intestinal membrane[1]. The purpose of this study was to investigate the interactions of steviol with efflux transporters BCRP (breast cancer resistance protein) and MDR1 (P glycoprotein) expressed along the human gastrointestinal tract and potential drug-drug interactions of steviol with trimethoprim. Studies were performed in MDCK II cells stably expressing transporters of interest. Competitive inhibitory studies in MDCK II cells stably expressing transporters of interest using a standard substrate (Hoechst 33342) were employed. Subsequently, tests on cytotoxicity with the same cell models were employed to prove steviol transport *via* the studied transporters and examine possible interaction between steviol and trimethoprim. In the competitive studies, steviol interacted with both MDR1 and BCRP and inhibited efflux of Hoechst 33342. Steviol had similar cytotoxic effect in the cells transfected with MDR1 and control cells. Significantly lower cytotoxic effect was found in the cells transfected with BCRP. Steviol exhibited a potency to increase the cytotoxicity of trimethoprim in MDR1 transfected cells. In conclusion, this pilot study shows that steviol may act as MDR1 inhibitor and BCRP substrate. In levels that could physiologically occur in the intestine after oral administration, steviol might be capable to inhibit the MDR1-mediated efflux and enhance the intestinal absorption of trimethoprim.

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VASODILATORY EFFECTS OF QUERCETIN AND ITS METABOLITES ON VASCULAR SMOOTH MUSCLE IN HEALTHY AND SPONTANEOUSLY HYPERTENSIVE RATS

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Oral administration of quercetin decreases arterial blood pressure in larger extent than intraperitoneal application.[1] We hypothesized that vasorelaxant effect is not caused only by quercetin but also by its metabolites formed by bacterial microflora in the colon. This study was designed to determine whether quercetin metabolites including small phenolic acids are able to decrease blood pressure.

The known quercetin metabolites were tested for relaxation of rat thoracic aorta rings precontracted by phenylephrine at concentration of 10^{-7} to 10^{-3} M. Subsequently the most active structure was selected for *in vivo* experiments, when the effect on blood pressure and heart rate was monitored after i.v. administration in dose range from 0.2 mg/kg to 25 mg/kg.

The most active structure, 3-(3-hydroxyphenyl)propionic acid, initiated *in vitro* vasorelaxation in concentration of 100 nM while quercetin at 500 nM. The major quercetin metabolite formed by human enzymes, 3-glucuronide, was almost inactive. During the *in vivo* study in Wistar-Han rats the 3-(3-hydroxyphenyl)propionic acid decreased systolic blood pressure even at dose of 1 mg/kg without having an effect on heart rate at this dose. Similar results were also achieved by experiments in spontaneously hypertensive rats.

The results suggest that some of metabolites may be responsible for vasorelaxant properties of oral quercetin administration.

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INTERACTION OF THE INTESTINAL TRANSPORTER hOATP2B1 WITH SELECTED NATURAL COMPOUNDS

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Although the intestinal absorption mechanisms of xenobiotics have been studied thoroughly, the interactions of many compounds with the important intestinal drug transporters have not been evaluated. It was shown that the transporters from the family organic anion transporting polypeptide (OATPs) contribute to the intestinal drug absorption.[1] The most expressed transporters from this family in human intestine are OATP2B1 and OATP1A2.

In this study we aimed to assess the potential inhibition of hOATP2B1 by natural compounds from the group of flavonoids (quercetin, myricetin, galangin, pinobanksin, pinocembrin, chrysin, fisetin) and diterpene steviol and its glycoside stevioside.

MDCK II cells transiently transfected with hOATP2B1 were used as the experimental model in the study. All mentioned flavonoids and steviol showed the inhibition of the hOATP2B1-mediated [^{3}H]-estrone 3-sulfate uptake, quercetin served as a control. Galangin and chrysin were the most potent inhibitors with IC_{50} of 15.0 μM and 18.2 μM, respectively. Stevioside showed no significant inhibition. According to the obtained results, these natural compounds could potentially affect the drug uptake by human OATP2B1 (e.g. statins) in the intestinal cells. Therefore, the food-drug interactions in humans based on this interaction cannot be excluded.

The study was supported by Charles University (SVV 260064 and PRVOUK).

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FUNCTIONAL AND MOLECULAR ASPECTS OF CHRONIC SUNITINIB CARDIOTOXICITY IN RATS

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Sunitinib is a modern multi-targeted tyrosine kinase inhibitor, which showed great promise in the treatment of metastatic renal cell carcinoma and some other solid tumors.

However, its chronic use may be associated with cardiotoxicity, which was not detected during preclinical testing and is poorly understood. Therefore, the aim of the present study was to investigate substantial molecular mechanisms responsible for the development of chronic sunitinib cardiotoxicity in Wistar Kyoto (WKY) rats.

For the induction of significant cardiac dysfunction, sunitinib (10 mg/kg) was administered daily for 8 weeks and after a wash out period (5 days), a re-challenge period followed for 2 and 3 weeks, respectively. Control rats received water in the same schedule.

Sunitinib treatment induced a significant left ventricular (LV) dysfunction (drop in FS by ~30%) and decline in heart rate. This was accompanied by increased LV BNP expression, increased BNP plasma concentrations and increased lung to body weight ratio. Interestingly, no treatment-related changes were detected in high sensitive plasma troponin T and myocardial fibrosis markers and only mild LV lipoperoxidation was observed. Noteworthy, sunitinib-induced cardiac dysfunction was accompanied by marked expression of numerous hypoxia-regulated genes and inflammatory molecules.

In conclusion, our findings suggest an important role of hypoxic signaling in the development of chronic sunitinib cardiotoxicity. Hence, with respect to the present data and available literature, involvement of "hibernating myocardium" may be implicated, which represents promising challenge for further study.

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INTERACTIONS OF ABACAVIR WITH PLACENTAL NUCLEOSIDE TRANSPORTERS

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Abacavir is a nucleoside analogue antiretroviral agent, which is commonly used in prevention of mother-to-child transmission (PMTCT) of HIV. Although its transplacental transport might be affected by fetus-protecting ABCB1/ABCG2 efflux transporters, the transfer of the drug from mother to fetus was found to be sufficient for adequate PMTCT. The exact mechanism of abacavir transplacental transport has not been fully elucidated so far. The aim of our study was to evaluate the potential of abacavir to interact with placental equilibrative nucleoside transporters (ENTs) and Na^+ dependent concentrative nucleoside transporters (CNTs) that are involved in the transcellular transport of endogenous nucleosides as well as nucleoside-derived drugs. Accumulation assays in human syncytiotrophoblast-derived BeWo cells revealed abacavir interactions with ENT1 and

CNTs. Similarly, employing the method of dually perfused rat term placenta we observed an effect of ENT1 inhibitor on abacavir transplacental clearance; however, no Na^+-dependency in abacavir transport could be detected. The uptake assays in human placental fresh villous fragments revealed decreased abacavir uptake in the presence of ENTs inhibitor and in Na^+-free buffer. In summary our data indicate possible involvement of ENT- and CNT-mediated transport in the penetration of abacavir across the placenta into the fetal compartment. Nevertheless, additional experiments are needed to elucidate this issue.

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COMBINATION OF HIGH SOLUBLE ENDOGLIN LEVELS AND HIGH FAT DIET AFFECTS AORTIC ENDOTHELIUM IN MICE

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Soluble endoglin (sEng) is a plasma protein, a cleavage product of the extracellular domain of tissue endoglin. sEng was supposed to be a biomarker in several cardiovascular pathologies, including endothelial dysfunction, hypertension, hypercholesterolemia and diabetes mellitus. However, the specific role of sEng in these disorders is still poorly understood. Therefore, we hypothesized whether high fat diet in combination with high levels of sEng may affect aortic endothelium *in vivo*.

Six-month-old transgenic female mice overexpressing human sEng on CBAxC57BL/6J background *(kindly provided by Prof. Lopez-Novoa)* were fed high fat diet for the following 3 months. Mice were divided into two groups according to plasma levels of sEng determined by ELISA (Sol-Eng+ group vs. control group). Functional parameters of aorta were assessed by means of wire myograph 620M. Western blot analysis of eNOS, peNOS, ICAM-1, P-selectin, FkB, iNOS, NOX-2, HO-1 expressions in aorta were performed.

Results of the study demonstrated that high plasma levels of sEng might induce a pro-inflammatory and oxidative stress phenotype of aorta, which is however compensated by an improved endothelial function in Sol-Eng+ group. Nevertheless, the mechanism of the compensatory response remains to be elucidated.

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ROLE OF ENDOPLASMIC RETICULUM STRESS IN MELANOMA RESISTANCE TO SMALL – MOLECULE INHIBITORS OF MAPKS PATHWAY

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Vemurafenib (VMF), Dabrafenib (DBF) and Trametinib (TMT), small-molecule inhibitors of mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAP/ERK) pathway, represent a new biological approach to treatment of malignant melanoma (MM). In spite of personalized targeted cancer therapy, successful treatment of MM remains a big scientific challenge, also due to fast development of drug resistance. One of the possible ways of overcoming MM resistance to treatment could be finding specific role of endoplasmic reticulum (ER) stress, as one of the most important mechanisms in cell homeostasis regulation, and consequent unfolded protein response (UPR) in cancer. We studied an effect of both inhibition and induction of ER stress on cell viability after treatment by VMF, DBF and TMT in human melanoma cell lines A375 – parental (A375-wt) and resistant (A375 R-VMF/R-DBF/R-TMT) using crystal violet cytotoxicity assay. For inhibition of ER stress, the tauroursodeoxycholic acid (TUDCA) was used, and thapsigargin (TG) was used as ER stress inducer. The effects of inhibition and induction in gene level were studied by qRT-PCR. XBP1 gene and its spliced form XBP1s were chosen as ones of the UPR important markers, follow by central regulator of ER function – GRP78, CHOP and ATF4, genes whose expression levels also play an important role as markers of ER stress/UPR.

The study was supported by GEFLUC-LR [grant number DC/MLP/09-011], HPC resources from GENCI-CINES (http://www.cines.fr) [grant number c2014035038] and LLP Erasmus.

EMTRICITABINE IS A SUBSTRATE OF MATE1, BUT DOES NOT INTERACT WITH P-GLYCOPROTEIN, BCRP, MRP2, OCT1 OR OCT2 TRANSPORTERS

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Emtricitabine, a nucleoside reverse transcriptase inhibitor, is often administered as an important component of combination antiretroviral therapy (cART) in HIV positive patients including pregnant women. Interactions of emtricitabine with membrane expressed

drug transporters could largely affect its pharmacokinetic behavior and transfer of the drug across the placenta and lead to drug-drug interactions with other antiretrovirals used in cART. The aim of this study was to evaluate whether or not transport of emtricitabine is affected by P-glycoprotein (ABCB1), BCRP (ABCG2), MRP2 (ABCC2), MATE1 (SLC47A1), OCT1 (SLC22A1) and/or OCT2 (SLC22A2) transporters, which are abundantly expressed mainly in excretory organs and in the placenta.

We used transfected MDCK cells stably expressing P-gp, BCRP, MRP2, MATE1, OCT1 and OCT2 for *in vitro* transcellular transport. Additionally, the method of dually perfused rat term placenta was used in closed-circuit arrangements to assess the contribution of drug transporters in transplacental transfer of emtricitabine.

As observed in our assays, neither conventional bi-directional (concentration gradient) transport experiments nor the concentration equilibrium method in MDCK cells indicated contribution of ABCB1, ABCG2, ABCC2, OCT1 or OCT2 in transport of emtricitabine. Nevertheless, transcellular transport of emtricitabine was significantly increased in MATE1 expressing cells. Correspondingly, MATE1 was responsible for significantly reduced intracellular accumulation of the drug in our assays. Addition of ritonavir or cimetidine, known MATE1 inhibitors, significantly reduced MATE1 mediated transcellular transport of FTC and increased its intracellular accumulation. The transport ratios which were calculated as the ratio between the apically directed transport and the basally directed transport measured at 2 h decreased with increasing apical pH, which indicates pH dependent transcellular transport and further confirms the role pH-sensitive MATE1 the transport of emtricitabine. Surprisingly, when employing in situ perfused rat term placenta, we did not observe any effect of either active transport or pH.

Based on our results, we therefore conclude that emtricitabine is a substrate of MATE1 but does not seem to interact with other studied transporters; nevertheless the impact of this finding for the pharmacokinetic behavior of emtricitabine in vivo remains to be further elucidated.

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IN VITRO INTERACTIONS OF ISOFLAVONOIDS WITH IRON AND COPPER

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Isoflavonoids represent a group of natural substances, particularly known for their oestrogenic effects. Positive cardiovascular action of these compounds has been proposed,

however, the results of epidemiological studies are equivocal.[1,2] Although the interaction with trace metals can influence antioxidant and pro-oxidant behavior of isoflavonoids, limited data are available in this field. In the present study, 13 structurally related isoflavones were tested for their iron- and copper-chelating and reducing properties by simple spectrophotometric approaches. The experiments were performed with emphasis on the structure-activity relationship. Isoflavones containing the 5-hydroxy-4-keto site were able to chelate ferric, ferrous and cupric ions, however, their affinity for mentioned ions was generally lower in comparison with flavonoids of similar chemical structures or standard chelators. No chelation of cuprous ions was observed. While ferric ions were not reduced by any of the tested compounds, all of them except for biochanin A and ononin were able to reduce cupric ions. The unsubstituted 4′-hydroxyl group was primarily associated with the reduction. The study may have clinical impact, since selective copper reduction may influence its absorption.

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NOVEL CLASS OF ACTIVATORS OF PHARMACOLOGICALLY IMPORTANT NUCLEAR RECEPTORS

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Several nuclear receptors (NRs), which are ligand-inducible transcription factors, have been shown to play a key role in a regulation of both endogenous and exogenous metabolism. Among the most studied NRs forming the regulatory network of xenobiotic-metabolizing enzymes (XMEs) such as cytochromes P450 (CYPs) belongs the constitutive androstane receptor (CAR, NR1I3), the pregnane X receptor (PXR, NR1I2) and the aryl hydrocarbon receptor (AHR). The NRs are activated by broad spectrum of compounds such as drugs, herbal compounds and environmental chemicals. The growing body of evidence suggests that activation of NRs mediated by ligands is not involved only in XMEs regulation but their ligands may be also utilized as therapeutics in the treatment of different disorders. Thus, there is an urgent need for specific and non-toxic NR ligands.

Recently, we reported a novel class (CHP) of human NR agonists (CHP4, CHP5 and CHP6) which were found by random screening of a compound library. The aim of present study was further characterize the three most potent activators of CHP class identified previously in our group to reveal their effects towards the other important NRs involved in xenobiotic metabolism.

In the transfection gene reporter experiments, we observed that all three used CHPs strongly increased activity either PXR- or AHR-dependent reporters in transient transfection experiments in HuH-7 and HepG2, respectively. Since, CHPs activated CYP reporter genes at a transcriptional level, it was also interesting to elucidate whether they might inhibit enzymatic activity of CYP enzymes. We showed that all three used CHPs inhibited CYP2C9 in the similar way to a model inhibitor fluconazole. CYP1A2 was also inhibited by CHPs but in less extent than that with model inhibitor α-naphthotoflavone. In the case of CYP3A4, we observed different effects among derivates indicating CHP4 had no effect on CYP3A4 compared to CHP5 and CHP6. The interaction of CHP4 with ligand binding pocket of PXR as target regions for molecular docking was further assessed by AutoDock Vina supporting our experimental results. Values of predicted Kd were 63.6 nM for the best binding mode of CHP4 to PXR.

Taken together, our results suggest that the compounds may interact with several NRs and have variable effects on major xenobiotic-metabolizing CYP enzymes. These compounds can be promising candidates for the treatment of some metabolic disorders.

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ACHE MODULATORS AFFECT CHOLINERGIC RECEPTORS

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Acetylcholinesterase inhibitors (AChEI) are used in the treatment of various disorders with impaired cholinergic transmission. They are considered as a treatment strategy for early and mild type myasthenia gravis (MG), an autoimmune disease characterized by fatigable weakness of voluntary muscles. The positive modulation of peripheral nicotinic receptors by AChEI could have an added value to the anti-AChE activity. AD is another disease whose treatment is linked to the cholinergic hypothesis. The classic one- the inhibition of AChE and relatively new one- the activation of M_1 receptors and neuronal nicotinic. Furthermore, reversible AChEI play an important role in the prophylaxis against nerve agents. Overstimulation of the cholinergic receptors (muscarinic and nicotinic) by excessive amounts of ACh causes several health problems and may even cause death.

It was examined if AChEI can simultaneously modulate cholinergic receptors. Their mechanism of action was studied on TE671 cell line which is medulloblastoma/ rhabdo-

myosarcoma cell line endogenously expressing human embryonic muscle type of nicotinic receptor $\alpha_1\beta_1\gamma\delta$ and on $\alpha_4\beta_2$ neuronal nicotinic receptor transiently expressed in COS cells (patch clamp technique). Then, on CHO cells stably expressing human recombinant M_1 muscarinic receptor, the calcium mobilization assay was used.

In summary, *in vitro* experiments showed that newly synthesized AChEI can inhibit AChE, mAChR and nAChR of both peripheral and central types. Thus, these promising compounds could be an effective way to diminish the effect of overstimulation cholinergic receptors during organophosphates poisoning. However, their real prophylactic potency and benefits must be definitively verified *in vivo.* On the other hand, such property is not in favour with our hypothesis concerning the treatment of MG and AD.

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DETERMINATION OF ATV TREATMENT EFFECTS ON ENDOGLIN AND ENOS EXPRESSION IN ENDOTHELIAL CELLS *IN VITRO*

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Endoglin (CD105), a TGF-β binding protein, was demonstrated to be strongly related to eNOS expression and activity *via* Smad2 dependent pathway *in vitro* and *in vivo*. Morevover statins were demonstrated to increase eNOS and endoglin expression *in vivo* in mouse atherosclerosis. In this study, we focused on endoglin and eNOS expression during inflammation and after atorvastatin treatment in HUVECs. We hypothesized whether statin induced eNOS expression depends on endoglin and Smad2.

HUVECs were exposed to TNFα (10 ng/ml) for 2, 6 and 16 h to mimic inflammation. Atorvastatin (ATV) was added for 30 minutes or 24 h at a concentration of 5 μM, DMSO 0.1% (v/v) was used as control. In atorvastatin pretreatment model, cells were treated 24 h by ATV, then rinsed and cultured with TNFα for 16 h. The protein expression HUVECs was determined by flow cytometry and Western blot analysis and soluble endoglin in medium by means of ELISA.

ATV treatment significantly upregulated endoglin and eNOS expression in HUVECs. Induction of inflammation by TNFα treatment significantly reduced endoglin expression, together with significant increase of soluble endoglin in medium. Pretreatment of ATV, before TNFα exposure, significantly prevented inflammation induced decrease of endoglin and eNOS expression, when compared to cells treated only by TNFα. Moreover, suppression of endoglin using small interfering RNA (siRNA), but not inhibition of TGF-β signaling with SB431542, abrogated ATV-induced eNOS expression. 30 minutes ATV treatment under starving conditions did not change the expression of phosphorylated Smad2 protein.

In conclusion, inflammation results in reduced expression of endoglin and eNOS in HUVECs, which could be prevented by atorvastatin treatment. Moreover, atorvastatin induced eNOS expression seems to be dependent on endoglin expression, but not on Smad2.

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SECTION OF PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS

FUMARIA OFFICINALIS ALKALOIDS AND THEIR BIOLOGICAL ACTIVITIES RELATED TO ALZHEIMER'S DISEASE

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Two new isoquinoline alkaloids named fumaranine and fumarostrejdine, along with 18 known alkaloids were isolated from aerial parts of *Fumaria officinalis*. The structures of the isolated compounds were elucidated on the basis of spectroscopic analysis and by comparison with literature data. The absolute configuration of the new compounds was determined by comparing their circular dichroism spectra with those of known analogues. Compounds isolated in sufficient amounts were evaluated for their acetylcholinesterase, butyrylcholinesterase, prolyl oligopeptidase and GSK-3β inhibitory activity.[1–3] Parfumidine and sinactine exhibited potent prolyl oligopeptidase inhibition activity (IC_{50} 99 ± 5 μM and 53 ± 2 μM, respectively).

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PHYTOCHEMICAL STUDY OF INDIVIDUAL PLANT SPECIES OF *BERGENIA* GENUS

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Bergenia, a genus included in the family *Saxifragaceae*, is a valuable source of healing matters. Scientific research is focused on five species mainly distributed in the mountains of Central and East Asia: *Bergenia ciliata* (Haw.) Sternb., *Bergenia stracheyi* Engl., *Bergenia crassifolia* (L.) Fritsch, *Bergenia ligulata* (Wall.) Engl. and *Bergenia himalaica* Boriss. These taxons belong to the widely used medicinal herbs in traditional Ayurveda medicine. Individual parts of plant demonstrate antibacterial, antiviral and cytoprotective effect. *Bergenia* is a valuable resource of interesting chemical compounds, contains polyphenol bergenin, its derivative norbergenin, arbutin, catechin, gallic acid, flavonoids. Our study is focused on the evaluation of arbutin, total tannin and bergenin contents, in connection with the research of biological and antioxidant activity of extracts prepared from green leaves of *Bergenia crassifolia, B. ciliata* and *B.* x *ornata.* One of the aims is also the study of the influence of meteorological data on the presence of phenolic compounds.[1] The highest content of bergenin and related highest antioxidant and antiradical activity (measured by FRAP, NADH, DPPH, ABTS) was found in the species *B. crassifolia* and *B.* x *ornata* (4.9–5.1 mg g^{-1}), the lowest content in the leaves of *B. ciliata* (3.1 mg g^{-1}). *B.* x *ornata* was first tested for the content of phenolic compounds. The dependence of contained metabolites on climatic conditions was revealed as well as the relationship between antiradical activity and the content of secondary metabolites. The presence of phenolic compounds has a clear influence on the biological activity.

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ALKALOIDS FROM *PEUMUS BOLDUS* MOL. AND THEIR BIOLOGICAL ACTIVITY

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Alzheimer's disease (AD) is neurodegenerative disease with specific neuropathological changes. Nevertheless, ethiopathogenesis is not still clear and therapy is only symptomatic. Natural products are source of potentially active compounds with neuroprotective effects[1].

Peumus boldus (boldo) leaves have been chosen for *in vitro* studies as source of alkaloids. Plenty of experimental studies have proven the effectiveness of main alkaloid boldine in preventing various free radical-mediated oxidative events in the ethiogenesis of various cardiovascular, tumoural, inflammatory and neurodegenerative pathologies[2].

The primary extract was acquired from dried boldo leaves by extraction with ethanol and then was treated by liquid extraction with different pH. Alkaloid extract was treated by standard chromatographic methods. Alkaloid structures were determined by spectroscopic methods (MS, NMR). 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).

Eleven alkaloids have been isolated: aporphines boldine, isocorydine, norisocorydine and laurotetanine, N-methyllaurotetanine; proaporphines pronuciferine and glaziovine; morphinanes pallidine and sinoacutine; benzylisoquinolines *N*-methylcoclaurine and reticuline. Any of isolated alkaloids did not inhibit HuAChE effectively. Potent inhibitors of HuBuChE were *N*-methylcoclaurine and reticuline with IC_{50} 15.02 ± 1.35 μM and 43.92 ± 1.19 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 boldo leaves were not potent compounds for AD treatment, although *N*-methylcoclaurine and reticuline could serve as lead structures for preparation of semi-synthetic cholinesterase inhibitors.

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ISOQUINOLINE ALKALOIDS FROM *FUMARIA OFFICINALIS* AND THEIR BIOLOGICAL ACTIVITIES RELATED TO ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most predominant cause of dementia in the elderly affecting more than 20 million people worldwide. In AD patients, deficit of the neurotransmitter acetylcholine (ACh) in the cortex results in a deterioration of the level of cholinergic functions, and this is responsible for the memory impairments. The principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh. However, not only AChE participates in the cholinergic regulation of central nervous system in humans, but also another enzyme, butyrylcholinesterase (BuChE), which is able to hydrolyze ACh, as well as other esters. In AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with the normal stage, while the concentration of BuChE increases. This fact has targeted BuChE as new approach to affect the progression of AD. Therefore, research into new inhibitors with dual enzymatic activity is required. Currently, acetylcholinesterase inhibition is the most therapeutic treatment for the symptoms of AD.

The summary ethanolic and diethylether extracts were prepared from the herbs of a plant *Fumaria officinalis* L. 201 fractions were obtained by column chromatography on the neutral Al_2O_3. Fractions 68–76 were processed by thin layer chromatography, and 3 substances were isolated in pure form (DH-1, DH-2, DH-3). The isolated compounds were identified as protopine, (+)-fumariline and *N*-methylcorydaldine by comparison with the literature data and results of MS and NMR studies. The alkaloids were screened for their biological activities related to AD (inhibition of HuAChE, HuBuChE, prolyl oligopeptidase (POP), glycogen synthase kinase-3β (GSK 3β)). Unfortunately the tested compounds did not show any significant inhibitory activities (IC_{50}, μM).

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IDENTIFICATION OF MRE IN PROMOTER OF FLAVONOL SYNTHASE

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A flavonol synthase (FLS) is enzyme, which catalyzes transformation of dihydroflavonols to flavonols. A production of enzymes for flavonoid synthesis is affected by the transcription factors (TF), which activate promoters. MYB12 is TF, which was found in *Arabidopsis thaliana*. It was proved that this MYB12 is responsible for activation of genes for flavonoid synthesis as chalcone synthase and FLS.[1] There are special sequences in promoter, which recognizes certain MYB transcription factors and they are called myb recognition elements (MRE).[2] This research is focused on identification of MRE in promoter of FLS1 from *Beta vulgaris*. Five constructs were prepared with promoters of different length from the plasmids with FLS1 promoter. The transfection reactions were made with protoplast of *A. thaliana* and AtMYB12 and BvMYB12 as effectors. The results were evaluated through

GUS activity of samples. The activity of AtMYB12 was bigger than BvMYB12 and more possible sequences of MRE were found for these transcription factors. Thus, further research has to be done for better identification of myb recognition element in FLS1 promoter.

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

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Vinca minor L. is an ornamental plant from Apocynaceae family commonly used in gardens, but it is also a source of alkaloids. So far, more than 45 alkaloids of indole type have been isolated from this plant. Indole type alkaloids are known as the source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease[1]. For example vinpocetine, a synthetic derivative of the *V. minor* alkaloid vincamine shows neuroprotective effects[2], but is ineffective in improving cognitive deficits and does not slow the rate of decline in individuals with Alzheimer's disease[3]. In our experiments alkaloidal extract showed high BuChE inhibitory activity (IC_{50} 7.75 ± 0.99 g/ml).

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

The mixture of the total alkaloids was divided by means of column chromatography into sixteen parts containing alkaloids. Chromatography was performed on alumina using gradually enriched petrol–chloroform and chloroform–ethanol mixtures for elution.

The combined fractions 73–110 obtained from column chromatography were further divided using flash chromatography on silica-gel. Two pure alkaloids were isolated from the fraction four by preparative TLC on silica-gel and tentatively identified by GC–MS as vincaminorine and vincaminorein[4].

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RAPID AUXIN METABOLITE PROFILING FOR HIGH-THROUGHPUT *ARABIDOPSIS* SCREENING

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The phytohormone auxin (indole-3-acetic acid; IAA) has a crucial role in plant growth and development. IAA precursors and degradation products have very different chemical properties (molecular weight, acidity and basicity). They occur in a wide concentration range, and some of them are highly labile in solution, which is challenging for sample preparation. Metabolite profiling has the potential to provide a deeper level of understanding of how auxin activity is regulated.

New high-throughput method based on solid phase extraction (SPE) using micro-pipette tips (μPT-SPE) with combination of two reverse phases (C18 and SDP-RPS) was developed for isolation and quantification of auxin and its metabolites. The procedure was completed by a single chromatographic analysis in 5 minutes (column: Kinetex C18 100A, 50 × 2.1 mm, 1.7 μm) coupled to tandem mass spectrometer (Agilent 6490 Triple Quadrupole LC/MS System).

The combination of microextraction with ultra-rapid high-throughput purification provides fast, simple, effective and cheap sample preparation for qualitative and quantitative measurements. This method enabled the analysis of a several hundred plant samples of Arabidopsis mutant lines and in combination with data obtained from genetic screening allows the explanation of the dynamics of auxin metabolism and activity in planta.

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ALKALOIDS OF SOME *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

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Alzheimer's disease (AD) is characterized by progressive and irreversible loss of neurons. For AD are characteristic decreased levels of neurotransmitter acetylcholine in the cortex, which is hydrolysed by acetylcholinesterase. In healthy brain, AChE is the most vital enzyme compared to others. In late AD stages butyrylcholinesterase is the main hydrolysing enzyme as its content will increase by up to 90% in comparison to normal state. BuChE cleaves ACh in a manner similar to that of AChE to terminate its physiological action[1].

Amaryllidaceae species are important for producing specific compounds, known as *Amaryllidaceae* alkaloids, which have interesting physiological effects such as antitumor, antiviral, antimalarial and acetylcholinesterase activity. Alkaloidal extracts of eight *Narcissus* species have been tested for their inhibitory effects on human erythrocytic acetylcholinesterase (HuAChE) and human serum butyrylcholinesterase (HuBuChE) and their alkaloid pattern. Fourty-two alkaloids were determined by GC/MS. Interesting biological activities were exhibited by extracts of *N. poeticus* cv. Pink Parasol IC50 HuBuChE = 3.3 ± 0.5 μg/ml (IC50 HuAChE = 191.3 ± 20.2 μg/ml).

N. poeticus cv. Pink Parasol was chosen for the phytochemical study and isolation of alkaloids. All isolated alkaloids were tested with respect to their HuAChE, HuBuChE and also prolyloligopeptidase (POP) inhibitory activity. POP is involved in key physiological functions, such as learning and memory, cell division and differentiation as well as in some psychiatric disorders. In recent years, POP has gained importance as a target for the treatment of schizophrenia, bipolar affective disorders and cognitive disturbances, such as those presented in AD. So far five alkaloids were isolated, and the best inhibitory activity was demonstrated by narwedine $IC_{50;\,POP}$ = 0.907 ± 0.087 mM.

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PHARMACEUTICAL TECHNOLOGY SECTION

STUDY OF FLOW PROPERTIES OF SORBITOL AND ITS FRACTIONS

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Flow and consolidation properties are fundamental during handling and processing particular materials in pharmaceutical technology. In the evaluation, a lot of standard methods are used (Ph. Eur 8.3, Methods of Pharmaceutical Technology). Out of them, determination of the rate of gravitational flow through an orifice of a hopper is often used.

In this work, the influencing of the mass flow rate (g/s) of excipient for direct compression – sorbitol (Merisorb 200) and its size fractions in the range from 0.080 to 0.400 mm by the diameter of the circular hopper orifice in the range from 6 to 15 mm is investigated. A conical testing hopper of the automatic tester (PTG S3, PHARMATEST, Germany) is used in this. The results are modelled using of the power law equation of Jones and Pilpel.[1]

The parameters of the flow equation are used for the prediction of the flow rate with the aim to recommend the suitable orifice of the hopper for achievement the most accurate estimation of the flow rate in routine testing of flowability.

Based on the experimental results, the orifice 10 mm can be recommended for testing of flowability of sorbitol and its fractions. Out of the used diameters of the hopper orifices, the lowest average deviation of 2.5% between of the measured flow rate and the estimated one from the generated equation was detected with this hopper orifice.

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COMPRESSIBILITY OF PELLETS MADE FROM MICROCRYSTALLINE CELLULOSE

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Tablets are the most frequently used pharmaceutical solid dosage form. This work deals with the preparation of tablets from pellets. Pellets are the particulate systems most commonly of spherical shape. They are often used as a filler of hard gelatin capsules.[1]

The pellets used in this work were Cellets® 100 and Cellets® 200 (HARKE Pharma) made from microcrystalline cellulose. These pellets are ideal for tableting and filling into capsules.[2] Other studies show that they have a very narrow particle size distribution, almost perfect sphericity and low friability.[3] Properties of these pellets and their compressibility were compared with the microcrystalline cellulose Avicel® PH-200.

It was found that the pellets have a narrower particle size distribution, contain less moisture and have higher flowability than microcrystalline cellulose. Evaluation of compaction by the force-displacement method showed lower total energy consumed during the compaction process in pellets. Parameters of the compaction equation showed that most of the energy used for the compression of the pellets is consumed by their fragmentation. Therefore tablets made of microcrystalline cellulose have many times higher tensile strength than tablets made from pellets.

The results of this study showed that the pellets Cellets® 100 can be used for the manufacture of tablets, but it is necessary to use higher compaction pressure. Pellets Cellets® 200 alone cannot be compressed into tablets with the optimal tensile strength.

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ORGANIZATION OF CERAMIDES WITH LONG (C16) AND VERY LONG (C24) ACYLS IN MODEL STRATUM CORNEUM MEMBRANES

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The barrier function of the skin is ensured by the outermost skin layer, the stratum corneum (SC). This layer consist of corneocytes embedded in lipid matrix composed of ceramides (Cer), free fatty acids (FFA) and cholesterol in lamellar arrangement. The number of carbons in Cer molecules seems to be also essential for skin barrier properties, e.g. increased levels of long Cer (NS16) at the expense of the very long Cer (NS24) have been found in skin of atopic dermatitis patients.

To understand the different behavior of the long (C16) and very long (C24) Cer we studied their properties in SC multilayer model membranes using infrared spectroscopy (IR) and SC monolayer models using Langmuir films at the air/water interface and on solid substrate by atomic force microscopy (AFM). Investigation by IR using unlabeled and deuterated compounds enables us to observe phase changes, differences in mixing, packing and conformation of lipid chains. In SC multilayer models Cer NS24 prefer an extended conformation in which FFA are associated with acyl chain of Cer, in contrast to membranes based on Cer NS16, in which FFA are mostly phase separated. Monolayers at air/water interface based on Cer NS24 form condensed phase with no apparent phase transition whereas molecules in monolayers with Cer NS16 occupy larger area and show a phase transition during compression. AFM images of monolayers containing Cer NS24 comprise continuous domains rich in FFA. The area of the FFA-rich phase in model containing Cer NS16 is smaller and rather discontinuous.

We confirmed that the length of acyl chain in Cer influences the arrangement of lamellae in SC model membranes.

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DEVELOPMENT OF ORODISPERSIBLE TABLETS I: INTRODUCTION STUDY

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European Pharmacopoeia defines orodispersible tablets (ODT) as uncoated tablets, which are intended to be placed in the mouth, where they disperse rapidly before being swallowed. To achieve this, disintegration within 3 minutes is the main requirement.[1] ODT possess many advantages of mouth dissolving drug delivery systems: ease of administration, accurate dosage, self-medication, patient compliance, and pain avoidance.[2] Common problem of many produced ODT is their poor mechanical properties.

ODT can be divided into 3 generations according to the production approach: freeze-dried tablets, molded tablets and directly compressed tablets.[2] Apart from the classical tablet excipients, two important groups are generally used in ODT´s formulation. First, superdisintegrants to facilitate disintegration of tablet[4], and second, taste masking excipients to improve palatability of the preparation[5].

For the development of ODT's formulation, four diluents were chosen – lactose, mannitol, sorbitol, and maize starch. Flow and compressibility properties of excipients were first studied. Pharmacopoeial methods such as particle size distribution analysis, angle of repose analysis, Hausner ratio analysis, and thermogravimetrical moisture analysis were used. All studied excipients are not directly suitable for further processing.

This study was supported by SVV 260 062 and it is dedicated to Assoc. Prof. RNDr. Milan Řehula, CSc.

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INFLUENCE OF LUBRICANTS ON VISCOELASTIC PROPERTIES OF TABLETS

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The principle of tablet compression process is the transformation of undeformed particles of compressed material to elastically and plastically deformed particles due to the action of compression force. To evaluate the viscoelastic properties, several methods can be used[1]. The force-displacement record and the stress relaxation test are the most commonly used methods.

This study evaluates the influence of two lubricants on viscoelastic properties of tablets made of three different fillers intended for direct compression, i.e. microcrystalline cellulose, lactose and dibasic calcium phosphate dihydrate. Magnesium stearate and micronized synthetic amorphous silica gel (Syloid) were used as lubricants at concentrations of 0.5% and 1%.

Viscoelastic properties are influenced by the type of used filler as well as by the type and the concentration of a lubricant. It was found that magnesium stearate affects primarily friction between particles but has a negative effect on the tablet tensile strength and plasticity. Syloid reduces elastic energy and has less effect on tensile strength of tablets.

Different effect of lubricants on plasticity of fillers was observed. Polymeric excipient, microcrystalline cellulose, which itself has the highest values of elasticity and plasticity was the most influenced filler in comparison to the other ones. Syloid increases the plasticity of cellulose tablets more than magnesium stearate. In opposite, Syloid decreases plasticity of tablets made of dibasic calcium phosphate dihydrate and/or lactose. The least effect of lubricants was noted with dibasic calcium phosphate dihydrate, the particles of which have very low elasticity as well as subsequent plasticity.

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PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

ANTHRACYCLINE-INDUCED DNA DAMAGE: METHODS OF DETECTION

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Cardiotoxicity is a serious life-threatening side effect of anthracycline antineoplastic drugs. Unfortunately, its mechanism has not been elucidated yet. Traditionally, oxidative stress is perceived to be the main cause of anthracycline cardiotoxicity. Nevertheless, there is increasing evidence questioning this traditional theory.[1] Number of studies now adress the role of topoisomerase II in this process.[2] Topoisomerase II is a nuclear enzyme modifying the linking number of DNA by breaking and resealing the DNA molecule. A group of topoisomerase II inhibitors, called "topoisomerase poisons" (eg. anthracyclines) are blocking the resealing of the DNA molecues, causing of DNA double strand break formation, which is believed to be the main mechanism of anthracycline antineoplastic effects. In higher eukaryotes, there are two isoforms of topoisomerase II – alpha and beta. The alpha isoform is indispensable for proliferation (eg. in tumor cells). On the other hand, beta isoform is present in the terminally differentiated cells (eg. cardiomyocytes).[3] Thus targeting the beta isoform of topoisomerase II in cardiomyocytes by antracyclines, with subsequent DNA damage, could represent a mechanism of antracycline cardiotoxicity. Here we discuss the possible methods to study DNA damage caused by anthracyclines in cardiac and cancer cells.

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PURIFICATION OF MEMBRANE-BOUND CARBONYL-REDUCING ENZYMES USING IN-HOUSE DEVELOPED AFFINITY CARRIER

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Approximately 30% of all human proteins are predicted to be membrane-bound proteins. Although they are involved in many physiological functions, there is still relatively little information because their study is demanding. The endoplasmic reticulum contains (among others) membrane-bound enzymes participating in metabolism of endogenous substances and drugs. There are also localized carbonyl-reducing enzymes, which are characterized mainly in relation to the metabolism of endogenous substrates (e.g. steroids or prostaglandins). Even though they are believed to play an important role in drug metabolism, knowledge on them is quite poor. Actually, until today there is only one well characterized microsomal carbonyl-reducing enzyme participating in metabolism of xenobiotic compound; 11-beta-hydroxysteroid dehydrogenase 1 (11β-HSD1). However, based on the research of anticancer drug oracin reduction stereospecificity[1], there were predicted microsomal carbonyl-reducing enzymes involved in its metabolism and inactivation, beside well known11β-HSD1, that are necessary to isolate and identify

Methods based on molecular recognition are currently the most powerful tool for separation and isolation due to their selectivity and recovery. Such a method based on the interaction of the enzyme with xenobiotic substrate oracin was developed in our laboratory[2]. Model anticancer drug oracin was immobilized on the surface of silica-coated magnetic microparticles and thus used as affinity carrier to isolate carbonyl-reducing enzymes from complex biological samples. Enzymes having affinity towards oracin were efficiently captured, gently eluted by 100 mM glycine buffer, pH 10.5 and subsequently identified by mass spectrometry.

The aim of this study was to implement in-house developed affinity chromatography protocol to the purification scheme of microsomal carbonyl-reducing enzymes presented by Škarydová[1]. A selected protein human liver fraction after initial Q-sepharose separation was subjected to affinity purification and three enzymes, DHRS1, RDH16 and 11β-HSD1 were isolated and identified. Further characterization of those enzymes could significantly extend our knowledge about membrane-bound carbonyl-reducing enzymes that metabolise xenobiotic substrates.

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POTENTIAL PROTECTIVE EFFECT OF NITRITES AND 3-MORPHOLINOSYDNONIMINE AGAINST ANTHRACYCLINE-INDUCED CARDIOTOXICITY *IN VITRO*

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Antracyclines (ANTs) are known for more than 50 years and they are still widely used for treatment of a number of hematological and solid malignancies.[1] ANTs inhibit tumor growth mainly by blocking the function of topoisomerase IIα.[2] Unfortunately, their clinical usefulness is hampered by their cardiotoxicity. Its pathogenesis is still poorly understood, although the reactive oxygen species-induced damage is generally believed to play the key role. As the treatment of ANT-induced cardiotoxicity is very difficult, preventive cardioprotective approaches are preferable.

Previously, the use of inorganic nitrites has been shown as cardioprotective in the myocardial ischemia-reperfusion injury probably due to NO release and/or S-nitrosylation of key myocardial proteins.[3] Since there is a lack of data on cardioprotective potential of these nitrites against ANT-induced cardiotoxicity, as well as their impact on ANT anticancer activity, we decided to assess these effects.

Cardiotoxicity and cardioprotection experiments were performed on H9c2 cells (derived from rat embryonal cardiomyoblasts) through incubation with daunorubicin (DAU; 1.2 μM), sodium nitrite and 3-morpholinosydnonimine (MSI; active metabolite of molsidomine) in wide range of concentrations and were assessed by MTT assay.

Sodium nitrite showed no significant own toxicity up to the 30 mM concentration, but it did not display any significant protective effect against DAU-induced cardiotoxicity. However, we noted some improvement of mitochondrial function using the JC1 probe staining.

MSI had no significant own cardiotoxic effect in a concentration range of 0.01–100 μM. In concentration of 100 μM, it showed significant protective effect against DAU-induced toxicity. However, this concentration of MSI also decreased anti-neoplastic effect of DAU on HL-60 leukemic cells.

This project is done in cooperation with Faculty of Medicine of the Charles University. Potential cardioprotective effects are also studied *in vivo* on well-established model of chronic anthracycline cardiotoxicity in rabbits.

The study was supported by the Internal Grant Agency of the Czech Ministry of Health No. NT/13457.

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AN INSIGHT INTO CELL DEATH PROCESS AFTER PHOTODYNAMIC TREATMENT OF CANCER CELLS WITH PHTHALOCYANINES AND THEIR AZA-ANALOGUES

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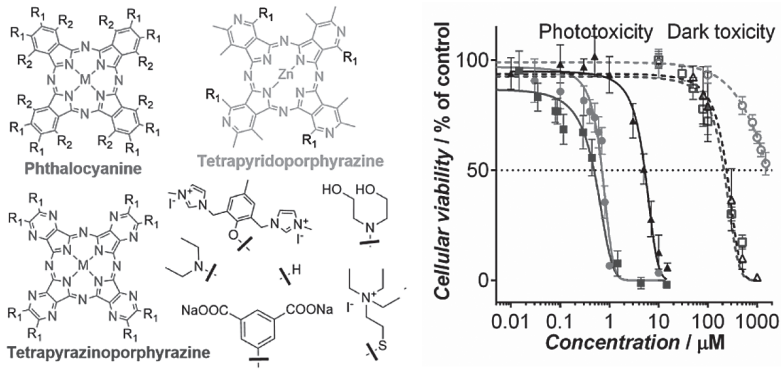
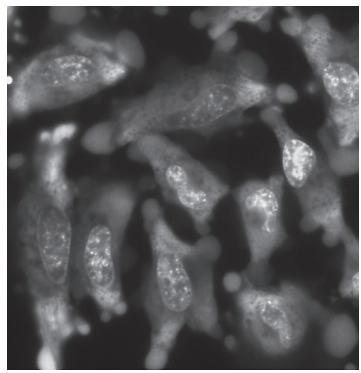
Photodynamic therapy (PDT), a simple but efficient way to treat localized and solid tumors, consists of three individual steps: (1) administration of photosensitizer (PS), (2) uptake of PS into tumor, (3) irradiation of tumor with visible red light. Such light penetrates deeper into tissues due to limited absorption caused by endogenous chromophores and water. PDT is efficient only in the presence of molecular oxygen. After irradiation of PS oxygen forms pathways leading to severe oxidative stress and subsequent damage to subcellular structures and even cell death. The major pathway of cell demise depends mostly on the structure of PS and its subcellular localization.

In this study we examined several water-soluble photosensitizers from the group of phthalocyanines, tetrapyrazinoporphyrazines and structurally new type of compounds – tetrapyridoporphyrazines. By the mean of fluorescence microscopy and specific fluorescent probes for distinct organelles, it was shown that all studied compounds are predominantly localized in lysosomes and/or endosomes. Uptake is relatively prompt in the first few hours, reaching plateau within 12 h for all studied compounds. After irradiation, PSs damage lysosomal membrane and are spread throughout the cell where they cause additional damage to other organelles. This leads to quick cell death with necrotic appearance, with activation of executioner caspases without previous activation of initial caspases probably by non-caspase proteases (with regard to lysosomal damage). Autophagy involvement in cell death and reactive oxygen species production in the entire process were studied as well.





Furthermore, several cationic water-soluble compounds showed promise as PSs for vascular-targeted photodynamic therapy on endothelial cells, where the PS administration immediately precedes irradiation.

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METABOLIC PATHWAYS OF ANTHELMINTIC DRUG MONEPANTEL IN SHEEP AND ITS PARASITE (*HAEMONCHUS CONTORTUS*)

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Monepantel (MOP) is a new anthelmintic drug intended for the treatment and control of gastrointestinal roundworms (nematodes) infection and associated disease in sheep. The aim of our study was to find out metabolic pathways of MOP in sheep *in vivo* and in its parasite *Haemonchus contortus ex vivo*. MOP biotransformation in two *H. contortus* strains with different sensitivity to anthelmintics was also compared. Ultra high-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) technique was used for the identification of MOP metabolites in ovine urine, faeces, and nematodes. MOP biotransformation study in sheep *in vivo* led to the identification of 13 MOP metabolites. The study of MOP biotransformation in *H. contorus ex vivo* revealed four MOP metabolites. The nitrile hydrolysis as a new biotransformation pathway in helminths *ex vivo* is reported here for the first time. Unlike sheep, *H. contorus* nematodes were not able to metabolize MOP *via* phase II biotransformation. Nematodes of resistant White river (WR) strain formed moretypes of MOP metabolites than nematodes of sensitive inbred susceptible Edinburgh (ISE) strain. Based on obtained results, schemes of metabolic pathways of MOP in sheep and nematodes are proposed.

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IRON METABOLISM OF THE TUMOUR-INITIATING CELLS

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Cancer is one of the major causes of death in the developed world. Most of the patients overcomes primary tumour, however, many patients progress to develop secondary tumours. The formation of the secondary tumour(s) is very likely caused by the tumour-initializing cells (TICs) or cancer stem cells (CSCs)[1,2]. TICs have distinct biological characteristics such as self-renewal capacity, predisposition to give rise to a tumour and higher resistance to clinically used chemotherapeutics or cytostatics. The presence of TICs within the tumour tissue may be the reason for the failure of many conventional cancer therapies because of their higher resistance to anti-cancer drugs and/or apoptotic stimuli. We are using a specific *in vitro* culture conditions which enable us to grow cancer cell as a small clumps of cells called "spheres", which are a model of TICs and exhibit higher expression of the "stemness" markers such as *ABCG2*, *CD44*, *CDH2* and *CD133*.

Iron is essential micronutrient involved in normal function of cellular metabolism, respiration, signalling and DNA repair[3]. It is known that there is higher iron requirement in proliferating cancer cells compared to normal ones but there are no data concerning

TICs. Our preliminary data show considerable differences in iron metabolism and in the expression of genes related to iron metabolism in TICs (*TFRC*, *FTH1*, *FTMT*, *QSOX1* and *TMPRSS6*). These cells also show higher sensitivity to iron chelators. It is well established that targeting iron metabolism with iron chelators can lead to apoptosis and death of cancer cells, while supplementing iron can block apoptosis induction and promote their growth. Thus understanding and manipulating the iron metabolism in TICs may affect the biology of TICs including TICs formation and maintenance and their sensitivity to apoptosis induction. We therefore aim to identify the differentially expressed genes related to iron metabolism in TICs and further analyse the role of these genes/proteins in the biology of TICs.

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NEUROPROTECTION BY IRON CHELATION IN CELLULAR MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease of humans; that affects dopamine(DA)-secreting neurons in the mesencephalon *substantia nigra*. Several cellular models were developed for *in vitro* study of PD. Among of them is the PC12 rat cell line derived from a pheochromocytoma of the rat adrenal medulla differentiable with neuron growth factor into DA-synthesizing sympathetic neurons. Neurotoxins, such as catecholamine 6-hydroxyDA (6-OHDA) may be used for experimental induction of cellular oxidative injury similar to damage of dopaminergic neurons in PD. This oxidative injury is tightly connected with local iron (Fe) disbalance, which may contribute to subsequent deterioration of damage in vicious circle. Therefore, Fe chelation seems to be appropriate approach to neuroprotection in PD.

The aim of present *in vitro* study was investigation of the role of DA and 6-OHDA in the PD. We focused on their spontaneous oxidation and toxic effects on PC12 cells and on the role of free Fe ions in connection with the possibility of neuroprotection by Fe chelation. Our experiments confirmed the ability of DA, 6-OHDA and their oxidized forms to induce

toxicity to PC12 cells and demonstrated the ability of strong cell-permeable Fe chelating agent salicylaldehyde isonicotioyl hydrazone (SIH) and its boronic ester pro-chelator BSIH to significantly suppress their toxicities.

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EVALUATION OF POTENTIAL REFERENCE GENES FOR REAL-TIME PCR STUDIES IN *HAEMONCHUS CONTORTUS*

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Data normalisation in Real-Time Polymerase Chain Reaction (qPCR) is a major step in gene quantification analysis. The reliability of any qPCR experiment can be improved by including an invariant endogenous control (reference gene) in the assay to correct for sample to sample variations in qPCR efficiency and errors in sample quantification. To identify reliable reference genes for toxicological studies in *Haemonchus contortus*, 11 potential genes were selected as candidates to evaluate their expression stabilities under experimental conditions. The relative transcription levels of genes encoding glyceraldehyde-3P-dehydrogenase (GAPDH), fatty acid and retinol binding protein (FARB), superoxide dismutase (SODc), large subunit ribosomal protein 2 (RP2), RNA polymerase II (large subunit gene) (AMA-1), β-actin (ACT), troponin T (TT), nuclear cap-binding protein subunit 2-like (NCBP), 18S ribosomal RNA (18S), elongation factor (EF-2) and phosphofructokinase (PFK) were quantified and compared in *H. contortus* adult worms of susceptible (ISE) and multi-resistant (WR) strain of both genders. The stability of these genes was analysed also in worms exposed to sub-lethal concentration of albendazole for 24 hours in liquid culture compared to non-exposed adult worms as a negative control. Expression stabilities of candidate genes were analysed using 4 independent evaluating approaches (BestKeeper, NormFinder, geNorm and the comparative delta-Ct method) followed by a comprehensive method. Our results showed that SODc and GAPDH genes were the most stable and can be used as reference genes for gene expression studies in different strains, genders and treatment conditions of *H. contortus*.

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THE EFFECT OF CATECHINS ON SELECTED BIOTRANSFORMATION ENZYMES

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Catechins are important group of naturally occurring flavonoids and they are the major polyphenolic compounds of green tea. Their ability to scavenge reactive oxygen species makes them valued compounds with a wide range of use in the health care. Consumption of dietary supplements with high concentrations of flavonoids is still increasing, but in addition to positive effects on human health it can also have side effects, especially influence drug metabolizing enzymes and thus may affect pharmacokinetic as well as pharmacodynamic profiles of co-administered drugs.

A human intestinal Caco-2 cell line is widely used model of intestinal absorption and metabolism of drugs and other substances more than twenty years. Cytochromes P450 were the most studied enzymes but our research is focused on modulation of enzymes of Phase II of drug biotransformation. We determined activity of four conjugation enzymes in this intestinal *in vitro* model. First of all, we defined differences between proliferative and differentiated Caco-2 cells and then their response to green tea catechins. We chose polyphenon 60 and epigallocatechin-3-gallate (EGCG) as a representative compounds.

We observed higher activity of sulfotransferase (SULT) in the proliferative cells than in the differentiated cells but there was no effect of catechins. Similar situation is with glutathione S-transferase (GST). Catechins had no effect on GST activity but slightly increased activities were measured in cytosolic fraction of differentiated cells unlike proliferating cells. Proliferating Caco-2 cell line showed an increase in catechol-O-methyltransferase activity after 96 hours of incubation with polyphenon 60 and EGCG, but after 24 hours of incubation the activity was reduced. The activity of UDP glucuronosyltransferase was not detected in microsomal fraction of all types of Caco-2 cells.

This study helped us to optimize Caco-2 cell line as *in vitro* model to investigate the effects on intestinal conjugation enzymes. Based on the results, normal consumption of green tea seems to be safe but extremely high doses of green tea extracts in dietary supplements could present some risk.

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DESCRIPTION AND EVALUATION OF DHRS8 BIOCHEMICAL PROPERTIES

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Human dehydrogenase/reductase (SDR family) member 8 (DHRS8) is membrane-bound enzyme belonging to the Short-chain dehydrogenases/reductases (SDR) superfamily. Members of SDR participate in metabolism of various endogenous and xenobiotic compounds and they also play an important role in serious diseases such as cancer or diabetes mellitus[1]. Nevertheless, there are still many uncharacterized or poorly characterized enzymes. One of them is DHRS8, also known as SDR16C2, 17β-HSD11, Pan1b or retSDR2.

DHRS8 was described for the first time as an enzyme with weak dehydrogenase activity to estradiol[1]. Further, catalytic activity toward 5α-androstane-3α, 17β-diol, subcellular localization and tissue expression at the mRNA level have been described so far[2–5]. On the other hand, a lot of information about the protein itself and particularly about its enzymatic activity is still lacking.

The aim of this study was to provide more detailed characterization of DHRS8 protein. Recombinant form of human DHRS8 was prepared using Bac-to-Bac Baculovirus expression system and Sf9 insect cells. It was demonstrated that the N-terminus of DHRS8 is plunging into the membrane and C-terminus containing active site is oriented into cytosol. Further, strong expression of DHRS8 protein in human adrenals, liver and small intestine was detected. According to obtained results DHRS8 is NAD^+-dependent oxidase that is involved in the metabolism of testosterone, estradiol and all-trans-retinol and in biotransformation of anabolic steroids such as methyltestosteron and nandrolon. This new knowledge about DHRS8 protein may lead to a better understanding of its role in the human organism.

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SECTION OF CLINICAL AND SOCIAL PHARMACY

JEWISH PHARMACISTS IN PHARMACIES IN THE INTERWAR CZECHOSLOVAKIA AND THEIR LIVES DURING WORLD WAR II

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In my contribution, which is part of my dissertation, I describe the status and fates Jewish pharmacists in the interwar Czechoslovakia and their fate during Second World War.

The term includes pharmacists, pharmacy owners and heaves (czech term was provisor, it means pharmacists, who cared for a pharmacy for pharmacy owners) and also employed (Czech term was kondicinující) pharmacists . After the establishment of Czechoslovakia in 1918 applicants could study pharmacy at the Czech Charles University and at the German University in Prague in 1920 the Faculty of Arts and from 1920 the Faculty of Science. If the student graduated in 1918, then the study of pharmacy would be recognized in other parts of the former Austro-Hungarian monarchy.

The turn of the twenties and thirties at both universities meant a significant increase in the representation foreign students, particularly from Poland and Romania. In the mid-thirties number of foreign students of pharmacy began to fall again.

The largest number of Jewish pharmacists was located on the territory of Carpathian Ruthenia, followed by Slovakia and ultimately historic lands – Bohemia, Moravia and Silesia. Most of them worked as pharmacy employees. But among the Jewish pharmacists were owners of pharmacy, too.

Some of them worked in pharmacies of their fathers or relatives, particularly in the east of the Republic. The Munich Agreement, the Second Republic and the beginning of World War II meant a tragedy for Jewish pharmacists . Only a few individuals of them emigrated in time. Largely finished in the Terezin ghetto (where it served as a hospital pharmacy), some killed right in the ghetto, some in the subsequent concentration camps (Dachau, Auschwitz and others). Pharmacies of Jewish owners were Aryanized. The fates of the Jews , who lived in Slovakia and Hungarian Kingdom (occupied Carpathian Ruthenia and south of Slovakia) were somewhat different, but their fate was no less tragic. Only a few individuals of them returned to post-war Czechoslovakia, but any of the them unnecessarily demanded an aryanised Estate.

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DELAYS IN TUBERCULOSIS DIAGNOSIS AND TREATMENT IN A HIGH-BURDEN COUNTRY: RISK FACTORS IN PATIENTS WITH DRUG-SUSCEPTIBLE AND MULTIDRUG-RESISTANT TUBERCULOSIS

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Low case detection rate is a major obstacle to effective management of tuberculosis (TB) in Uzbekistan. Enhancing early diagnosis is a priority to reduce TB transmission.

We aimed to identify factors associated with patient and diagnostic delays by comparing characteristics of drug-susceptible TB patients with multidrug-resistant TB patients. We examined association between the extent of delay and patient characteristics, including HIV-status and socio-demographic variables.

A cross-sectional survey of 600 pulmonary TB patients was conducted in four hospitals in Tashkent and Nukus, between August 2013 and January 2014 using an adopted version of the WHO questionnaire. The characteristics associated with patient and diagnostic delays were evaluated using univariable and multivariable logistic regression analyses.

The median patient delay for all patients included in the study was 27 days (IQR, 6–62 days), the median health system delay of 7 days (IQR, 1–32 days), and the median total delay was 50 days (IQR, 22–92 days). Factors associated with longer delay included cough, self-medication, seeking initial care from a primary or a private health care facility, and HIV infection.

TB diagnostic and treatment delay should be reduced to the least possible time interval. There is a need to decrease TB stigma and promote public awareness of TB curability and the importance of early referral to health services. A high index of suspicion of TB should be maintained in public and private practitioners and an appropriate diagnostic work-up should be performed.

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PREVALENCE OF DRUG-RELATED PROBLEMS AMONG PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background: In most industrialized countries chronic kidney disease (CKD) is a major public health problem, with particularly high social and human costs. In the Czech Republic, approximately 8,000 people were hospitalized with chronic kidney disease in 2013. Patients suffering from renal dysfunction have often multiple medical conditions either as a cause or as a consequence of their renal disease. Impairment of the kidney function alters the pharmacokinetics of many prescribed medications. Therefore doses of drugs metabolized or eliminated by the kidney must be adjusted to renal function.

Objective: The objective of this study was to determine the nature and extent of drug-related problems (DRPs) in renally compromised patients and the potential for clinical pharmacists to contribute towards resolving or preventing some of these DRPs.

Methods: This prospective, descriptive, observational study has been carrying out at the Institute of Clinical and Experimenal Medicine, the Department of Nephrology, Prague since 2012. Patients with chronic kidney disease and renal transplant patients hospitalized in this department were included. Patterns of the DRPs were identified using an adapted the Pharmaceutical Care Network Europe (PCNE) classification of the DRPs.[1]

Results: Anti-infective agents (34%) were the most common therapeutic classes of medications implicated in causing DRPs. The most common DRP identified in this classes was overdose (48%). Pharmacists provided 202 therapeutic drug monitoring (aminoglycosides, vancomycin) to 1,300 inpatients.

Conclusion: Renal compromised patients especially renal transplant patients have an increased risk of infection because they use immunosuppressive therapy. Monitoring of their therapy especially of the anti-infective agents is the principal task of pharmacist.

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ANALYSIS OF MEDICATION ADHERENCE AND USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES AMONG CHRONICALLY TREATED PATIENTS

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Monitoring of medication adherence should always be part of the routine care of patients with chronic diseases. It is also necessary to take into account other patients' attitudes to their treatment as well as overall health. There are various models analyzing factors influencing adherence, however, fewer studies focused on a relationship between use of

complementary and alternative medicine (CAM) and medication adherence have been conducted. The aim of the present study is to determine the prevalence of CAM among chronically treated patients and analyze the adherence to the conventional therapy (CT). We addressed patients who attended Czech pharmacies in the period from April to December 2014 and were treated with any prescribed drug for at least 3 months. Patients were asked to fill in the questionnaire including items about CAM use and the Czech validated instruments evaluating adherence. Data obtained from 548 respondents were analyzed by descriptive statistics. Preliminary results of 344 respondents (median of age 57 ± 16.3 years; 235 females; mean number of prescribed drugs per patient was 3.7) showed that 253 (73.5%) patients reported high adherence to CT. Two hundreds and eighteen (63.4%) respondents reported using any CAM (mean number of CAM types per patient was 1.7) and the majority of them (71%) were from the group of patients with high adherence to CT. Apart from 2, all CAM users considered CAM as a supplement to CT. In conclusion, our results predicted that active involvement of the patients in CAM may reflect their positive approach in care of their own health which could be associated with better long-term medication adherence.

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ADHERENCE TO CALCIUM AND VITAMIN D SUPPLEMENTATION IN POSTMENOPAUSAL WOMEN – BASELINE AND FOLLOW-UP

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Supplementation with calcium and vitamin D significantly increases the effect of antiresorptive medication for osteoporosis and the overall treatment effect is not simply the sum of the individual effects. While adherence to bisphosphonates is at least partially monitored in clinical practice, adherence to supplementation therapy escapes attention.

The objective of the study was to evaluate treatment adherence to calcium and vitamin D in a sample of postmenopausal women (over 55 years) treated for osteoporosis or osteopenia in clinical practice, further, to monitor adherence over time and to compare adherence which was found out by 3 different methods.

This was a observational study. Data were obtained in osteocenter in University hospital in Hradec Králové (Department of Clinical Biochemistry). Adherence was measured by a unique combination of methods: electronic bottles type Medication Events Monitoring System (MEMS), self-reported questionnaire (QNR) and tablet count (TC). The measuring of the adherence covered a period of 90 days in each patient. Adherence was measured in the same patient cohort two times – 1st round in 2013 and 2nd round in 2014. High adherence is defined as medication possession ratio of 75% to 100%.

The mean age of the sample (N = 26) was 71 years). Based on MEMS 54% of the patients were highly adherent. Adherence measured by MEMS in the 1st round correlates

with adherence measured in the 2nd round. Based on MEMS 65% of the patients showed stability in adherence. Results from QNR and TC did not correlate with the results from MEMS.

Based on MEMS the overall compliance was 69% in the 1st round and 64% in the 2nd round. More than half of the patients were highly adherent. Most patients showed stability in adherence. QNR and TC highly overestimated adherence compared to MEMS.

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