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Social Happenings

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

HORSTKOTTE, B., CHOCHOLOUŠ, P., BEŠŤÁK, O., SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Kr*á*lov*é*,
Charles University, Czech Republic

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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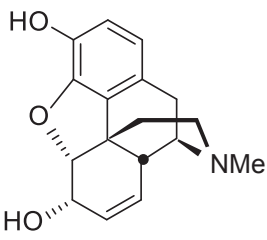
BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

STUDIES TOWARDS THE SYNTHESIS OF MORPHINE PRECURSOR

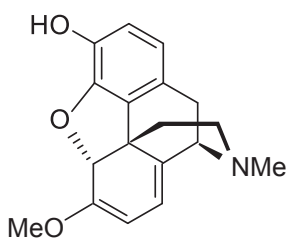
GHAVRE, M.,[1] VARGHESE, V.,[2] HUDLICKÝ, T.[2]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Chemistry Department and Centre for Biotechnology, Brock University, St. Catharines, ON L2S 3A1, Canada
e-mail: ghavrem@faf.cuni.cz

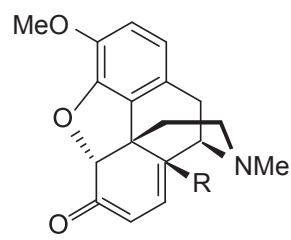
Morphinans are known to be excellent analgesics since centuries. Especially, morphine is referred to as a gold standard in the management of severe pain. These opiate structures have continued to attract the organic chemists for their challenging syntheses. A plethora of publications have been reported 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

HOLAS, O., SLOVÁK, F., DOLEŽAL, M.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: ondrej.holas@faf.cuni.cz

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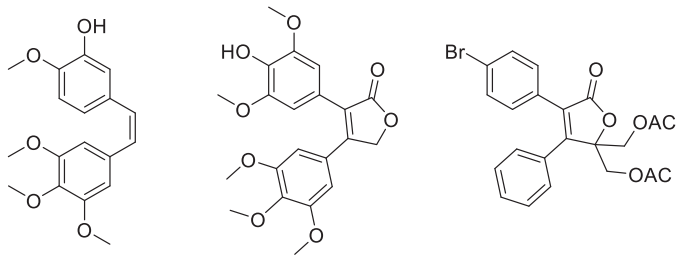
SYNTHESIS OF NOVEL A,B-DIPHENYL FURANONES AS POTENCIAL ANTITUMOROUS AND ANTIMICROBIAL AGENTS

HORKÝ, P.,[1] VORÁČOVÁ, M.,[1] VACEK, J.,[2] POUR, M.[1]

[1] Department of Organic and Inorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Medical Chemistry and Biochemistry, Palacky University in Olomouc, Czech Republic
e-mail: horkyp@faf.cuni.cz

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

HROCH, L.,[1,2] BENEK, O.,[2,3] AITKEN, L.,[4] GUNN-MOORE, F.,[4] MUSÍLEK, K.[5]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] University Hospital, Biomedical Research Centre, Hradec Králové, Czech Republic
[3] Department of Toxicology, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[4] School of Biology, Medical and Biological Sciences Building, University of St. Andrews, North Haugh, St. Andrews KY16 9TF, United Kingdom
[5] Department of Chemistry, Faculty of Science, University Hradec Králové, Hradec Králové, Czech Republic
e-mail: lukashroch@gmail.com

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.

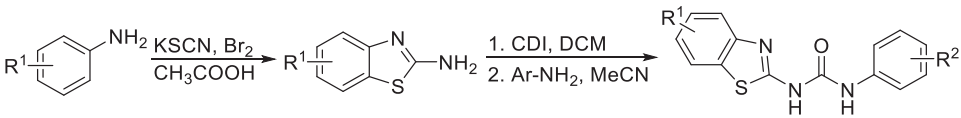


Fig. 1. Preparation of substituted benzothiazolylureas.

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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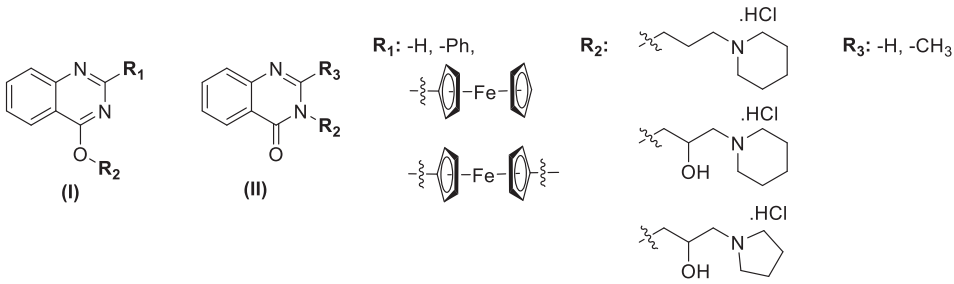
PREPARATION OF QUINAZOLINE COMPOUNDS WITH BRONCHODILATORY ACTIVITY

HRUŠKOVÁ, Z. R.,[1] JEŽKOVÁ, V.,[1] VOPRŠÁLOVÁ, M.,[2] ŠPULÁK, M.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Rrepublic
e-mail:hruskovz@faf.cuni.cz

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

JANĎOUREK, O.,[1] PATEROVÁ, P.,[2] KRÁLOVÁ, K.,[3] DOLEŽAL, M.[1]

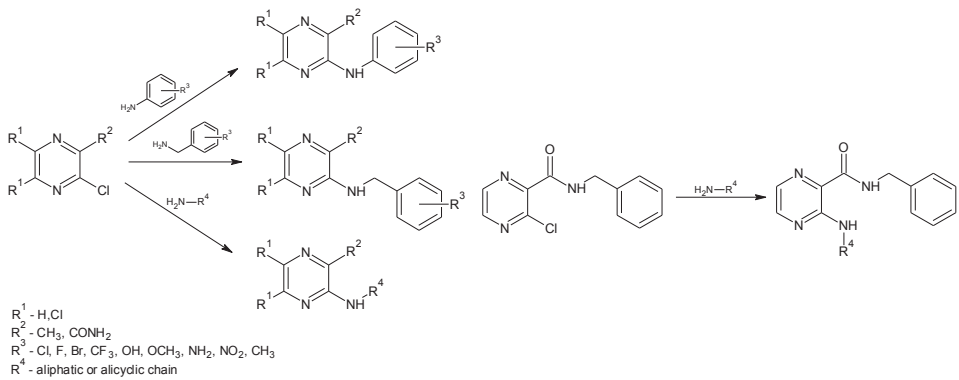
[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Slovakia
e-mail: jando6aa@faf.cuni.cz

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]

[1] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences in Hradec Králové, University of Defence, Brno, Czech Republic
[2] Biomedical Research Centre, University Hospital, Hradec Králové, Czech Republic
[3] Department of Chemistry, Faculty of Science, University of Hradec Králové, Czech Republic
e-mail: korabecny.jan@gmail.com

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.

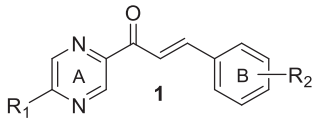
The work was supported by the Grant Agency of the Czech Republic (No. P303/11/1907), by Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011) and by MH CZ – DRO (University Hospital Hradec Králové, No. 00179906).

HALOGENATED PYRAZINE-BASED CHALCONES AS POTENTIAL ANTIMICROBIAL AGENTS

KUČEROVÁ-CHLUPÁČOVÁ, M., VYŠKOVSKÁ-TYLLOVÁ, V., RICHTEROVÁ-FINKOVÁ, L., OPLETALOVÁ, V.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: marta.kucerova@faf.cuni.cz

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

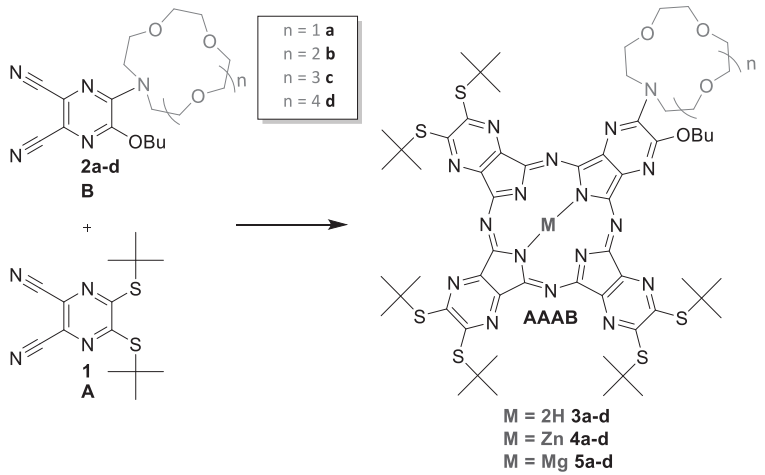
LOCHMAN, L.,[1] ŠVEC, J.,[1] ZIMČÍK, P.,[1] NOVÁKOVÁ, V.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: lochmanl@faf.cuni.cz

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.

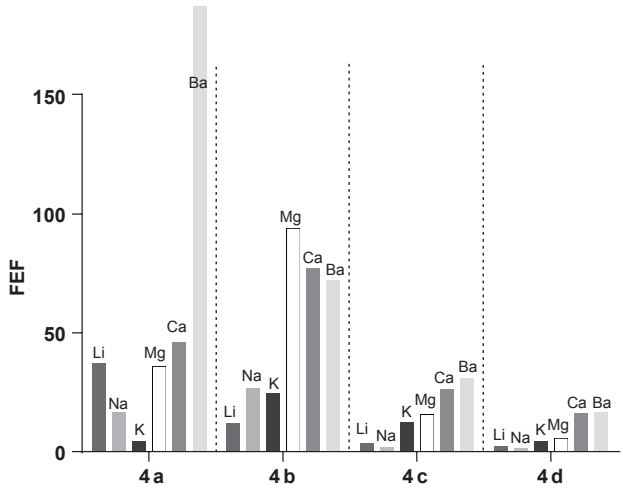


Fig. 1. Fluorescence titration experiment.

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)

MIKUŠEK, J., MATOUŠ, P., KOLENIČ, M., JANOUŠEK, M., POUR, M.

Department of Organic and Inorganic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: mikuj6aa@faf.cuni.cz

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.

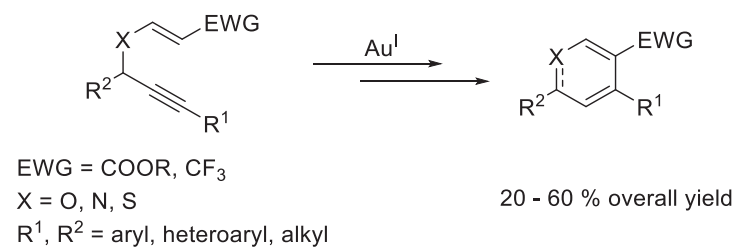

Fig. 1.

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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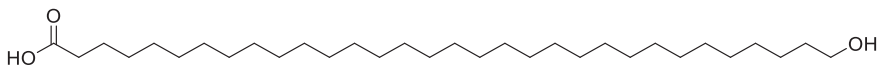
COMPLETE SYNTHESIS OF ULTRA LONG HUMAN SKIN CERAMIDES

OPÁLKA, L., VÁVROVÁ, K.

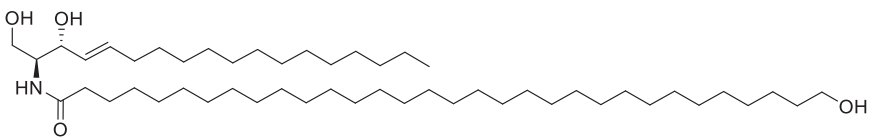
Department of Inorganic and Organic Chemistry (Skin Barrier Research Group), Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: opall6aa@faf.cuni.cz

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.

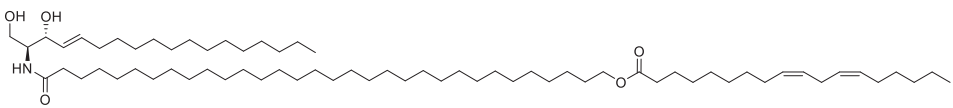
The aim of our work was to prepare ceramides with ultra long chain (also known as acylceramides or ceramides of the O and EO classes), because these molecules are not commercially available, but they are essential for the proper skin barrier function.



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.

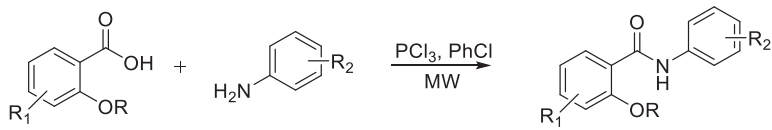
This work was supported by the Czech Science Foundation (13-23891S) and by Charles University (SVV 260 062).

RECENT OUTCOMES OF NOVEL MODIFICATIONS ON THE STRUCTURE OF SALICYLANILIDES

PARASKEVOPOULOS, G., MONTEIRO, S. E. A,, VINŠOVÁ, J.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: paraskeg@faf.cuni.cz

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

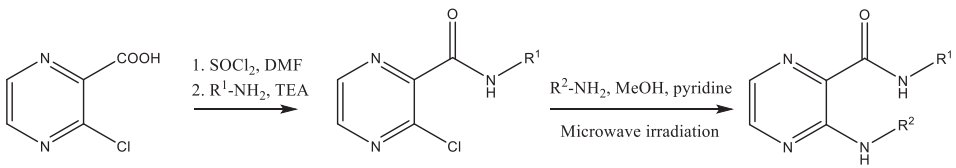
SEMELKOVÁ, L.,[1] ZÍTKO, J.,[1] PATEROVÁ, P.,[2] DOLEŽAL, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: semelkol@faf.cuni.cz

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

VALÁŠKOVÁ, L.,[1] ROH, J.,[1] KARABANOVICH. G.,[1] NĚMEČEK, J.,[1] STOLAŘÍKOVÁ, J.,[2] VÁVROVÁ, K.,[1] KLIMEŠOVÁ, V.,[1] HRABÁLEK, A.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Bacteriology and Mycology, Laboratory for dg. of mycobacteries, Regional Institute of Public Health, Ostrava, Czech Republic
e-mail: valaskl1@faf.cuni.cz

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

VAŇÁSKOVÁ. B.,[1] ZÍTKO, J.,[1] PATEROVÁ, P.,[2] DOLEŽAL, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Clinical Microbiology, University Hospital in Hradec Králové, Czech Republic
e-mail: barbora.servusova@faf.cuni.cz

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.

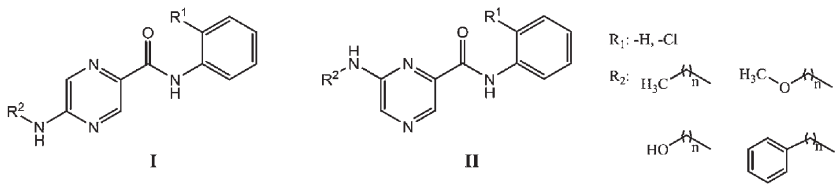

Fig. 1. Final structures **I** and **II**.

The publication is co-financed 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 and by the European Social Fund and the state budget of the Czech Republic. Project No. CZ.1.07/2.3.00/30.0022. This study was also supported by the Grant Agency of the Charles University (B-CH/710312), by the Ministry of Health of Czech Republic (IGA NT 13346) and by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 062).

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

CIDLINA, A.,[1] NOVÁKOVÁ, V.,[2] ZIMČÍK, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: CIDLA6AA@faf.cuni.cz

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.

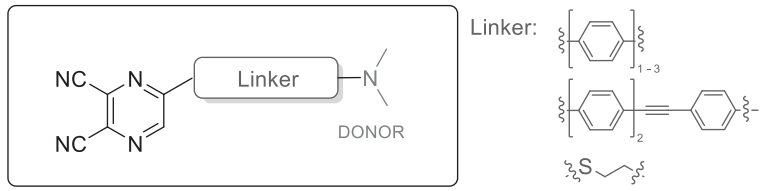

Fig. 1. Pyrazines with π-extended linkers.

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)

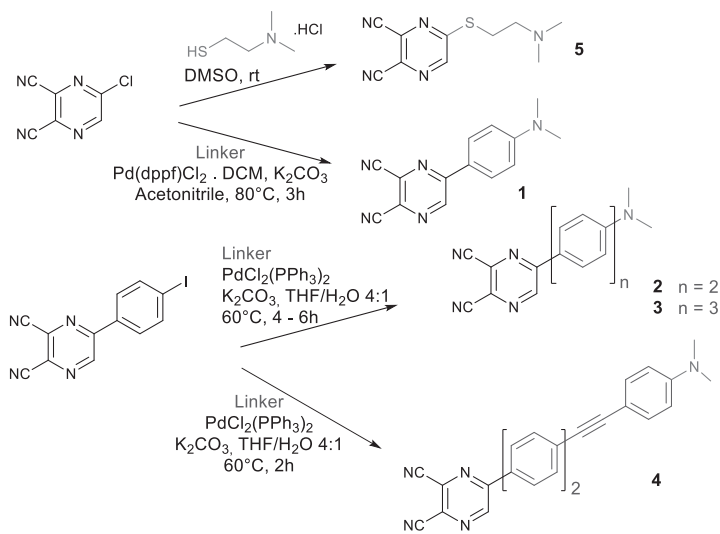

Fig. 2. The synthetic pathway for prepared precursors.

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

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EVALUATION OF TRANSDERMAL PERMEATION ENHANCERS BASED ON TERPENES

KOPEČNÁ, M.,[1] MACHÁČEK, M.,[2] ROH, J.,[1] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: kopem7ba@faf.cuni.cz

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

KOVÁČIK, A.,[1] PULLMANNOVÁ, P.,[1] ZBYTOVSKÁ, J.,[1,2]
PALÁT, K.,[1] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Chemical Technology, Prague, Czech Republic
e-mail: kovacika@faf.cz

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

ŠVEC, J.,[1] NOVÁKOVÁ, V.,[2] ZIMČÍK, P.,[1] STUZHIN, P. A.[3]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Ivanovo State University of Chemical Technology, RF-153460 Ivanovo, Russia
e-mail: svecjan2@faf.cuni.cz

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

VOSÁTKA, R., KRÁTKÝ, M., VINŠOVÁ, J.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: vosatkar@faf.cuni.cz

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

AUFARTOVÁ, J.,[1,2] MARTÍN-FERNÁNDEZ, B.,[2] SÁNCHEZ-PANIAGUA LÓPEZ, M.,[2] SOLICH, P.,[1] LÓPEZ-RUIZ, B.[2]

[1] Department of Analytical Chemistry, Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic
[2] Departamento de Química Analítica, Facultad de Farmacia, Universidad Complutense, Pz. Ramón y Cajal s/n, 28040 Madrid, Spain
e-mail: aufartova@gmail.com

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

BUREŠ, J.,[1] FILIPSKÝ, T.,[2] MLADĚNKA, P.,[2] KLIMEŠ, J.,[1] KOVAŘÍKOVÁ, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: burej7ba@faf.cuni.cz

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

ČERVINKOVÁ, B.,[1,2] KUJOVSKÁ- KRČMOVÁ, L.,[1,2] HUTNÍKOVÁ, M.,[1] SOLICHOVÁ, D.,[2] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, University Hospital, Hradec Králové, Czech Republic
e-mail: cervb7aa@faf.cuni.cz

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

GOTTVALD, T.,[1] PILAŘOVÁ, V.,[1] JAKUBEC, P.,[1] BENEŠOVÁ, K.,[2] BĚLÁKOVÁ, S.,[2] NOVÁKOVÁ, L.,[1] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Research Institute of Brewing and Malting, Malting Institute Brno, Brno, Czech Republic
e-mail: Gottvalt@faf.cuni.cz

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

JAVORSKÁ, L.,[1,2] KUJOVSKÁ- KRČMOVÁ, L.,[1,2] KUČEROVÁ, K.,[1]
SOLICHOVÁ, D.,[2] KAŠKA, M.,[3] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, University Hospital, Hradec Králové, Czech Republic
[3] Academic Department of Surgery, Medical Faculty in Hradec Králové, Charles University,
University Hospital, Hradec Králové, Czech Republic
e-mail: javorskl@faf.cuni.cz

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

JENČO, J.,[1,2] KUJOVSKÁ- KRČMOVÁ, L.,[1,2] NETRIOVÁ, J.,[3] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, University Hospital in Hradec Králové, Czech Republic
[3] Department of Clinical Biochemistry and Hematology, St. Michaels Hospital
in Bratislava, Slovak Republic
e-mail: jencojaf@cuni.cz

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

KASALOVÁ, E.,[1,2] AUFARTOVÁ, J.,[1,2] KUJOVSKÁ- KRČMOVÁ, L.,[1,2]
SOLICHOVÁ, D.,[2] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] III. Internal Gerontometabolic Clinic, University Hospital, Hradec Králové, Czech Republic
e-mail: kasalove@faf.cuni.cz

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

KLIVICKÝ, M., KUČERA, R., KLIMEŠ, J.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: klivm6aa@faf.cuni.cz

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

LHOTSKÁ, I., ŠATÍNSKÝ, D., HAVLÍKOVÁ, L., SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: lhotski@faf.cuni.cz

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

LOPES, E.,[1] ZELENÁ, L.,[2] SKLENÁŘOVÁ, H.,[2] SEGUNDO, M.,[3] PÁVEK, P.[4]

[1] Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal
[2] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
[4] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: ea_lopes@hotmail.com

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

NEJEDLÝ, T., KLIMEŠ, J.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: nejet6aa@faf.cuni.cz

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

STARIAT, J.,[1] KLIVICKÝ, M.,[1] KUČERA, R.,[1] PAWLISZYN, J.,[2] KOVAŘÍKOVÁ, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Chemistry, University of Waterloo, Waterloo, Canada
e-mail: jan.stariat@faf.cuni.cz

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

SVOBODA, P.,[1] COMBES, A.,[2] PETIT, J.,[2] NOVÁKOVÁ, L.,[1] PICHON, V.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Analytical, Bioanalytical Sciences and Miniaturization (LSABM), UMR CBI 8231 ESPCI-ParisTech/CNRS, PSL Research University, ESPCI-ParisTech, Paris, France
e-mail: svobodp1@faf.cuni.cz

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

ŚWIT, P.,[1,2] CHOCHOLOUŠ, P.,[2] WIECZOREK, M.,[1] KOZÁK, J.,[1] SOLICH, P.,[2] KOŚCIELNIAK, P.[1]

[1] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Poland
[2] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: swit.pawel@gmail.com

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

Šesták, V.,[1] Stariat, J.,[1] Mičuda, S.,[2] Chládek, J.,[2] Štěrba, M.,[2] Roh, J.,[3] Bureš, J.,[1] Richardson, D. R.,[4] Kovaříková, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[3] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[4] Department of Pathology and Bosch Institute, University of Sydney, Australia
e-mail: sestv7aa@faf.cuni.cz

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

ŠRÁMKOVÁ, I., HORSTKOTTE, B., SKLENÁŘOVÁ, H., ŠATÍNSKÝ, D., CHOCHOLOUŠ, P., SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: srami5aa@faf.cuni.cz

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

VLČKOVÁ, H.,[1] SVOBODA, P.,[1] NOVÁK, O.,[2] NOVÁKOVÁ, L.,[1] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic
e-mail: vlckh3aa@faf.cuni.cz

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

ZAHÁLKA, L.,[1] ZAHÁLKOVÁ, O.,[1] MATYSOVÁ, L.,[1] KLOVRZOVÁ, S.,[2,3] ŠKLUBALOVÁ, Z.,[2] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Hospital Pharmacy, University Hospital in Motol, Prague, Czech Republic
e-mail: zahal6aa@faf.cuni.cz

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

ZELENÁ, L.,[1] ŘEZNÍČEK, J.,[2] ČEČKOVÁ, M.,[2] SKLENÁŘOVÁ, H.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: zelenal@faf.cuni.cz

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

BÁRTA, P.,[1,2] STERBERG, J.,[2] SPIEGELBERG, D.,[2] NESTOR, M.,[2] TREJTNAR, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biomedical Radiation Sciences, Uppsala University, Sweden
e-mail: pavel.barta@faf.cuni.cz

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)

CARAZO, A.,[1] SMUTNÝ, T.,[1] HYRŠOVÁ, L.,[1] BERKA, K.,[2] PÁVEK, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Palacký University in Olomouc, Czech Republic
e-mail: carazofa@faf.cuni.cz

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

ČEČKOVÁ, M.,[1] NEUMANOVÁ, Z.,[1] ČERVENÝ, L.,[1] GLAZIER, J.,[2] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Maternal and Fetal Health Research Centre, Institute of Human Development, University of Manchester, St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester M13 9WL, UK
e-mail: martina.ceckova@faf.cuni.cz

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

ČEPA, A.,[2] RÁLIŠ, J.,[2] PAVELKA, A.,[3] SEIFERT, D.,[2] SIEGLOVÁ, I.,[4] KRÁL, V.,[4] POLÁŠEK, M.,[2] PAUROVÁ, M.,[5] LEBEDA, O.,[2] LÁZNÍČEK, M.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Radiopharmaceuticals, Nuclear Physics Institute, The Academy of Science, Czech Republic, v.v.i.
[3] ÚJV Řež a. s., Czech Republic
[4] Laboratory of Structural Biology, Institute of Molecular Genetics, The Academy of Science, Czech Republic, v.v.i.
[5] Department of Inorganic Chemistry, Faculty of Science, Charles University, Czech Republic
e-mail: cepaa7aa@faf.cuni.cz

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

ČERVENÝ, L., NEUMANOVÁ, Z., KARBANOVÁ, S., HAVLOVÁ, I., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: cervenyl@faf.cuni.cz

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

ČÍHALOVÁ, D., ČEČKOVÁ, M., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: cihad6aa@faf.cuni.cz

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

FIKROVÁ, P., VAŘEJČKOVÁ, M., JEŽKOVÁ, K., RATHOUSKÁ, J., DOLEŽELOVÁ, E., NĚMEČKOVÁ, I., NACHTIGAL, P.

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: petra.fikrova@faf.cuni.cz

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

FILIPSKÝ, T.,[1] KARLÍČKOVÁ, J.,[2] ŘÍHA, M.,[1] MACÁKOVÁ, K.,[2]
HRDINA, R.,[1] MLADĚNKA, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: tomas.filipsky@faf.cuni.cz

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

HYRŠOVÁ, L.,[1] GERBAL-CHALOIN, S.,[2] PÁVEK, P.[1]

[1] Department of Pharmakology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Hepatic Differentiation of Stem cells and Biotherapy of Liver Disseases, INSERM U1040, Institut de Recherche en Biothérapie, Montpellier, France
e-mail: hyrsl7aa@faf.cuni.cz

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

JEŽKOVÁ, K.,[1] RATHOUSKÁ, J.,[1] NĚMEČKOVÁ, I.,[1] SERWADCZAK, A.,[2] CHLOPICKI, S.,[2] NACHTIGAL, P.[1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland
e-mail: jezkk6aa@faf.cuni.cz

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

KADOVÁ, Z.,[1,2] DOLEŽELOVÁ, E.,[3] CERMANOVÁ, J.,[2] ŠTAUD, F.,[1] MIČUDA, S.[2]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[3] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: kadoz6aa@faf.cuni.cz

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

MANDÍKOVÁ, J., VOLKOVÁ, M., NAVRÁTILOVÁ, L., PÁVEK, P., TREJTNAR, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: mandj3aa@faf.cuni.cz

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

NAJMANOVÁ, I., VOPRŠÁLOVÁ, M., MLADĚNKA, P.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: najmanoi@faf.cuni.cz

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

NAVRÁTILOVÁ, L., MANDÍKOVÁ, J., TREJTNAR, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: navratl2@faf.cuni.cz

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

NĚMEČKOVÁ, I.,[1] LENČOVÁ, O.,[2] JIRKOVSKÝ, E.,[2] ADAMCOVÁ, M.,[3] GERŠL, V.,[2] NACHTIGAL, P.,[1] ŠTĚRBA, M.[2]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[3] Department of Physiology, Faculty of Medicine in Hradec Králové, Czech Republic
e-mail: ivana.nemeckova@faf.cuni.cz

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

NEUMANOVÁ, Z.,[1] ČERVENÝ, L.,[1] ČEČKOVÁ, M.,[1] GREENWOOD, S. L.,[2] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Maternal and Fetal Health Research Centre, Institute of Human Development, University of Manchester, St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester M13 9WL, UK.
e-mail: neumz6aa@faf.cuni.cz

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

RATHOUSKÁ, J.,[1] JEŽKOVÁ, K.,[1] NĚMEČKOVÁ, I.,[1] SERWADCZAK, A.,[2] BERNABEU, C.,[3] LOPEZ-NOVOA, J. M.,[4] CHLOPICKI, S.,[2] NACHTIGAL, P.[1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland
[3] Centro de Investigaciones Biologicas, Madrid, Spain
[4] Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain
e-mail: jana.rathouska@faf.cuni.cz

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

ROZKYDALOVÁ, L.,[1] VINCENT, L. A.,[2] CUQ, P.,[2] MIČUDA, S.,[3] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Laboratoire de Toxicologie du Médicament, Institut des Biomolécules Max Mousseron (UMR5247), UFR des Sciences Pharmaceutiques et Biologiques, Université Montpellier I, 15 avenue Charles Flahault, BP14491, 34093 Montpellier Cedex 05, France
[3] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
e-mail: lucka.rozkydalova@gmail.com

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

ŘEZNÍČEK, J., ČEČKOVÁ, M., NEUMANOVÁ, Z., ČERVENÝ, L., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: reznj7ba@faf.cuni.cz

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

ŘÍHA, M.,[1] MACÁKOVÁ, K.,[2] KARLÍČKOVÁ, J.,[2] FILIPSKÝ, T.,[1] HORŇASOVÁ, V.,[1] HRDINA, R.,[1] MLADĚNKA, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: riham6aa@faf.cuni.cz

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

SMUTNÝ,T.,[1] FERNÁNDEZ, A. C.,[1] ŠPULÁK, M.,[2] POUR, M.,[2] PÁVEK, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: smutt6aa@faf.cuni.cz

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

ŠEPSOVÁ, V.,[1] ŽĎÁROVÁ, J. K.,[1] SOUKUP, O.[1,2]

[1] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences Hradec Králové, University of Defence, Brno, Czech Republic
[2] Biomedical Research Center, University Hospital Hradec Králové, Czech Republic
e-mail: vsepsova@gmail.com

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*

VAŘEJČKOVÁ, M., ZEMÁNKOVÁ, L., FIKROVÁ, P., JEŽKOVÁ, K., RATHOUSKÁ, J., NĚMEČKOVÁ, I., NACHTIGAL, P.

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: varem7aa@faf.cuni.cz

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

CHLEBEK, J.,[1] NOVÁK, Z.,[2] OPLETAL, L.,[1] HOŠŤÁLKOVÁ, A.,[1] LOČÁREK, M.,[1] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jakub.chlebek@faf.cuni.cz.cz

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

HENDRYCHOVÁ, H., TŮMOVÁ, L.

Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: hendryh@faf.cuni.cz

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

HOŠŤÁLKOVÁ, A.,[1] KUNEŠ, J.,[2] NOVÁK, Z.,[2] HRABINOVÁ, M.,[3] OPLETAL, L.[1]

[1] ADINACO Research Group, Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Institute of Organic Chemistry and Biochemistry of the AS CR, Prague, Czech Republic
e-mail: hosta4aa@faf.cuni.cz

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

HULCOVÁ, D.,[1] CHLEBEK, J.,[1] NOVÁK, Z.,[2] OPLETAL, L.,[1] KUNEŠ, J.,[2] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: hulcovd@faf.cuni.cz

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

KUBEŠ, J.,[1] STRACKE, R.,[2] TŮMOVÁ, L.,[1] WEISSHAAR, B.[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] The Chair of Genome Research, Faculty of Biology, Bielefeld University, Germany
e-mail: kubej7aa@faf.cuni.cz

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

LOČÁREK, M., OPLETAL, L.

Department of Pharmaceutical Botany and Ecology, ADINACO Research Group, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: locarekm@faf.cuni.cz

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

PILAŘOVÁ, V.,[1] NOVÁK, O.,[2,3] PĚNČÍK, A.,[2,3] CASANOVA-SAEZ, R.,[3] LJUNG, K.,[3] NOVÁKOVÁ, L.,[1] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University & Institute of Experimental Botany AS CR, Czech Republic
[3] Umea Plant Science Centre, Swedish University of Agricultural Sciences, Sweden
e-mail: pilarovv@faf.cuni.cz

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

ŠAFRATOVÁ, M.,[1] OPLETAL, L.,[1] BENEŠOVÁ, N.,[1] NOVÁK, Z.,[2] HRABINOVÁ, M.,[3] CAHLÍKOVÁ, L.[1]

[1] ADINACO Research Group, Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Institute of Organic Chemistry and Biochemistry of the AS CR, Prague, Czech Republic
e-mail: safratom@faf.cuni.cz

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

HURYCHOVÁ, H., ŠKLUBALOVÁ, Z.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: hurychh@faf.cuni.cz

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

ONDREJČEK, P., SVAČINOVÁ, P., STONIŠ, J., JIRÁSKOVÁ, J., RABIŠKOVÁ, M.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: ondrp3aa@faf.cuni.cz

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

ŠKOLOVÁ, B.,[1] PULLMANNOVÁ, P.,[1] HUDSKÁ, K.,[1] SLEPIČKA, P.,[2] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Solid State Engineering, Institute of Chemical Technology Prague, Czech Republic
e-mail: skolb5aa@faf.cuni.cz

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

STONIŠ, J., ŠKLUBALOVÁ, Z., ONDREJČEK, P., SVAČINOVÁ, P.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: stonisj@faf.cuni.cz

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

SVAČINOVÁ, P., ONDREJČEK, P., STONIŠ, J., JIRÁSKOVÁ, J., RABIŠKOVÁ, M.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: svacp3aa@faf.cuni.cz

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

JIRKOVSKÁ, A.,[1] FIKROVÁ, P.,[2] ŠIMŮNEK, T.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jirkovan@faf.cuni.cz

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

ANDRÝS, R.,[1] ZEMANOVÁ, L.,[1] LENČO, J.,[1,2] WSÓL, V.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Molecular Pathology, Faculty of Military Health Sciences in Hradec Králové, University of Defence, Brno, Czech Republic
e-mail: andrysr@faf.cuni.cz

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*

JANSOVÁ, H., VOSTATKOVÁ, L., ŠIMŮNEK, T.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: jansovah@faf.cuni.cz

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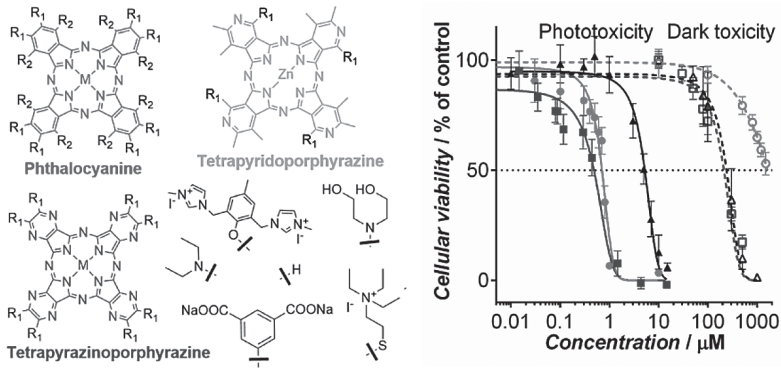
AN INSIGHT INTO CELL DEATH PROCESS AFTER PHOTODYNAMIC TREATMENT OF CANCER CELLS WITH PHTHALOCYANINES AND THEIR AZA-ANALOGUES

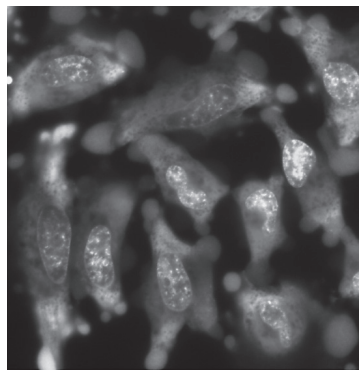
MACHÁČEK, M.,[1] BRÁZDOVÁ, P.,[1] NOVÁKOVÁ, V.,[2] CIDLINA, A.,[3] RUDOLF, E.,[4] ZIMČÍK, P.,[3] ŠIMŮNEK, T.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[4] Department of Biological Sciences, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
e-mail: machamil@faf.cuni.cz

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*)

STUCHLÍKOVÁ, L.,[1] JIRÁSKO, R.,[2] VOKŘÁL, I.,[3] VALÁT, M.,[1] LAMKA, J.,[3] SZOTÁKOVÁ, B.,[1] HOLČAPEK, M.,[2] SKÁLOVÁ, L.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Czech Republic
[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: stuchli2@faf.cuni.cz

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

RYCHTARČÍKOVÁ, Z.,[1,2] LETTLOVÁ, S.,[2] TRUKSA, J.[2]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Laboratory of Tumour Resistance, Institute of Biotechnology, Academy of Science of the Czech Republic, Prague, Czech Republic
e-mail: rychtarcikovazuzana245@gmail.com

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

HAŠKOVÁ, P., JANSOVÁ, H., ŠIMŮNEK, T.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: haskp2aa@faf.cuni.cz

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*

LECOVÁ, L., RŮŽIČKOVÁ, M., MATOUŠKOVÁ, P., SKÁLOVÁ, L.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: lecoval@faf.cuni.cz

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

LNĚNIČKOVÁ, K., SZOTÁKOVÁ, B.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: lnenickk@faf.cuni.cz

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.

The study was supported by Czech Science Foundation, Grant No. P303/12/G163 and by the Grant Agency of Charles University, Grant No. 2014/1874214.

DESCRIPTION AND EVALUATION OF DHRS8 BIOCHEMICAL PROPERTIES

LUNDOVÁ, T.,[1] ZEMANOVÁ, L.,[1] ŠTAMBERGOVÁ, H.,[1] HAVRÁNKOVÁ, J.,[1]
ŠAFR, M.,[2] WSÓL, V.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Institute of Legal Medicine, Faculty of Medicine, Charles Universityin Prague
and University Hospital in Hradec Králové, Czech Republic
e-mail: lundovat@faf.cuni.cz

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.

The study was supported by the Grant Agency of Charles University (Grant No. 677012/C/2012), the European Social Fund and the state budget of the Czech Republic (TEAB, project no. CZ.1.07/2.3.00/20.0235) and the Charles University project SVV 260 065.

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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

ARNDT, T.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: arndttomas66@gmail.com

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.

The study was supported by SVV 260 066.

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

BELKINA, T.,[1] KHODJIEV, D.,[2] TEBBENS, J.,[3] VLČEK, J.

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] National Center for Tuberculosis and Lung Diseases, Tashkent, Uzbekistan
[3] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: belkinat@faf.cuni.cz

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.

The study was supported by Charles University (Project SVV 260 066).

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

DVOŘÁČKOVÁ, E.,[1,2] STARÁ, D.,[2] VLČEK, J.[1]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Hospital Pharmacy, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
e-mail: dvore4aa@faf.cuni.cz

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.

The study was supported by SVV 2014 260 066.

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

LADOVÁ, K.,[1] DRAPÁKOVÁ, T.,[1] MATOULKOVÁ, P.,[2] KOTLÁŘOVÁ, J.,[1] POKLADNÍKOVÁ, J.[1]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Regulatory and Pharmacovigilance, Sanofi Generics Division, Zentiva Group a.s., Praha
e-mail: ladok5aa@faf.cuni.cz

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

TOUŠKOVÁ, T., VYTŘÍSALOVÁ, M.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: touskovt@faf.cuni.cz

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.

The study was supported by project SVV 260 066.

23rd NATIONAL STUDENTS' SCIENIFIC CONFERENCE OF THE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ (CZ), CHARLES UNIVERSITY (CZ), *HRADEC KRÁLOVÉ 22–23 APRIL 2015*

SECTION OF CHEMICAL SCIENCES

DESIGNING A METHOD FOR SALT-ASSISTED DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION IN A "LAB-IN-SYRINGE" SYSTEM

BEŠŤÁK, O., HORSTKOTTE, B., CHOCHOLOUŠ, P, SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: BestakO@faf.cuni.cz

The sequential injection analysis (SIA) is a technique derived from flow injection analysis technique. The system generally consists of a computer controlled syringe pump, a selection valve and a detector, all connected by inert plastic tubing. It is used to automate laboratory procedures. The "Lab-In-Syringe" is a modified SIA used to carry out parts of the experiment inside the used syringe pump. Using a PTFE-coated magnetic-propelled stirring bar inside the syringe[1] allows, for example, to homogenously mix the syringe contents and perform dispersive liquid-liquid micro-extraction (DLLME)[2].

In this work, the approach to perform salt-assisted in-syringe DLLME was explored and evaluated for the first time. Starting with a one-phase system, the analyte was extracted from water into n-propanol. For this, a highly-concentrated solution of magnesium sulfate was used to increase the polarity of the aqueous phase. The high polarity causes the separation of the two normally miscible liquids.

Measuring the absorbance in the organic phase was studied both in-syringe and at the outlet and yields precise analysis of the sample content. Astraphloxine and riboflavin were used as model analytes and various conditions, i.e. salt concentration and water/solvent ratio were tested. The method performance and parameters were studied, evaluated and improved for the highest preconcentration factor and the fastest phase separation.

The highest achieved preconcentration factor was 6.68. The fastest phase separation took < 5 sec. The reproducibility of 3 repetitive extractions was generally < 1% RSD.

Using n-propanol, even compounds of moderate polarity can be extracted with high-efficiency. Furthermore, n-propanol is a HPLC compatible solvent, so the extract can be optionally analyzed on-line in modern HPLC systems.

In conclusion, the salt-assisted DLLME presents an interesting approach to perform a fast, precise, and automated extraction in small scale for the analyte preconcentration using an environment-friendly and HPLC compatible solvent.

The study was supported by SVV 260 184.

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DEVELOPMENT OF MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY METHOD FOR THE ANALYSIS OF ILLEGAL FAT-SOLUBLE FOODSTUFF DYES

BRADOVÁ, J., PETRŮ, K., POLÁŠEK, M.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: PETRK3AA@faf.cuni.cz

A microemulsion electrokinetic chromatography (MEEKC) method was developed and proposed for the determination of fat-soluble dyes (Sudan I, Sudan II, Sudan III, Sudan Red 7B, Sudan Orange G, and Methyl Red) illegally used in foodstuffs.

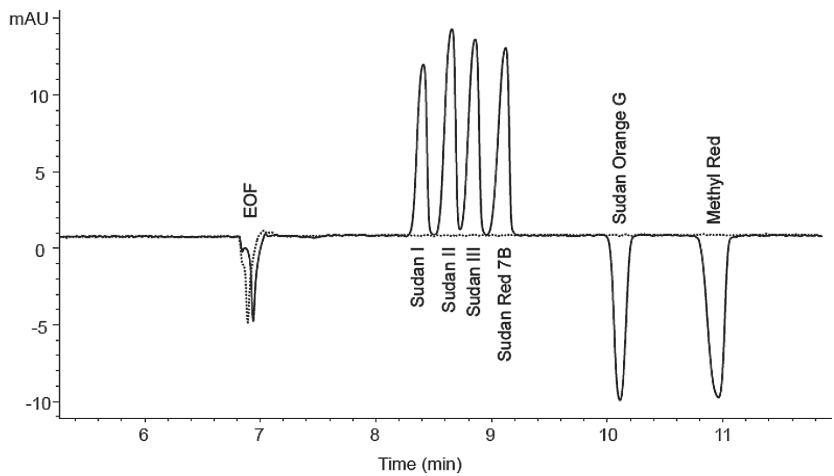

Fig.1. MEEKC separation of standard mixture of illegal foodstuff dyes under optimum conditions.

The effect of surfactant, co-surfactant, organic modifier and oil as well as the capillary length were examined in order to optimize the separation. Final background electrolyte (solution of the microemulsion) for MEEKC was composed of 30mM phosphate buffer (pH 7.5), 1.2% (w/v) sodium dodecylsulfate, 1.2% (v/v) of n-hexane, 15% (v/v) of butan-1-ol, and 20% (v/v) of acetonitrile.

A baseline separation of these six dyes was achieved within 11 min by using fused-silica capillary with 75 μm i.d. and effective length 36.5 cm. The applied voltage was 20 kV and temperature 25 °C was maintained. The VIS detection wavelengths were 500 and 400 nm.

The repeatability of the migration times and peak areas were characterized by RSD values ranging from 0.3 to 0.9% and 1.7–2.7% (n = 5), respectively. The calibration curves were linear for all analytes ($R^2 \geq 0.9990$) and the limits of detection ranged from 0.19 μg/ml (for Sudan III) to 1.27 μg/ml (for Sudan Red 7B).

The method devised is suitable for the analysis of suspected foodstuffs after appropriate sample pretreatment to eliminate matrix effects and to achieve sample pre-concentration.

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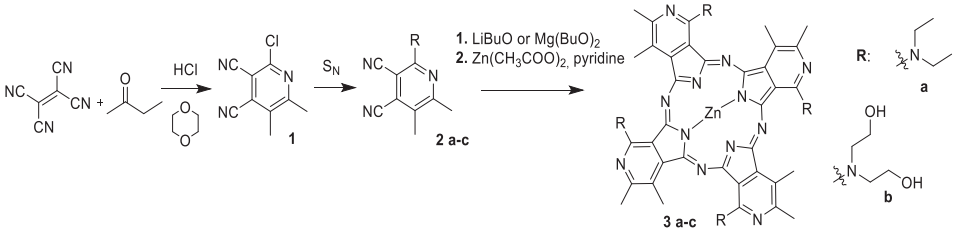
PREPARATION AND PHOTOPHYSICAL EVALUATION OF TETRA-3,4-PYRIDOPORPHYRAZINES SUITABLE FOR THE PHOTODYNAMIC THERAPY

ČERMÁK, P., NOVÁKOVÁ, V.

Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: cermakp8@faf.cuni.cz

Tetra-3,4-pyridoporphyrazines (TPyPz) are aza-analogues of phthalocyanines. Their large system of conjugated bonds enables them to absorb light in the red part of the absorption spectrum. Due to their ability to produce singlet oxygen, they can be potentially used as photosensitizers in photodynamic therapy (PDT). Its mechanism is based on co-functioning of three elements – photosensitizer, light and oxygen. Photosensitizer excited by light absorption transfers its energy into tissue oxygen, thus, creating cytotoxic singlet oxygen. This method is beneficial for its high selectivity, low toxicity, minimal invasion and fast effect.

The aim of this work was to synthetize and study water-soluble TPyPz suitable for PDT. Water solubility was achieved by introduction of hydrophilic non-charged substituents (OH), quarternized amines (structure of target TPyPzs see below), forming of salts or using suitable delivery systems (hydrophilic emulsion). Firstly, appropriate precursors for TPyPz (i.e., 2-substituted-5,6-dimethylpyridine-3,4-dicarbonitriles) were prepared by nucleophilic substitution according to the scheme below. Then, cyclotetramerization of **2 a–c** with butoxide as an initiator of the reaction gave required macrocycles. Obtained TPyPz were transferred into metal free derivatives under acidic condition and zinc was then coordinated



into the center. All prepared TPyPz were characterized by physico-chemical properties and biological activity.

The study was supported by SVV 260 183.

PREPARATION AND PHOTOPHYSICAL EVALUATION OF TETRA-3,4-PYRIDOPORPHYRAZINES CARRYING CHARGED SUBSTITUENTS ON THE PERIPHERY

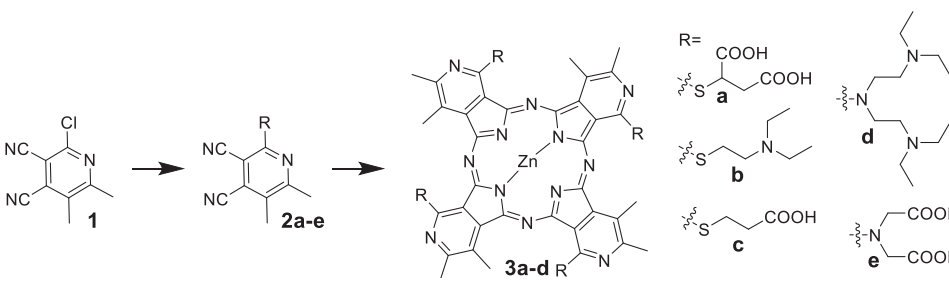
DEMUTH, J., NOVÁKOVÁ, V.

Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: demuthj1@faf.cuni.cz

Phthalocyanines are planar organic molecules, which have a metal cation coordinated in their center. This work deals with their aza-analogues – tetra-3,4-pyridoporphyrazines (TPyPz). TPyPz can absorb light in red part spectrum and then produce singlet oxygen. Due to this ability, they may be used in photodynamic therapy (PDT) of cancer. PDT's mechanism is based on three components: photosensitizer, light and singlet oxygen. Photosensitizer transfers energy of absorbed light to oxygen making, thus, cytotoxic singlet oxygen.

The goal of this project was to synthesize water soluble TPyPz absorbing in red part of spectrum. TPyPzs bearing different charged substituents will be compared within the series.

The synthesis consisted of preparation of 2-chloro-5,6-dimethylpyridine-3,4-dicarbonitrile (**1**), which was the starting precursor for other reactions. Nucleophilic substitution of **1** was used for the introducing of hydrophilic substituents **a–e**. Prepared precursors **2a–e**



underwent cyclotetramerization leading to final TPyPz **3a–d**. Structures were characterized by physico-chemical properties and in *in vitro* testing.

The study was supported by SVV 260 183.

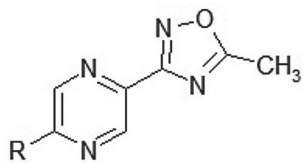
OXADIAZOLES AS POTENTIAL DRUGS

DZÁMOVÁ, P., KUČEROVÁ-CHLUPÁČOVÁ, M., OPLETALOVÁ, V.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy
in Hradec Králové, Charles University, Czech Republic
e-mail: dzamovpa@faf.cuni.cz

Due to the increasing resistence of bacteria and fungi against conventional drugs, it is imperative to design and develop new antibacterial or antifungal agents[1]. Some derivatives of 1,2,4-oxadiazoles exert antibacterial activity and they are known as the compounds with promising future in this direction. The 1,2,4-oxadiazole ring is located in some biologically active compounds such as muscarinic receptor agonists, tyrosine kinase inhibitors, anti-inflammatory agents, antitumor agents, selective H_3 receptor antagonists, monoamine oxidase inhibitors, anticonvulsant and anti-HIV agents[2]. The 1,2,4-oxadiazoles are bioisosteres for amides and esters with higher hydrolytic and metabolic stability[3].

In the experimental part of this study, six new oxadiazole derivates have been synthesized. First, six pyrazine-2-carbonitriles have been prepared differently alkylated in position 5. Afterwards the nitriles were converted to corresponding amidoximes using hydroxylamine hydrochloride. In the last step amidoxime reacted with acetic anhydride in xylene to form corresponding oxadiazoles.



R = *terc*-butyl, isobutyl, propyl, isopropyl, pentyl, hexyl

Six novel 3-(pyrazil-2-yl)-1,2,4-oxadiazoles were obtained characterized by melting points, IR and NMR spektra. Their purity was checked by TLC and elemental analysis. The compounds were tested *in vitro* for their antifungal and antibacterial activity.

This study was supported by project SVV 260 183.

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STUDY OF IN-SYRINGE ANALYSIS FOR THE AUTOMATION OF IMMERSED SINGLE-DROP MICROEXTRACTION AS A SAMPLE PRETREATMENT TECHNIQUE

FIKAROVÁ, K., HORSTKOTTE, B., ŠRÁMKOVÁ, I., SKLENÁŘOVÁ, H.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: fikaroka@faf.cuni.cz

Lead belongs to the most toxic elements with respect to human health due to its negative effect on metabolism of human beings. Moreover, lead has proven to be a carcinogen. The recommended limit of lead in tap water by the World Health Organization[1] is 10 μg L^{-1}. In this work, an immersed single-drop microextraction for the spectrophotometric determination of lead in tap water is presented for the first time.

Dithizone was used as a reagent, which creates a rose-coloured complex with lead. Mixture of toluene and n-hexanol was used as a solvent for the pre-concentration of the complex into the single drop. Ammonium-acetate buffer was used for keeping basic pH conditions. The method took place in the void of an automated syringe pump. A magnetic stirring bar was used for mixing the syringe's content continuously. The parameters of the method were optimized including the toluene/n-hexanol ratio, drop size, pH value of buffer, volumes of the dithizone reagent, buffer, and sample, extraction time and rotation speed of the stirring bar.

The calibration curve was linear over the range of 100–700 nmol L^{-1} with a correlation coefficient of $r^2 = 0.999$. The limits of detection (LOD = 39.2 nmol L^{-1}) and quantification (LOQ = 130.6 nmol L^{-1}) were also evaluated. Repeatability for the concentration range of 100–700 nmol L^{-1} was proven and the relative standard deviation (RSD) was 2.8%. Using a pre-concentration time of 300 s, the whole analysis took about 500 s. The extraction efficiency was in the range of 25%.

Important advantages of the proposed method are a small instrumentation size and thus its portability, so it can be used for on-site analysis and a high sensitivity.

The study was supported by the specific research, No. SVV 260 184.

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SYNTHESIS OF POTENTIAL ORGANOCATALYSTS BASED ON QUINAZOLINE ALKALOIDS

GÓRECKI, L., MIKUŠEK, J., KUNEŠ, J., POUR, M.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: goreckil@faf.cuni.cz

A series of substances derived from vasicine-type alkaloids was synthesized. The compounds were prepared using different α-hydroxy carboxylic acids (lactic and mandelic) (Fig. 1) and α-amino carboxylic acids (phenylglycine, alanine, proline and valine) (Fig. 2). These derivatives are currently being tested for their organocatalytic activity on a series of reactions, such as asymmetric enamine catalyzed aldolisation and conjugate addition of aldehydes to nitroalkenes.

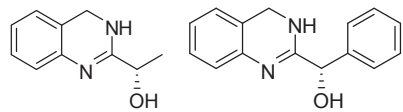

Fig. 1.

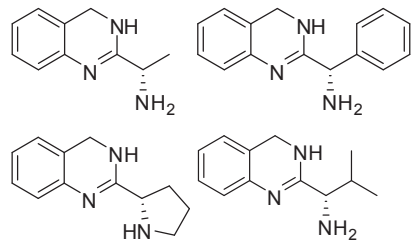

Fig. 2.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. 15-07332S) and Charles University Research (SVV-260-183).

ON-LINE SPE HPLC METHOD OPTIMIZATION FOR DETERMINATION OF PATULIN MYCOTOXIN IN APPLE DRINKS

HOLZNEROVÁ, A.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: holznera@faf.cuni.cz

The issue of food contamination with mycotoxins is a serious problem worldwide. These substances are highly toxic to humans and chronic effects on the human organism

in very low doses may cause long-term medical complications. Some mycotoxins are also substances stable and persistent in the environment, from where they can get through agricultural (crop and livestock) production further into the human food chain. From this standpoint it is therefore necessary to monitor whether the limits are not exceeded for individual mycotoxins and for this purpose to develop sensitive and selective analytical techniques for their detection. In our work we focus on one of the most common dietary mycotoxins – patulin, which is found in apples and related products (especially apple juices). High performance liquid chromatography (HPLC) coupled with on-line solid phase extraction (SPE) using a column switching technique for sample treatment was developed for determination of mycotoxin patulin in apple drinks and juices. A volume of 250 µl of juice sample was injected directly into the on-line SPE-HPLC system. After injection of the sample the extraction of patulin from juice matrix was carried out on SPE precolumn. SPE precolumn 25 × 3 mm was filled with Supel MIP Patulin sorbent, which is a specific "molecularly imprinted polymer" (MIP) designated for the selective extraction of patulin from an apple matrix. As the washing solution for removing ballast matrix was selected 1% solution of $NaHCO_3$, which flowed through MIP precolumn at flow rate 0.75 ml/min for 2.5 minutes. After this period a valve was switched and the residual ballastmatrix, retained on the extraction precolumn, together with patulin were further separated on an analytical column Kinetex Biphenyl 150 × 4.6 mm (particle size 5 µm). The mobile phase composition of 20% ethyl acetate in acetonitrile with water in ratio 20 : 80, flow through the column at 1 ml/min in gradient elution. Detection was performed by UV-VIS detector at a wavelength of 276 nm. The total analysis time of one juice sample, including its online pretreatment, was less than 9 minutes. The detection limit of this method was found at level 50 µg/l, which is the value corresponding to the maximum allowed levels of patulin in apple juices according to EU standards.

The study was supported by the Charles University project no. SVV 260 184.

DEVELOPMENT OF AN ABSOLUTE METHOD FOR DETERMINATION OF SINGLET OXYGEN QUANTUM YIELDS OF PHTHALOCYANINES

HRUBÁ, L., NOVÁKOVÁ, V.

Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: hrubale2@faf.cuni.cz

Photodynamic therapy (PDT) with a singlet oxygen as an essential agent is believed to be a promising way of cancer treatment or treatment of some cutaneous diseases. Thanks to its high selectivity, harmful adverse effects are significantly decreased. The principle of PDT is based on excitation of a photosensitizer by light absorption, followed by transfer of energy to oxygen ($^3O_2$) forming cytotoxic singlet oxygen ($^1O_2$)[1]. The efficiency by which photosensitizer transforms absorbed energy to singlet oxygen production is characterized by *singlet oxygen quantum yields (Φ_Δ)*.

The aim of this study was to develop and optimize absolute method for determination of Φ_Δ. In comparison to a relative method, no reference is needed in this case, which enables accurate results with lower error. Verification of the new method was performed in *N*,*N*-dimethylformamide with zinc phthalocyanine as a model photosensitizer because of its well-known singlet oxygen quantum yield and with 1,3–diphenylisobenzofuran (DPBF) as a chemical quencher of $^1O_2$.

Different sources of light for excitation and different set-ups of the instrumentation were tried and compared. Efficient and accurate method for absolute determination of Φ_Δ was successfully developed. This method will be used for Φ_Δ measurements of the new compounds prepared in our research group.

The study was supported by SVV 260 183.

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SEPARATION OF TOCOPHEROLS USING HPLC TECHNIQUE

HUTNÍKOVÁ, M.,[1] ČERVINKOVÁ, B.,[1,2] KUJOVSKÁ-KRČMOVÁ, L.,[1,2] SOLICHOVÁ, D.,[2] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, University Hospital, Hradec Králové, Czech Republic
e-mail: hutnikom@faf.cuni.cz

Vitamin E represents eight related compounds α-, β-, γ-, δ-tocopherols (saturated phytyl side chain) and α-, β-, γ-, δ-tocotrienols (unsaturated phytyl side chain). α-Tocopherol was the most studied isomer in the past and its anti-inflammation and proliferative effect was described. Therefore most of cancer prevention trials have been focused on α-tocopherol. Present research studies have described the important role of γ- and δ-tocopherols in cell proliferation, anti-inflammation and tumor burden[1]. Also it has been demonstrated that γ-tocopherol inhibits colon, prostate, mammary and lung tumorigenesis in animal models, suggesting to have a high potential in the prevention of human cancer[2].

In this study the novel sensitive method for analysis of α-, β-, γ-, δ-tocopherols was developed using the High Performance Liquid Chromatography (HPLC) technique. All the measurements were carried out on the chromatographic system HPLC Prominence LC 20, Shimadzu (Kyoto, Japan).

During the method development core-shell columns (Kinetex) with different stationary phases (HILIC, Biphenyl, Pentafluorophenyl) were tested under various conditions such as temperature, injection volume, flow rate and composition of mobile phase. The best results were achieved using Kinetex Pentafluorophenyl column (4.6 × 100 mm, 2.6 μm) and mixture of methanol and water as a mobile phase.

The newly developed analytical method will be used for analysis of breast and gastrointestinal cancer patients. Results of this research can provide closer knowledge about the tocopherols metabolic pathway and their role in human body.

The authors thank for financial support to the project SVV 260 184, the European Social Fund and the state budget of the Czech Republic, TEAB, project no. CZ.1.07/2.3.00/20.0235 and IRP UK 2015 "Využití mikrotitračních destiček pro zpracování a chromatografickou analýzu biologických vzorků v klinickém výzkumu".

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DEVELOPMENT OF SPE AND UHPLC-MS/MS METHOD FOR THE DETERMINATION OF QUERCETIN AND ITS 9 METABOLITES

CHRENKOVÁ, L., PLACHKÁ, K., PILAŘOVÁ, V., MIŠÍK, J., VLČKOVÁ, H., NOVÁKOVÁ, L.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: chrenklu@faf.cuni.cz

Quercetin (Fig. 1) is one of the major flavonoids belonging to subgroup of flavonols. It is one of the most potent plant antioxidants which is distributed in edible plants (tea, red wine, fruits and vegetables). Consumption of quercetin may be associated with a decreased risk of coronary, bacterial and viral diseases.[1] However, in some newly published works the correlation of plasma levels of quercetin in adults and antioxidant capacity has not been confirmed.[2]

The aim of this study was to develop and validate a new extraction method for the preparation of biological samples for the determination of quercetin and its nine potential

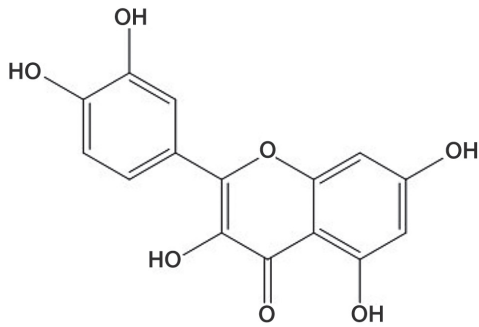

Fig. 1. The structure of quercetin.

metabolites: phloroglucinol, 3,4-dihydroxyphenylacetic acid, homovanilic acid, 3-hydroxyphenylacetic acid, 3-(3-hydroxyphenyl)propionic acid, rutin, quercetin-3-glucuronide, tamarixetin and isorhamnetin. These metabolites differ substantially in physicochemical properties (pKa, molecular weight, log P, chemical structures etc).

For the first screening in extraction procedure, the silica-based cartridges with C8, C18 ligand and polymer-based MAX (Mixed- mode Anion-eXchange) sorbents were used. MAX is mixed-mode polymeric sorbent that has been optimized to achieve higher selectivity and sensitivity for extracting acidic compounds with anion-exchange groups, which is the case of small acid metabolites of quercetin.

Due to the best retention and subsequent elution of all tested analytes the MAX cartridge was chosen. Other sorbents demonstrated poor retention of small polar acidic molecules. The optimization of elution solvent included optimization of methanol concentration (60–95%) and the choice of organic acid (formic acid 0.5–10% and trifluoracetic acid 0.1–1%). The mixture of 95% methanol and 0.5% trifluoracetic acid demonstrated the best recovery (60.9–105.7% with RSD 0.4–9.4%). Therefore it was chosen as optimal solvent for elution. The acidic pH was defined as a critical factor for the retention of analytes. Subsequently the wash solvent was optimized. The wash procedure consisted of two steps – the washing with water component (0.1–5% ammonium hydroxide, water, 0.01 M ammonium formate buffer with pH 5.0) and the washing with organic solvent (1–10% methanol). Due to good selectivity, the lowest losses of analytes, the best clean-up and removing of interfering compounds the combination of 0.01 M ammonium formate buffer with pH 5.0 and 1% MeOH was chosen.

The optimized extraction method was used for the determination of quercetin and metabolites in biological samples. The determination of quercetin and its 9 metabolites was performed by developed chromatographic method using ultra high performance liquid chromatography (Acquity UPLC) with tandem mass spectrometry (Quattro Micro triple quadrupole mass spectometer). The best selectivity between the critical pair of analytes (tamarixetin and isorhamnetin) was achieved using column BEH Shield RP C18 (2.1 × 100 mm; 1.7 μm) and gradient elution with methanol and 0.1% formic acid. The ionization was performed in electrospray polarity switching mode and the quantification by selected reaction monitoring (SRM). The method will be validated in terms of linearity, limit of detection and quantification, accuracy, precision, selectivity and matrix effects.

The advantages of newly developed SPE method for the preparation of biological samples prior to UHPLC-MS/MS analysis are simultaneous analysis and extraction of the compounds with different polarity, good recovery and repeatability.

The study was supported by SVV/2015/260184.

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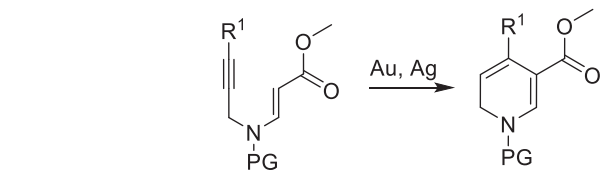
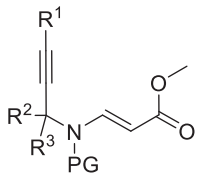
SYNTHESIS OF SUBSTITUTED PYRIDINES USING TRIS(2-FURYL)PHOSPHINE GOLD(I) CATALYST

JANOUŠEK, M., MIKUŠEK, J., POUR, M.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: janousm4@faf.cuni.cz

Synthesis of various types of heterocycles is possible from 1,5-enyne precursors using cationic gold(I) species as a catalyst. In order to extend previous research[1] we focused on cyclisation of 1,5-enynes with various aryls and silyls in the position R^1. The influence of various protecting groups and substituents in positions R^2 and R^3 was also investigated.





R^1 = phenyl, 3-methoxyphenyl, thienyl, 4-chlorophenyl
R^2,R^3 = H, alkyl
PG = *tert*-butylcarbamate, 4-methoxybenzenesulfonamide

The study was supported by SVV 260 183, GAČR P207-15-073325.

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5-ALKYLAMINO-*N*-PHENYLPYRAZINE-2-CARBOXAMIDES AS POTENTIAL ANTITUBERCULARS

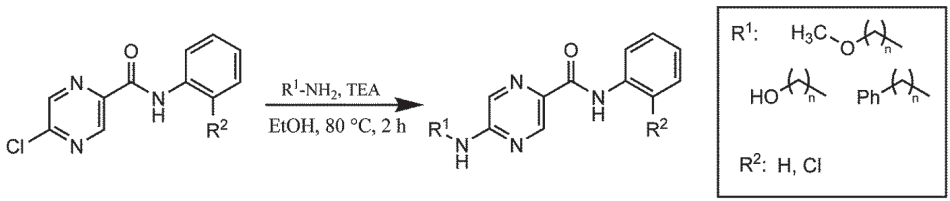
JANOUTOVÁ, A., PATEROVÁ, P., ZÍTKO, J.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy
in Hradec Králové, Charles University, Czech Republic
e-mail: jan.zitko@faf.cuni.cz

This study is focused on new derivatives of pyrazinamide (PZA) prepared as potential antituberculars. PZA itself is a well-established first-line antitubercular agent and a constituent of all basic tuberculosis treatment regimens. The design of final compounds was based on the previously synthesized 5-alkylamino-*N*-phenylpyrazine-2-carboxamides[1], which possessed promising *in vitro* antimycobacterial activity with MIC ranging from

0.78 to 3.13 μg/mL. The object of this study was to test the activity of derivatives with alkylamino chain modified with terminal phenyl, hydroxyl or methoxy group.

Final compounds were prepared by nucleophilic substitution of chlorine with respective amines in refluxing EtOH (Scheme 1). Reaction yields, after all purification steps, were 5887%. Compounds were characterized by ^{1}H and ^{13}C NMR, IR, elementary analysis and melting point.



Scheme 1. Synthesis of the final compounds.

Final compounds were tested for *in vitro* antimycobacterial, antibacterial and antifungal activity. Only six substances, out of total of 16 newly prepared, showed moderate activity against *M. tuberculosis* H37Rv and *M. kansasii* (MIC =12.5–50 μg/mL, $MIC_{(PZA)}$ = 6.25 μg/mL). All compounds were ineffective against *M. avium* and other tested pathogens. All compounds with R^2 = Cl were inactive. Detailed structure-activity relationships will be discussed.

This publication is a result of the project implementation: 'Support of establishment, development, and mobility of quality research teams at the Charles University', project number CZ.1.07/2.3.00/30.0022, supported by The Education for Competitiveness Operational Programme (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic. Additional support was provided by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 183), and Ministry of Health of the Czech Republic IGA NT 13346 (2012).

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SYNTHESIS OF ON THE RING SUBSTITUTED PHENYLGUANIDINES WITH BIOLOGICAL ACTIVITY

JAREŠOVÁ, D.,[1] PALÁT, K.,[1] VEJSOVÁ, M.,[2] JÍLEK, P.,[2] DUFKOVÁ, I.[2]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jaresovd@faf.cuni.cz

The increasing frequency of systematic mycoses caused by opportunistic fungi resistant to antifungal drugs is a great health problem in the last years. It leads to high mortality especially of immunocompromised patients. Therefore it is necessary to find new substances with antifungal activity.

Series of four on the ring substituted phenylguanidines (I) were sythesized for their potential antibacterial, antifungal, and antimycobacterial activities in this study.

$R^1 = -C_{15}H_{31}, -C_{14}H_{29}$

(I) $R^2 = -H, -CH_3$, R3 = $-H, -CH_3, -CH_2CH_3$

Products were synthetized in the four-step synthesis[1,2]. Alkylarylsulfides were prepared by the reaction between alkylthiols and 4-chloro-3-nitrotoluene or *p*-chloronitrobenzene with active copper as a catalyst in the first step. The nitro group on the ring was reduced to amino group by the reaction with stannous chloride under nitrogen atmosphere in the second step. Sulfanylphenylamines were then transferred by the reaction with gaseous hydrogen chloride to ammonium chlorides. Phenylguanidines were prepared by the reaction of these salts with cyanamide or dialkylcyanamides in the last step.

All compounds were evaluated *in vitro* for antimicrobial activity by the broth microdilution method against representative human pathogenic fungi: *Candida albicans*, *Candida tropicalis, Candida krusei, Candida glabrata, Trichosporon asahii, Aspergillus fumigatus*, *Absidia corymbifera, Trichophyton mentagrophytes*; and bacteria: *Staphylococcus aureus*, *Staphylococcus aureus* Methicilin resistant, *Staphylococcus epidermidis, Enterococcus sp., Escherichia coli, Klebsiella pneumoniae, Klebsiella pneumoniae ESBL positive, Pseudomonas aeruginosa*.

The substance 1,1-dimethyl-3-[5-methyl-2-(tetradecylsulfanyl)fenyl]guanidine has significant antifungal activity. Its activity against some strains of fungi was higher than activity of ketoconazole.

This study was financially supported by the grant GA14-08423S.

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SYNTHESIS OF POTENTIAL CHOLINESTERASE INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE – TACRINE DERIVATES

JAROŠOVÁ, M.,[1,2] NEPOVIMOVÁ, E.,[2] SPILOVSKÁ, K.,[2] KORABEČNÝ, J.,[2] OPLETALOVÁ, V.,[1] KUČA, K.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Science, Hradec Králové, University of Defence, Brno, Czech Republic
e-mail: jarosom3@faf.cuni.cz

Alzheimer's disease (AD) affects currently about 6.1 million people in Europe. The threefold increase of this number can be expected due to population aging.

AD manifests as memory loss, social and spatial disorientation and gradual deterioration of intellectual capacity.

Although AD is described since 1907, the effective treatment has not been found yet. The reason is the most likely a multifactorial origin of AD.

To date, the pharmacotherapy of AD is relies on acetylcholinesterase inhibitors (AChEIs) – tacrine, rivastigmine, donepezil and galanthamine. More recently memantine, an antagonist of *N*-methyl-D-aspartate receptor, has been approved for moderate to severe stages of AD.

New approaches for AD therapy have emerged in recent years. One of them, multi-targed-directed ligands (MTDLs) capable of interaction with multiple target are currently being extensively investigated.

In our contribution we would like to introduce series of newly designed molecules based on MTDLs. These MTDLs combine tacrine, 6-chlortacrine or 7-methoxytacrine (7-MEOTA) as AChEIs with some amino acids using different linkers. It can be assumed that the molecules are capable to simultaneously bind peripheral anionic site (PAS) as well as catalytic anionic site (CAS) of AChE. The coumpounds exhibited very good inhibitory profile with IC_{50} values ranging from micromolar to sub-nanomolar concentration scale.

Supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), Long Term Development Plan – 1011 and by the Grant Agency of the Czech Republic (No. P303/11/1907). No. SV/FVZ 201409, No. SVV 260 183.

DESIGN AND SYNTHESIS OF HYBRID COMPOUNDS BASED ON TACRIN/RESVERATROL DERIVATIVES

JEŘÁBEK, J.,[1] KORÁBEČNÝ, J.,[2] BOLOGNESI, M.,[3] ROBERTI, M.,[3] ŠEPSOVÁ, V.,[2] JUN, D.,[2] KUČA, K.,[2] DOLEŽAL, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Biomedical Research Centre, Faculty Hospital Hradec Králové, Czech Republic
[3] Department of Pharmacy and Biotechnology, University of Bologna, Italy
e-mail: jerabej@faf.cuni.cz

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder, in which a progressive dementia appears. The cause of AD is currently unknown, however, scientific research has revealed several pathological hallmarks – β-amyloid plaques and neurofibrillary tangles. These changes cause gradual disintegration of nerve cells and they change the metabolism in the brain. The current drugs are not able to treat the cause of the disease, being able only to delay the onset of severe symptoms. The basic drugs for AD treatment are acetylcholinesterase (AChE, E.C. 3.1.1.7) inhibitors and, more recently approved, *N*-methyl-*D*-aspartate (NMDA) receptor antagonist memantine. These drugs are able to increase cholinergic activity or preventing glutamate excitotoxicity in the patient's brain, thus improving cognitive functions and delaying severe stages of the disease. One of the emerging approaches in drug synthesis represents multi-target-directed ligands (MTDLs). Apart from the ability to inhibit AChE, they can also target more pathological processes at once. As such, they are able to bring an added value in a single molecule. In this work, we turned our attention to the preparation of hybrid compounds based on tacrine and resveratrol moieties. Tacrine scaffold act as cholinesterase inhibitor, whereas resveratrol is a strong antioxidant, naturally occurring in the vine. We assumed that coupling of these moieties could lead to the derivatives affecting multiple pathological targets of the disease and consequently represent new leads for AD therapy.

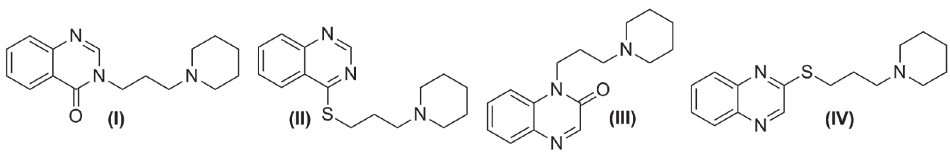
Supported by SVV 260 183 and by Erasmus program.

PREPARATION OF BENZODIAZINES WITH BRONCHODILATORY ACTIVITY

JEŽKOVÁ, V.,[1] HRUŠKOVÁ, Z. R.,[1] VOPRŠÁLOVÁ, M.,[2] POUROVÁ, J.,[2] ŠPULÁK, M.[1]

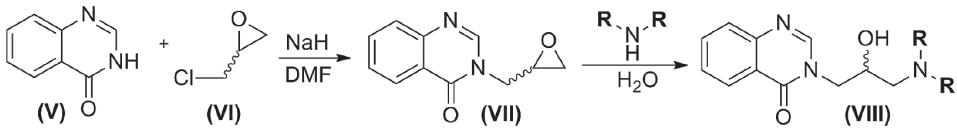
[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jezkovav@faf.cuni.cz

The most active compounds from previous screening contained (piperidine-1-yl)propyl moiety attached to quinazoline (**I**, **II**) or quinoxaline rings (**III**, **IV**).[1,2] The goal of this project was to introduce hydroxyl into the three-membered carbon linker with a possibility of further modification.



Bronchodilatory activity: ED_{50} = 3 – 25 μmol/L

The synthesis was carried out employing the reaction of commercially available quinazolinone (**V**) and racemic epichlorohydrine (**VI**) leading to epoxide (**VII**) which undergoes nucleophilic attack resulting in hydroxyquinazolinones (**VIII**). Similarly, this reaction sequence was applied on quinoxalinones.



However, the attempts with sulphur analogues of quinazolines and quinoxalines failed.

The relationship between the bronchodilatory effect and the prepared compounds will be discussed.

This work was supported by Charles University (SVV-260-183).

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SYNTHESIS AND SKIN PERMEATION-ENHANCING EFFECTS OF OF 6-((7-NITROBENZO[C][1,2,5]OXADIAZOL-4-YL)AMINO)HEXANOIC ACID DERIVATIVES

KUČERA, O., KOPEČNÁ, M., KOVÁČIK, A., VÁVROVÁ, K.

Department of Organic and Inorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Transdermal permeation enhancers are used to increase absorption of drugs through skin or, more importantly, through the stratum corneum, which is the uppermost layer of the skin. Mechanism of action of enhancers is not fully understood. In general the most active enhancers consist of hydrophilic and hydrophobic parts. Fluorescent dye 7-nitrobenzo[c][1,2,5] oxadiazol (later only NBD) is fairly hydrophilic, so we thought that it could act as a hydrophilic head of the potential enhancer. Such fluorescent enhancers could help us understand more about the mechanism of action, since it enables imaging of its penetration pathways in the skin.

We synthesised compounds containing the NBD as the hydrophilic head and ester-linked C8–C12 alkyls as hydrophobic tails. Aminocaproic acid reacted with 4-chloro-NBD and then with a series of alcohols to give us different lengths of alkyl chains. Then we applied these enhancers to human skin in Franz diffusion cells using two model drugs theophylline and hydrocortisone in two different media. We measured the concentrations of the drugs and also the enhancers beneath the skin in time to yield the flux values as well as concentrations in the skin after the test.

We found that the drug permeation was two to three times higher in the presence of 1% ester enhancers (NBD-acid was inactive) in comparison with control (only drug without the NBD-enhancer). Significant enhancer concentrations were found in epidermis and dermis and we also observed significant ester hydrolysis in the skin. Since these esters show strong fluorescence, they can provide interesting visual data, where in the skin are these enhancers located, and what structures they possibly interact with. Thus, the next step would be imaging the skin after the application of a selected enhancer using fluorescent microscopy.

The study was supported by The Charles University, No. SVV 260 183.

IN SILICO SCREENING OF SIRT6 INHIBITORS

KUČERA, T.,[1] LAHTELA-KAKKONEN, M.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Finland
email: kucerat2@faf.cuni.cz

SIRT6 is called NAD-dependent protein deacetylase sirtuin-6 and it is a member of sirtuin protein family. Its deacetylase activity is targered on histone H3K9Ac and H3K56Ac and it modulates acetylation of H3 histone during the S phase. The SIRT6 enzyme is an interesting drug target because of its role in DNA replication, glycolysis and inflammation – that is why, the design of SIRT6 inhibitors is relevant in context of diabetes melitus, arthritis and cancer.[1,2]

There are about 9 known SIRT6 inhibitors published by Kokkonen 2014 and Parenti 2014. The aim of the work was to find new possible ligands of this enzyme using methods of computational chemistry and molecular modeling. We tried to find specially some new lead structures with possibility to be optimized in next phases of the drug discovery process.

The 9 known inhibitors and crystal structure of SIRT6 (PDB code 3K35) were used as input data during the modeling. The screening was done on the databases Enamine and Chembridge. Pharmacophoric and molecular similarity search were selected from the group of ligand-based methods. The pharmacophore was defined after structural alignment of four known ligands and tested on set of ligands and non-ligands. As pattern molecules for molecular similarity search (BIT_MACCS fingerprint), known ligands and their fragments were used.

All molecules from ligand-based screening were docked into SIRT6 crystal structure with several algorithms in software MOE, Glide and InducedFit and scored with different methods (London dG, GBVI/WSA dG and Glide score).

After final selection, 32 molecules were recommended for *in vitro* testing.

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DEVELOPMENT OF HPLC METHOD FOR DETERMINATION OF VANCOMYCIN IN CLINICAL RESEARCH – PART I

KUČEROVÁ, K.,[1,2] JAVORSKÁ, L.,[1,2] KUJOVSKÁ-KRČMOVÁ, L.,[1,2] SOLICHOVÁ, D.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, University Hospital, Hradec Králové, Charles University, Czech Republic
e-mail: kucerok1@faf.cuni.cz

Vancomycin, a glycopeptide antibiotic, provides bactericidal effect against gram-positive bacteria such as methicillin resistant *Staphylococcus spp.*, *Streptococcus spp.* and *Enterococcus spp.* It is useful mainly in serious bacterial infectious disease when resistances or allergies to penicillin or oxacillin in patients were indicated. Optimizing of vancomycin therapy is beneficial because of narrow therapeutic index and potential toxicity in high serum level.

Aim of this study was development of a clinical routine practice suitable method for determination of vancomycin levels in biological fluids (serum, urine and body effusion fluid).

In the first part of the method development separation of vancomycin standard solution and cefuroxime (internal standard) was done. The best chromatographic conditions were carried out by Kinetex™ C18 column, 2.6 μm particle size, 100 A, 50 × 4.6 mm (Phenomenex, Torrance, USA) in combination with potassium phosphate buffer (pH 4.5) and acetonitrile (90:10, v/v) as the mobile phase. For analytes determination UV detection at 220 nm wavelength was applied. Real patient serum samples, after simple protein precipitation with zinc sulphate (4%) and methanol, were analyzed.

This new HPLC-UV method will be applied in clinical study interested in optimizing antibiotic dosing in surgical patients with Systemic Inflammatory Response Syndrome (SIRS) caused by multi-trauma or serious bacterial infection and accompanied with fluid sequestration.

The study was supported by the project SVV 260 184; the European Social Fund and the state budget of the Czech Republic, TEAB project no. CZ.1.07/2.3.00/20.0235. and IGA Ministry of Health project NT14089-3/2013.

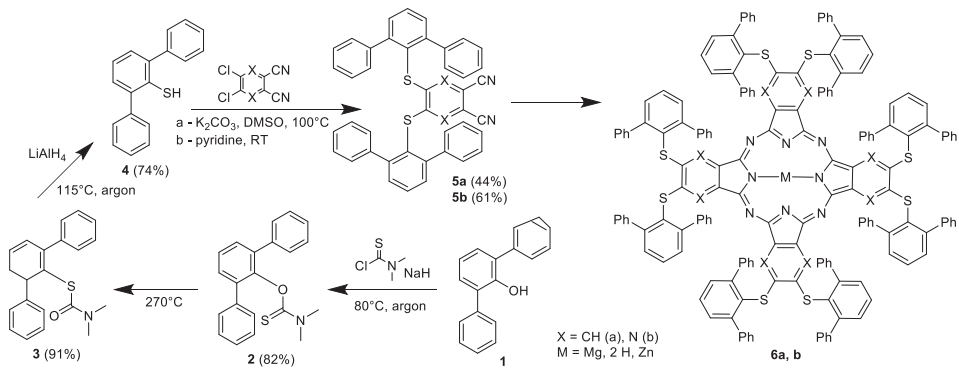
PHTHALOCYANINES AND THEIR AZA-ANALOGUES WITH BULKY DIPHENYLPHENYLSULFANYL SUBSTITUENTS

MÁLKOVÁ, A., ZIMČÍK, P.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: malkova1@faf.cuni.cz

Phthalocyanines (Pcs) and their aza-analogues (AzaPc), chemical substances used in photodynamic therapy, are characterized by interesting photophysical properties which may substantially vary in dependence on the character of peripheral substituents. For example, sum of singlet oxygen and fluorescence quantum yields reaches typically a value of one for Mg and Zn complexes while is significantly decreased for metal-free derivatives. It has been suggested from several previous experiments that this effect can be influenced by bulkiness of the peripheral substituents. The aim of this work is therefore the synthesis of bulky 2,6-diphenylphenylsulfanyl substituted Pcs and AzaPcs and afterwards their photophysical characterisation.

The synthesis started from 2,6-diphenylphenol (**1**), a commercially available substance, which was converted to *O*-carbamothioate **2** with dimethylcarbamoyl chloride. Isomeric *S*-carbamothioate **3** was prepared from *O*-carbamothioate using Newman-Kwart rearrangement at high temperatures and then reduced to thiol **4** with $LiAlH_4$. The thiol was used for the nucleophilic substitution of two dicarbonitrile precursors with pyrazine (**5b**) and a benzene ring (**5a**). Subsequent cyclotetramerisation and following exchange of the central cations led to the Pc and AzaPc macrocycles **6a,b** bearing different central cations (Mg, 2H, Zn) that were subject of the following photophysical study.



The study was supported by SVV 260 183.

NMR SPECTROSCOPY – THE IDENTIFICATION OF THE ISOLATED SUBSTANCE FROM *NERINE BOWDENII*

MAŘÍKOVÁ, J., NOVÁK, Z., KUNEŠ, J.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: marikoj2@faf.cuni.cz

The sample was obtained from the Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Hradec Králové and was isolated from plant *Nerine bowdenii* (Amaryllidaceae). The family of Amaryllidaceae is well known for the presence of alkaloids, especially large group of isoquinoline-derived alkaloids.

The unknown substance was characterized employing basic ^{1}H and ^{13}C NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC and gCOSY. After identifying the substructural fragments, the final skeleton (Fig. 1) of molecule was constructed.

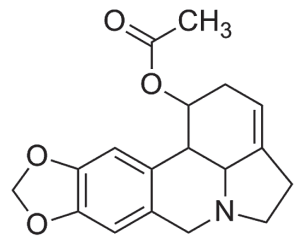


Fig. 1.

The resultant isoquinoline alkaloid has not been yet fully characterized in the literature.

This work was supported by the Charles University (SVV-260-183).

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HIGH THROUGHPUT METHOD FOR DETERMINATION OF CAFFEINE IN COFFEE DRINKS

MIHALČÍKOVÁ, L., BOONJOB, W., SKLENÁŘOVÁ, H., SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic

Caffeine is a xanthine alkaloid acting like a stimulant of heart and central nervous system. Quantification of caffeine in coffee drinks is significant to show how much of caf-

feine was in each cup which has been taken per day prior to prevent a caffeine overdose. The development of high-throughput sequential injection analysis (SIA) spectrophotometric assay for the determination of caffeine in coffee drinks was performed. Sample was treated with carrez reagent for matrix suppression followed by filtration thereafter analyte was isolated from organic acids by a short monolithic column. The flow rate was 10 μL s^{-1} with 10% *v/v* of methanol as the elution solvent. Caffeine was detected directly at 274 nm. The influence of main parameters affecting the quantification of caffeine were optimized. Under optimal conditions, the method was successfully applied to determine caffeine in different real samples including the soluble coffee, coffee from espresso machine and brewed-coffee drinks. The limit of detection (LOD) and limit of quantification (LOQ) were 0.01 and 0.03 mg L^{-1}, respectively. Linear range was 0.03–15 mg L^{-1} and determination coefficient (r^2) was 0.9969. The relative standard deviation (RSD) was 4.5% (n = 3).

The study was supported by the specific research, No. SVV 260 184.

DESIGN AND SYNTHESIS OF NOVEL CENTRALLY ACTING CHOLINESTERASE REACTIVATORS

NGUYEN, T. D.,[1,2] KORABEČNÝ, J.,[2,3] NEPOVIMOVÁ, E.,[2,3] KUČEROVÁ-CHLUPÁČOVÁ, M.,[1] KUČA, K.[3]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[3] Biomedical Research Center, University Hospital, Hradec Králové, Charles University, Czech Republic
e-mail: nguyentd@faf.cuni.cz

Exposure to the organophosphates (OP), which are used in the form of pesticides (e.g. paraoxon, malaoxon) or as warfare nerve agents (OPNAs, e.g. tabun, sarin, soman) can have fatal consequences. The toxic effect involves irreversible inhibition of acetylcholinesterase (AChE) that causes an accumulation of acetylcholine in central and peripheral synapses leading to overstimulation of the cholinergic receptors, seizures and ultimately respiratory arrest and death. Current treatment for OPNAs intoxication combines an antimuscarinic drug, anticonvulsant drug and AChE reactivator based on pyridinium aldoxime scaffold.

The design strategy, that we introduce, combines tacrine moiety (itself or its structural modifications: 7-MEOTA, 6-chlorotacrine, 7-phenoxytacrine) and pyridine-4-aldoxime *via* various linkers. The major advantage of such reactivators is that the binding of tacrine moiety to the peripheral anionic site allows the oxime better access to the catalytic anionic site of AChE. We assume that despite the presence of permanently charged pyridinium moiety, the molecule will still be lipophilic enough to cross the blood-brain barrier and be able to reactivate OP-inhibited brain AChE. Combinations of structures like tacrine with pyridine-4-aldoxime represent a promising approach for further drug development in this field.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011), specific research (No. SV/FVZ201409) and SVV 260 183.

EVALUATION OF STABILITY OF NOVEL AROYLHYDRAZONES IN PLASMA USING HPLC

PANAGIOTIS, M.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: mingasp@faf.cuni.cz

Aroylhydrazone iron chelators are potential drug candidates which both *in vitro* and *in vivo* pharmacodynamics studies demonstrated promising antioxidant and cardioprotective properties. Salicylaldehyde isonicotinoyl hydrazone (SIH) is the compound of the aroylhydrazone class that was the most thoroughly studied. The results that were obtained showed that its hydrazone bond makes SIH susceptible to hydrolysis in biological materials, decreasing its biological half-life. Various novel derivatives were developed in the direction of improving the stability of SIH in biological materials. The main aim of the research was firstly, to develop suitable chromatographic methods for the analysis of 8 novel iron chelators of the aroylhydrazone class (H21, H22, H23, H24, H25, H26,H32, H54) and secondly, utilize those methods to evaluate their stability in plasma *in vitro*.

The appropriate separation of all compounds, their precursors and the different internal standards was achieved on reversed stationary phase (Ascentis C18, 100 × 3 mm, 3 μm, Sigma-Aldrich) protected by the same type of guard column. The mobile phase was composed of a mixture of 10 mM of phosphate buffer (with the addition of 2mM EDTA) with either methanol or methanol/acetonitrile in various ratios. Flow rate of 0.3 ml/min, a column temperature of 25 °C and an injection volume of 20 μl were utilized. The UV detection was performed at a maximum absorbance for each compound.

The stability of the eight different compounds in rabbit plasma *in vitro* (100 μM, 37 ± 0.5 °C, 10 hours) displayed different results. Most of the tested chelators demonstrated clearly superior stability comparing to SIH, whose concentration decreased to 10% of its initial concentration after 3 hours of incubation. A drop in concentration down to 66.9% in 4 hours followed by further decrease to 40.6% after 10 hours was observed for H26 chelator which belongs to the compounds that decomposed quickly. For H23 chelator a decrease to 77.9% after 2 hours of incubation and a further decomposition of 33.7% was observed after 10 hours. The rest of the compounds demonstrated a smaller degree of decomposition.

The work was supported by SVV 260 183.

TACRINE-HYNIC HETERODIMERS – ANTICHOLINESTERASE AND ANTIOXIDANT LIGANDS WITH GOOD TOXICOLOGICAL PROFILE

PHAM, N. L.,[1,2] NEPOVIMOVÁ, E.,[2,3] KORABEČNÝ, J.,[2,3] OPLETALOVÁ, V.,[1] KUČA, K.[3]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[3] Biomedical Research Center, University Hospital Hradec Králové, Charles University, Czech Republic
e-mail: phama92@gmail.com

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by general cognitive impairment such as memory loss, disorientation and behavioral issues that gradually lead to dementia. Multifactorial nature of AD includes loss of cholinergic function, protein misfolding and aggregation, oxidative stress and free radical formation, mitochondrial abnormalities, and neuroinflammation. Also the etiology of the disease has not been fully understood yet. All these facts make the therapy very difficult. In addition, the therapeutic options on the market are severely narrow: acetylcholinesterase (AChE) inhibitors – tacrine, donepezil, galantamine, rivastigmine; *N*-methyl-*D*-aspartate (NMDA) receptor antagonists – memantine. These drugs are only able to hit a single target in organism and that is one of the reasons why pharmacotherapy is not sufficient. Therefore, the drugs which are able to affect multiple targets have a great potential in the treatment of neurodegenerative diseases; these are so called multi-target-directed ligands (MTDLs).

In this work, we focused on the design of new tacrine (THA) heterodimers with antioxidant activity, specifically tacrine-6-hydrazinylnicotinamide (THA-HYNIC) compounds. THA was the first AChE inhibitor launched to the market against AD. Additionally, due to its synthetic accessibility it remains the cornerstone in AD drug discovery. Involvement of HYNIC moiety as a derivative of vitamin B_3 was expected to provide antioxidant properties. *In vitro* assays performed on the whole series have shown that these compounds inhibit cholinesterases in micro-nanomolar concentration scale. Moreover, DPPH assay revealed that these heterodimers exhibit even better antioxidant properties compared to standard antioxidants (trolox, *N*-acetylcystein). Finally, the acute toxicity of three selected candidates demonstrated better toxicological profile than tacrine. Therefore, pursuant above-mentioned results we may assume that THA-HYNIC heterodimers could be interesting candidates for further studies as potential AD drugs.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by the University of Defence (Long Term Development Plan – 1011), specific research (No. SV/FVZ201409) and Charles University SVV 260 183.

OPTIMIZATION, VALIDATION AND COMPARISON OF UHPSFC AND UHPLC METHODS FOR THE DETERMINATION OF AGOMELATINE AND ITS IMPURITIES

PLACHKÁ, K.,[1] CHRENKOVÁ, L.,[1] DOUŠA, M.,[2] NOVÁKOVÁ, L.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Zentiva, k.s. Praha 10, Czech Republic
e-mail: plachkka@faf.cuni.cz

Agomelatine is a synthetic compound with chemical structure N-(2-(7-methoxynapht-1-yl)ethyl)acetamide (Fig. 1). It is an analogue of epiphysis hormone melatonin and the first antidepressant from a new group of melatonin agonists and selective serotonin antagonists (MASSA). By influencing MT_1, MT_2 and $5\text{-}HT_{2C}$ receptors agomelatine regulates circadian rhythms and the release of noradrenaline and dopamine. This effect allows its indication for treatment of depression disorders in adults.

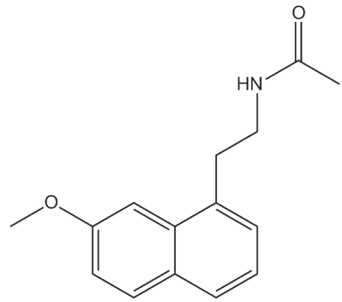

Fig. 1. The structure of agomelatine.

The aim of this study was to develop and validate UHPSFC and UHPLC methods with UV detection for the separation and the determination of the group of structurally similar substances, agomelatine and its six impurities: (7-methoxynapht-1-yl)ethylamine hydrochloride, (7-methoxynapht-1-yl)acetonitrile, bis[2-(7-methoxynaphtalen-1-yl)-ethyl] amine, (7-methoxynapht-1-yl)acetamide, (7-methoxynapht-1-yl)acetonitrile, (7-methoxynapht-1-yl)acetic acid.

Although these substances are structurally close to agomelatine, their physicochemical properties differ substantially. Therefore, one of the main goals was to compare the retention and selectivity not only in supercritical fluid chromatography system and liquid chromatography system but also under different separation conditions. The UHPSFC separations were accomplished using four different stationary phases (Acquity UPC^2 BEH, Acquity UPC^2 BEH 2-EP, Acquity UPC^2 CSH PFP and Acquity UPC^2 HSS C18), all of them with 100 × 3.0 mm dimension and with particles sizes 1.7 μm. Gradient elution was performed using modified CO_2 with gradient program started at 5% of modifier and ended at 30% in 3 minutes. Methanol with different additives including 20mM ammonium acetate, 20mM ammonium formate and 20mM ammonium formate with addition of 5%

of water was used. Flow rate was set at 2 ml/min, the temperature at 40°C and BPR at 2000 psi. The UV detection was performed by Acquity UPC^2 PDA detector at 275 nm. The column BEH 2-EP and gradient elution with 20mM ammonium formate with the addition of 5% of water were chosen due to the best selectivity and resolution results.

The stationary phases for UHPLC system included Acquity UPLC CSH C18, Acquity CSH Fluoro-Phenyl, Acquity UPLC BEH Shield RP18, Acquity UPLC BEH Phenyl and Acquity UPLC BEH C18 with column dimension 2.1 × 50 mm × 1.7 µm or 2.1 × 100 mm × 1.7 µm. Methanol, acetonitrile and a mixture of acetonitrile and methanol in the ratio 1:1 were tested as a organic component of mobile phase using gradient elution with different gradient slopes, gradient curves and buffers (pH 2.0, 3.0, 9.0, 9.5 and 10.0). The column temperature was 30°C and the UV detection was performed at 275 nm. The final conditions were chosen as follows: column BEH Shield RP18, gradient elution with a mixture of methanol and acetonitrile (ratio 1 : 1) and buffer with pH 9.5 started at 5% and increased up-to 70% within 5 minutes under gradient curve number 4.

Both developed methods were properly validated according to ICH guidelines. The methods were validated in terms of linearity, sensitivity (LOD, LOQ), accuracy and precision. The UHPSFC method was linear in the range 0.7–70 µg/ml for all analytes with accuracy and precision ≥ 95.5% and RSD ≤ 2.4 for impurities and ≥ 97.6% and RSD ≤ 0.9 for API. The UHPLC method was linear in the range 0.1–10 µg/ml with accuracy ≥ 95.7% and RSD ≤ 2.6 for impurities and ≥ 95.2% and RSD ≤ 1.5 for API.

The measurement of real samples of agomelatine tablets was performed and the methods were compared in the selected parameters as shown in Fig. 2.

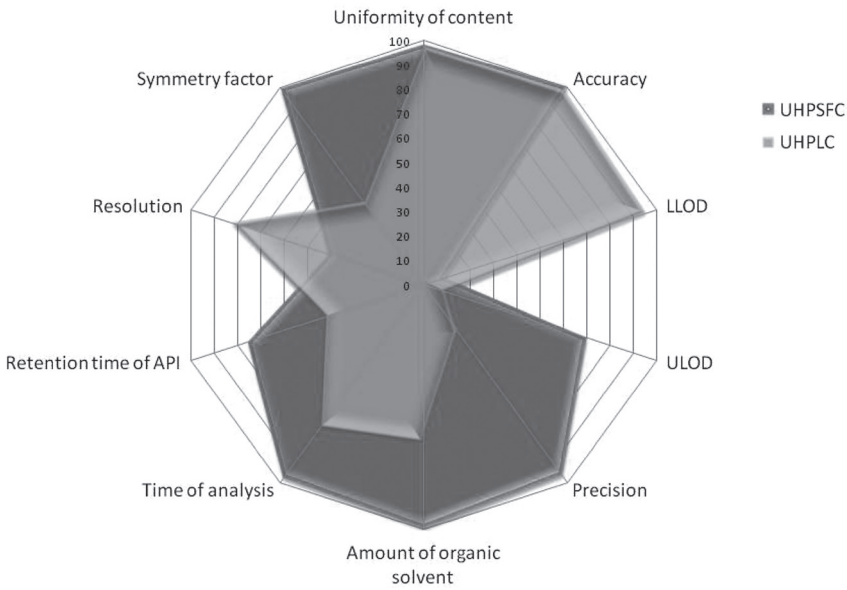

Fig. 2. The comparison of UHPSFC and UHPLC methods in selected parameters.

The study was supported by SVV/2015/260184.

THE FAST HPLC METHOD FOR DETERMINATION OF ARGININE AND ITS METABOLITES IN MONITORING OF WOUND HEALING PROCESS

PISKÁČKOVÁ, H.,[1] AUFARTOVÁ, J.,[1,2] KUJOVSKÁ-KRČMOVÁ, L.,[1,2]
SOLICHOVÁ, D.,[2] SOLICH, P.,[1] SOBOTKA, L.[1]

[1] III. Internal Gerontometabolic Clinic, University Hospital Hradec Králové,
Charles University, Czech Republic
[2] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: piskacha@faf.cuni.cz

Wound healing is characterized by three phases – inflammatory, proliferative, and maturation. Nevertheless, the relationship among these phases is not always linear since this process can progress forward and backward. The healing process depends on intrinsic and extrinsic factors. Long-acting negative influences may disrupt this process and lead to chronic condition. Each phase is characterized by certain events that require specific components[1,2,3]

Wound healing is multi-factorial process, however, the nutritional factor have a basic role in their development. One of these factors is the level of arginine. Arginine is the sole precursor of nitric oxide, a signal molecule, among others, involved in immune responses, angiogenesis, epithelization and formation of granulation tissue, all essential aspects accompanying wound healing[4]. According to previous studies, the ratio of arginine and its metabolites, ornithin and citrulline, is expected to help in the treatment of chronic wounds as an indicator of the healing process[1,5].

The aim of this study was to develop fast and sensitive chromatographic method for analysis of arginine, citruline, and ornithin in wound exudates. Analytical determination was performed using HPLC system Prominence LC 20 Shimadzu (Koyto, Japan) with fluorescence detector, since low concentrations in complex matrix required the use of derivatization reagent. The mobile phase of sodium acetate buffer (pH 7.3) and mixture of acetonitril and methanol (9 : 2, v/v), respectively and a monolithic column Chromolith HighResolution, RP-18e, 100 × 4.6 mm (Merck, Darmstadt, Germany) were used. Sample preparation was performed by ultrafiltration using Microcon Centrifugal Filters (Merck, Millipore, Darmstadt, Germany).

The new HPLC-FD method for determination of arginine and its metabolites in wound exudates was developed. After full validation will be used for monitoring of chronic wounds treatment of patients at Internal Gerontometabolic Clinic in University Hospital, Hradec Králové.

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THE UTILIZATION OF THE NEAR INFRARED SPECTROSCOPY IN THE EVALUATION OF THE HOMOGENEITY OF THE POWDER BLEND

PLOT, J.,[1,2] MORÁVKOVÁ, K.,[1], PALÁT, K.,[2] ŠKLUBALOVÁ, Z.[1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: plotja@faf.cuni.cz

Working with the powder blends, particularly their mixing, is very commonly used method in pharmaceutical technology. In this area, the biggest problem is reaching for the homogeneity of the mixtures and its measurement. Mixing of active substances and excipients is one of the key factors in the preparating.

The main aim of the study was to introduce a suitable method for the evaluation the homogeneity of the powder blends by using near infrared spectroscopy (NIR). After that, it was monitored the appropriateness of this method with respect to its application during the routine mixing in Pharmaceutical Technology.

To study the homogeneity, the mixture of acetylsalicylic acid (ACS) and microcrystalline cellulose (MCC) with a concentration of active substance of 20% was prepared at different experimental conditions. The total amount of mixture 40 and/or 200 g was homogenized at speed 17 and 34 rpm in the mixing cube Erweka KB 15S. Five samples of mixture were taken at time of 0, 5, 10, 20, 40, 80 and 160 seconds. To prepare tablets, each sample was compressed on the material tester Zwick/Roell Z050. Infrared spectra were measured on a spectrometer Nicolet 6700 at wavenumbers in the range 10000 to 4000 cm^{-1}. In order to evaluate the concentration of ACS, the calibration tablets containing 0–20% of ACS were prepared and measured the same way. The area under the curve (AUC) was used in evaluation of the NIR spectra.

Testing the calibration samples, the best strip for a range of wavenumbers 9020–8750 cm^{-1} by using a horizontal baseline 9032 cm^{-1} was selected and used in evaluation of the homogeneity of powder blends. It was detected that the best homogenization was achieved with a larger amount of the powder blend at the faster speed; the homogeneity was completed after 10 seconds.

The study was supported by the student grant SVV 260 183.

HPLC METHOD DEVELOPMENT FOR ARTIFICIAL COLORANTS DETERMINATION IN GREEN BEER

STACHOVÁ, I.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: stachoi@faf.cuni.cz

Food colorants are an important class of food additives. They are widely used in drinks, juices, meat products and sweets to preserve or restore the natural color of food products and enhance appeal. Natural food dyes have been used more and more for consumer preference, however, synthetic food dyes are still widely used in food and feed industry because of their low cost and high stability. Most of the synthetic dyes show good resistance against degradation and pose little threat to human and animal health. But some of these substances and their metabolites pose potential health risk to human beings and may even be carcinogenic, especially when they are consumed in excessive amounts. Therefore, the use of synthetic dyes in foodstuff is strictly controlled by legislation throughout the world[1].

HPLC method was used and validated for the simultaneous determination of synthetic water-soluble dyes: E 102 – tartrazine, E 104 – Quinoline Yellow, E 110 – Sunset Yellow, E 131 – E 132 Patent blue – Indigo carmine, E 142 – Green S, E 133 – Brilliant Blue FCF and E 143 – Fast Green FCF. The method was applied for direct determination of these dyes in samples of green beers Jarní pivo 11° (Primátor, Náchod), Krasličák 14° (Ježek, Jihlava), Zelený král Vratislav 12° (Konrád, Vratislavice), Velikonoční speciál 14° (Starobrno, Brno), Velikonoční ležák 12° (Radniční pivovar, Jihlava).

Analytical Chromolith Performance CN 100 × 4.6 mm and guard column Chromolith CN 5 × 4.6 mm Merck were used and mobile phase contained 40% (v/v) methanol / 2% (v/v) acetic acid buffer with addition of ammonia for pH adjustment to value 7.0. Successful separation was obtained for all the compounds using an optimized gradient elution within 12 minutes. Analysis was carried out at temperature of 30 °C and the flow rate 2 ml/min, the injection volume was set at 10 µl. The diode-array detector (DAD) was used for monitor the dyes at the 3 wavelenghts 420 nm ((Tartrazine a Quinoline yellow), 482 nm (Sunset yellow) a 625 nm (Indigo carmine, Green S, Brilliant blue FCF, Patent blue a Fast green FCF).

The study was supported by: SVV 260 184.

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PHYSICO-CHEMICAL PROPERTIES OF DRUGS – MEASUREMENT OF DISSOCIATION CONSTANT AND USAGE IN PRACTICE

SUCHÝ, M.

Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: suchymi1@faf.cuni.cz

To test physico-chemical properties of new molecules is necessary during drug development. It could be helpful to understand or predict the pharmacocinetic parametres of a new drug *in vivo*/*in vitro* experiments.

One of this parameters is a dissociation constant (pK). Dissociation constant is defined as "Number on pH scale, wherein is just fifty percent of molecule in a ionization condition". In real case this number can help us to know where in the gastro-intestinal tract (GIT) the drug will be absorbed. In GIT only molecules exhibiting pK from 3 to 11 could be absorbed. Out of this range it is not possible.

In this work I would like to introduce the ways of experimental measurement of pK values. I am working with two methods to measure the pK values of water-soluble compounds. The spectrophotometric method and the potentiometric one. Water-insoluble compounds can be experimentally measured, too. The dissociation constant is affected by functional groups in the molecule. So I also tried to compare a variety of functional groups on pyrazine heterocyclic to show how a pK value is changed by introduction of various functional groups. I also studied if using of both method to measure every compounds is possible, or not.

The study was supported by Ing. Vladimir Kubiček, CSc. SVV – 260 183.

TACRINE-BENZOTHIAZOLE HYBRIDS NOVEL MULTITARGET AGENTS TO COMBAT ALZHEIMER'S DISEASE

SVOBODOVÁ, L.,[1] NEPOVIMOVÁ, E.,[2,3] KORABEČNÝ, J.,[2,3] KUČEROVÁ-CHLUPÁČOVÁ, M.,[1] KUČA, K.[3]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[3] Biomedical Research Center, University Hospital Hradec Králové, Charles University, Czech Republic
e-mail: luca.svobodova@gmail.com

Alzheimer's disease (AD) is a progressive fatal neurodegenerative disorder and the most common type of dementia. It is manifested by a variety of neuropsychiatric symptoms such as memory loss, visuospatial deficits etc. Ethiology of the disease has a multifactorial character and is not well known. Among the major pathological features belong: presence of extracellular amyloid plaques, mainly represented by amyloid-beta peptide, intracellular aggregates of hyperphosphorylated tau protein and neuronal loss, especially of cholinergic

neurons. Also the oxidative stress of the neuronal cells contributes to the patophysiology of the disease. Because AD is affected by the multiple factors, the main strategy of the treatment is to affect the multiple targets in the brain as well. Such drugs are denoted as multitarget-directed ligands (MTDLs) and they target the different molecular abnormalities of AD.

In our contribution we would like to introduce tacrine-benzothiazole hybrids combining tacrine with the benzothiazole moiety. Linkers of different lenghts were used to connect these two scaffolds. Tacrine was the first drug approved for AD treatment by FDA. Its mechanism of action is based on inhibition of cholinesterases and thereby increasing the levels of synaptic acetylcholine. On the other hand, benzothiazole, as a planar molecule, could prevent the protein–protein interactions and thus could have anti-amyloid effect. Moreover, benzothiazole moiety represents the core of inhibitors of amyloid-binding alcohol dehydrogenase (ABAD). ABAD is a mitochondrial enzyme that contributes to oxidative stress in AD progression. Therefore its inhibition could avoid ROS production and act neuroprotectively. Pursuant above-mentioned facts, molecules bearing tacrine and benzothiazole motif could become promising drug candidates in AD therapy. Nevertheless, just *in vitro* and *in vivo* determination of biological activity will reveal their real value in the field of Alzheimer's disease.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011) specific research (No. SV/FVZ201409) and by Charles University, Faculty of Pharmacy, specific research (No. SVV 260 183).

SYNTHESIS OF SULFONAMIDE ANALOG OF CARDIOPROTECTIVE DRUG DEXRAZOXANE

ŠKODA, J.,[1] ROH, J.,[1] VÁVROVÁ, K.,[1], ŠIMŮNEK, T.[2]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: skodajo@faf.cuni.cz

Anthracyclines such as daunorubicin or doxorubicin are widely used anticancer drugs. However, the administration of anthracyclines is connected with cardiotoxicity leading to irreversible damage and congestive heart failure. The reason of their toxicity is unknown yet, there are two main theories. It is assumed that the complexation of anthracyclines with intracellular iron ions catalysis the formation of reactive oxygen species. The second theory involves inhibition of topoisomerase II. The only known drug effective against the anthracycline cardiotoxicity is dexrazoxane (DEX). The mechanism of cardioprotection is also unknown yet. One theory involves chelation of iron ions, the second involves interaction with topoisomerase II in heart. In this study we deal with the synthesis of a new analog of DEX and with the study of its cardioprotective effect (Fig. 1).

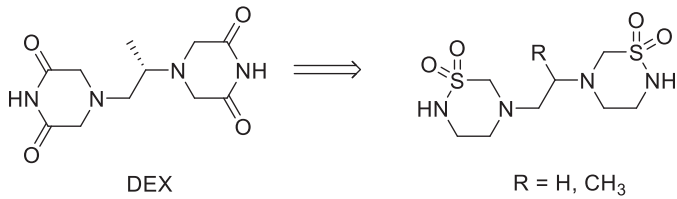

Fig. 1. Structure of dexrazoxane (DEX) and its sulfonamide analog.

The new analog was designed to have sulfonamide group, which mimics the original imide group in DEX and importantly has similar acidity. In the case when R = H synthesis started from triethylenetetramine. In the case when R = CH_3, 1,2,5-thiadiazinane-1,1-dioxide was prepared and subsequent reaction with 1,2-dibromopropane would provide target compound.

The study was supported by the Charles University (Charles University Research Centre UNCE 204019/304019/2012 and project SVV 260 183).

WHEN –CONH– BECOMES –NHCO–: PYRAZINYL BENZAMIDES AS POTENTIAL ANTITUBERCULARS

VALÁŠEK, O., PATEROVÁ, P., ZÍTKO, J.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jan.zitko@faf.cuni.cz

Rising incidence of mycobacteria resistant to known antitubercular drugs opens new space for the search for new antitubercular compounds. Our work was aimed at new derivatives of pyrazinamide, more specifically on derivatives of 5-chloro-*N*-phenylpyrazine-2-carboxamides (**I**) with various substituents on the phenyl ring, which were previously shown to possess significant *in vitro* antimycobacterial activity (MIC = 0.78–3.13 μg/mL *M. tuberculosis* H37Rv).[1] Chemical modifications or model compound (**I**) were focused on the carboxamide moiety, which was inverted from CO–NH to NH–CO.

Final compounds **II** were prepared by aminolysis (Fig. 1) of commercially available benzoyl chlorides by 5-chloro-2-aminopyrazine in dichloromethane as a solvent, maximiz-

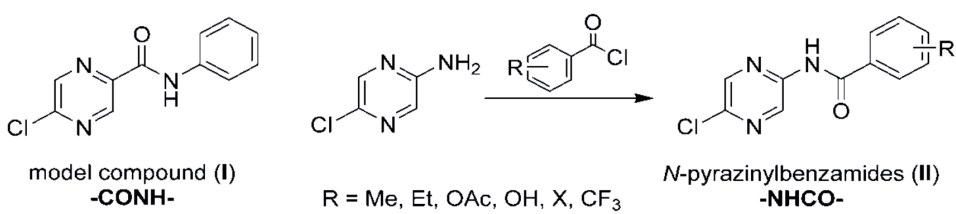

Fig. 1. Model compound I and synthesis of final compounds II.

ing the yields by working in non-aqueous environment. Compounds with R = OH were obtained using the acetyl protected chlorides of hydroxybenzoic acids. Final compounds were characterized by ^{1}H and ^{13}C NMR, IR, elementary analysis and melting point.

Final compounds will be tested for *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv, *M. kansasii, M. avium* and *M. smegmatis*. Additionally, compounds will be tested for activity against selected bacterial and fungal strains of clinical importance. The results and structure-activity relationships will be discussed.

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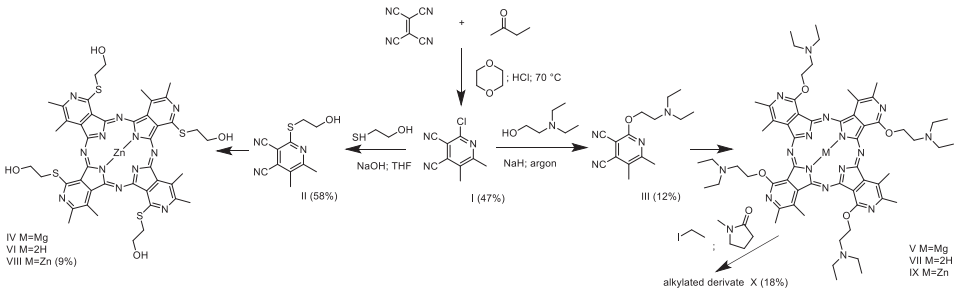
SUBSTITUTED TETRA(3,4-PYRIDO)PORPHYRAZINES AS POTENTIAL PHOTOSENSITIZERS

VAVREČKOVÁ, M.,[1] ZIMČÍK, P.,[1] MACHÁČEK, M.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: vavrecma@faf.cuni.cz

Substituted tetra(3,4-pyrido)porphyrazines represent new structural type of potential photosensitizers with interesting properties in the area of photodynamic therapy (PDT). The aim of this work was to synthesize two types of tetra(3,4-pyrido)porphyrazines with hydrophilic substituents as potential photosensitizers. Photosensitizers are substances with an ability to produce singlet oxygen, the key toxic species in PDT, after irradiation.

2-Chloro-5,6-dimethylpyridine-3,4-dicarbonitrile (I) was prepared in the first step by condensation of tetracyanoethylene and butan-2-one. In the next step, an hydrophilic substituent was attached by nucleophilic substitution. Compound II was prepared by reaction of I with 2-mercaptoethanol in aq. NaOH. Similarly, compound III was prepared by reaction with *N*,*N*-diethylaminoethanol in the presence of NaH. The third step involved cyclotetramerization with magnesium butoxide as initiator that gave Mg complexes (IV, V). Mg complexes were converted to metal-free derivatives (VI, VII) and then to Zn complexes (VIII, IX). Complex IX was subsequently quaternized by ethyl iodide to the final



compound (X). Zn complexes VIII and X were tested for photodynamic activity and toxicity on tumor HeLa cells.

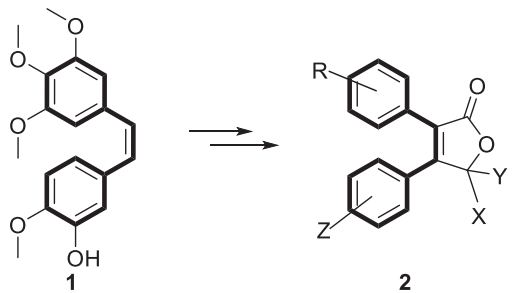
The study was supported by SVV 260 183 and Czech Science Foundation 13-27761S.

SYNTHESIS OF COMBRETASTATIN ANALOGUES AS POTENTIAL ANTITUMOR AND ANTIMICROBIAL AGENTS

VORÁČOVÁ, M.,[1] HORKÝ, P.,[1] VACEK, J.,[2] POUR, M.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Czech Republic
e-mail: voracom@faf.cuni.cz

Combretastatins are naturally occurring molecules possessing remarkable *in vitro* cytotoxicity against human cancer cell lines. These compounds, such as combretastatin A-4 **(1)**, are known to disrupt mitosis through the inhibition of tubulin assembly. Furthermore, some of them also exhibit antivascular and antiangiogenic effects.[1] The compounds, however, are highly lipophilic and insufficient in terms of chemical stability. Our aim was, therefore,



to synthesize a library of α,β-diphenyl furanones analogous to combretastatins with improved pharmacological properties and subject it to biological activity screening.

Variously functionalized α,β-diphenyl furanones are possible to be obtained from commercially available acetophenones and phenylacetic acids in good to excellent yields. Derivatizations of aromatic cores as well as of γ-position are subsequently performed in order to improve the solubility in aqueous media.

To date, a series of compounds (**2**) was prepared and screened for cytostatic and antimicrobial activity. Our molecules display interesting both antineoplastic and antibacterial activities in micromolar concentrations. Structural modifications responsible for boosting antimicrobial effects were observed.

The study was supported by Charles University (SVV-260-062 and GA UK 1906214) and Czech Science Foundation (P207/10/2048).

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SECTION OF OTHER PHARMACEUTICAL SCIENCES

CONSISTENCY OF THE SEMISOLID PREPARATIONS

HÁNOVÁ, A., ŠNEJDROVÁ, E.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: hanovaa@faf.cuni.cz

Semi-solid preparations are intended for local or transdermal delivery of active substances, or for their emollient or protective action. They consist of a simple or compound base in which, usually, one or more active substances are dissolved or dispersed. The base is not inert carrier of an API but influences the effect of the preparation. Commonly used hydrophobic bases are yellow and white soft paraffin, purified mixture of semisolid saturated hydrocarbons obtained from petroleum. Only for these bases the measurement of consistency by penetrometry as control of quality is required in the pharmacopoeia. The question is why among all hydrophobic viscosifiers just only soft paraffin must be tested and how its consistency should be changed not to comply with pharmacopoeial requirement.

In our study, white soft paraffin was mixed either with liquid paraffin or with solid paraffin to change its consistency. The samples for penetrometry measurement were prepared either by methods A or method C according to Ph.Eur., and measured at 25 ± 0.5 °C. The consistency of the samples was significantly affected by the chosen method of preparation. The value of consistency of samples prepared in semisolid state (method A) is 215, the consistency of the samples prepared by melting (method C) is 143 i.e. by 44% less. The addition of 10% of liquid paraffin increased the value of consistency in 290, near to the

upper pharmacopoeial limit in case of method A, while in case of method C the value of consistency was not influenced. The value of consistency of white soft paraffin with 10% of solid paraffin was significantly decreased, samples with 20% of solid paraffin prepared by melting even did not comply with pharmacopoeial requirement.

The result of the test of consistency by penetrometry i) can be influenced with method of preparation of the samples, ii) is required only for yellow and white soft paraffin, and iii) limit is in very wide range. Rheological characteristics as consistency coefficient and flow index, or yield stress and viscosity on yield stress can be suggested as more suitable and more useful.

The study was supported by SVV 260 183.

ONLY ONE THIRD OF PATIENTS WITH COPD IS FULLY ADHERENT TO INHALATION THERAPY

HRUZDOVÁ, H.,[1] TOUŠKOVÁ, T.,[1] VYTŘÍSALOVÁ, M.,[1] ŠVANCARA, J.,[2] NOVOTNÁ, B.,[3] KOBLÍŽEK,V.[3]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Pulmonary Department, Faculty of Medicine and University Hospital in Hradec Králové, Charles University, Czech Republic

Patient adherence to treatment in chronic obstructive pulmonary disease (COPD) is essential to optimise disease management. Poor adherence is common and results in increased rates of morbidity, healthcare expenditures, hospitalisations and possibly mortality, as well as unnecessary escalation of therapy and reduced quality of life. The major problem is failure to adhere to inhaler technique. The aims of this project were to assess inhaler adherence for different types of devices and to analyze various aspects of adherence in a cohort of patients with severe COPD in the Czech Republic.

An observational multicentre study with the participation of 12 centres in the Czech Republic was conducted in cooperation with the Czech Multicentre Research Database of COPD (http://chopn.registry.cz). The inclusion critera are: severe COPD without fibrosis and FEV1 < 60%. The assessment was structured into five steps to be followed while using an inhaler. Adherence to each step was assessed in a dichotomous manner (performed properly/improperly). Each respondent was asked to report how often he/she rinses his/her mouth after using the corticosteroid inhaler (always: 75–100%, sometimes: 25–75%, never: 0–25%).

Three hundred and forty-three patients were enrolled in the study (mean age of 67 years). They used various types of inhalers, sometimes in combination. The most often used devices were pressurized metered-dose inhaler (pMDI) (N = 171) and dry powder inhalers (DPIs) – Handihaler (N = 151), Aerolizer (N = 146) and Diskus (N = 68). The assessment of the adherence to inhalation technique revealed that less than 35% of the study cohort adhered properly to each of the five steps. Correct adherence to each inhaler was following: pMDI (31.9%), Handihaler (28.2%), Aerolizer (27.2%) and Diskus (12.7%).

For all types of inhalers, the highest rate of failure to was observed for step 3 (failure to breathe out completely in one breath before taking the medicine with the next breath). After using an inhaler containing corticosteroid, 62% of respondents always rinse out their mouth, 29% sometimes, and 9% never. Patients who fully adhere to inhaler use technique rinse out their mouth every time after inhalation of corticosteroids in 71% of cases while those who fail to use the inhaler properly only in 57% of cases ($p = 0.036$).

Patients failed to adhere to inhaler technique more often while using aerosol inhalers. Powder inhalers are used improperly by more than half of the respondents. The most common error was complete exhalation before inhaling the drug. Patients who adhere to inhaler technique rinse out their mouth after inhalation of corticosteroids more often than those who failed to adhere to one or more steps.

The study was supported by project SVV 260 187. The COPD project is registered in Clinical Trials.gov with the identifier NCT01923051.

PREPARATION OF MICROPARTICLES BY MICROFLUIDIC METHOD

HUSÁROVÁ, P.,[1,2] TOURNÉ-PÉTEILH, C.,[2] ŠKLUBALOVÁ, Z.[1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Technology, Faculty of Pharmacy in Montpellier, Université de Montpellier, France
e-mail: husarovp@faf.cuni.cz

The aim of this work was to prepare hydrogel microparticles based on silated-hydroxypropyl methylcellulose (HPMC-Si). The microparticles are expected to be obtained by self-hardening of HPMC-Si from the microdroplets formed by the emulsification in the continuous phase.

The microparticles were prepared by microfluidic method. This method is based on phase separation of a droplet in a non-miscible continuous phase by using microchannels. A 3% w/w solution of HPMC-Si in the sodium hydroxide solution 0.2M (pH 13.2), the HEPES buffer (pH 3.5) and a fluorescent dye FITC-Si were utilized to form microparticles. Sesame oil was tested as a continuous phase.

In order to form microparticles of controlled size and to improve their stability, various experimental conditions were tested. Parameters like temperature, speed rate of the continuous and dispersed phase, length of the microchannel and the use of the surfactant Plurol in a concentration of 1% to 3% were investigated.

The results showed that the particles were well spherical and more uniform by choosing suitable values of speed rate. By influencing the temperature and the length of the outlet microchannel, the stability of particles was ameliorated minimizing the fusion of particles. Although the results are preliminary, this research proved that it is possible to prepare microparticles and encapsulate FITC-Si using microfluidic method.

The study was supported by student grant SVV 260 183 and Erasmus+ programme.

PHARMACEUTICAL CARE AND CONTINUAL PROFESSIONAL DEVELOPMENT IN GREECE

KARAGEORGIOU, E., KOLÁR, J.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: karageoe@faf.cuni.cz

The political line of the health systems in EU is patients oriented and demands increased rates of training and well understanding of pharmaceutical services and pharmaceutical care. Either in hospital pharmacies or in community pharmacies. Almost half of the annual pharmaceutical expenses accrued by the so called expensive drugs special category. Almost half of the annual turnover of community pharmacies is of the OTCs and the negative list of medicines. So examine the aspects of Continuing Professional Development (CPD) within deepened economic crisis in Greece in order to find professional solutions for the near future as well as by means of survival.

We deal with European surrounding and the present situation in basic education and continual training of community pharmacies in Greece taking into account the assumption that community pharmacies are part of the primary health care. We trace the real needs of well-trained community pharmacists to improve value for both the benefit of patients and the Health Care System.

It is the outcome of collaboration with the Institute for the Continual Training and CPD (IDEEAF) of PanHellenic Pharmaceutical Association which merely performs the unique thorough effort on the matter in Greece under the auspices and financing by pharmaceutical companies. The method of study includes a disseminated simple questionnaire to all those freely participating to IDEEAFs lessons.

The anonymous responds give a first impression and we can conclude in the following points.

a) The urgent dramatic changes – as main proposal – of the basic educational program within pharmacy faculties in Greece.
b) The direct collaboration of the PanHellenic Pharmaceutical Association (through IDEEAF) with international institutions specialized on social pharmacy.
c) The evaluation of pharmaceutical services by ETESTA the company of the PanHellenic Pharmaceutical Association for the research statistics and analysis of pharmaceuticals as basic pharmacoeconomic support.
d) The ongoing procedure of questionnaires in a second phase proposed by PGEU at a more centralized way.
e) The immediate establishment of an accreditation system certified by the PanHellenic Pharmaceutical Association.
f) A separate part of therapeutic categories of services for the emergency and/or the "heroic" drugs.

THE ANALYSIS OF THE CARE OF PHARMACY CLIENTS WITH THE RISK OF ARTERIAL HYPERTENSION

KOTLANOVÁ, L., MALÝ, J.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: kotlanol@faf.cuni.cz

Arterial hypertension (AH) is still very serious worldwide health problem. AH belongs among the most important risk factors for coronary heart disease, stroke, or peripheral arterial disease. Since, the detection and treatment of AH received considerable attention, in the clinical practice, satisfactory results have not been achieved yet. This problem can be improved by the involvement of pharmacists in the care of pharmacy clients as shown by published data. The aim of this work is to present the results of the analysis focused on the involvement of pharmacists in the care of pharmacy clients with the risk of AH.

Data were collected in one community pharmacy in the town with 3,500 inhabitants in Pilsen region from September 2014 to January 2015. Blood pressure measurement was included in each interview with the selected pharmacy client. The following data were recorded: socio-demographic characteristics; opinion on blood pressure measurement in a pharmacy; risk factors of AH or atherosclerosis; illness in anamnesis; using drugs including dietary supplements; the result of blood pressure measurement; interventions of pharmacist or identified drug-related problems. The measurement of blood pressure was carried out in accordance with valid current recommendations. The obtained data were evaluated using frequency analysis. Drug problems were classified according to the modified Pharmaceutical Care Network Europe classification V5.01.

Analysed data were obtained from 199 pharmacy clients (55.3% women; mean age 49.7 years). 30 clients, of which 12 clients were without diagnosed AH, had blood pressure above 140/90 mm Hg. Further, there were identified 43 drug-related problems related to pharmacotherapy of AH. Each client received the recommendation to promote the adherence to healthy life style or pharmacotherapy. If necessary, the recommendations, how to use medications, were provided. Some clients were recommended to visit their general practitioners.

The blood pressure measurement as a part of counselling in a pharmacy can be regarded as the suitable method for identification of persons with undiagnosed AH. Particular attention should be given to smokers or persons with a higher BMI. More detailed knowledge of anamnesis can contribute to optimization of treatment plan, e. g. by the identification and solution of drug-related problems.

The study was supported by SVV 260 187.

INFLUENCE OF EXCIPIENTS ON THE MECHANICAL PROPERTIES OF ORODISPERSIBLE TABLETS

MATOUŠKOVÁ, Z.,[1,2] STASIAK, P.,[2] ŠKLUBALOVÁ, Z.[1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Zentiva, k.s., Praha, Czech Republic
e-mail: jungrovz@faf.cuni.cz

Orodispersible tablets are modern solution to the administration of drugs, mainly characterized by a short disintegration time and rapid onset of drug effect. Due to the required disintegration in the oral cavity, a necessary step in the formulation is also a choice of suitable sweeteners and flavours to influence palatability.

This work studies the mechanical properties of orodispersible tablets containing an active ingredient (VF) in confrontation with the used pre-compression/compression force and selected excipients. Tablets were produced by a direct compression method using two combinations of pre-compression/compression forces. The effect of the addition of hypromellose 5–10% (Methocel E5) and/or crospovidone 5–15% (Polyplazdone XL) on tablet friability, disintegration time and strength was studied.

Experimental results shown that mechanical strength and disintegration time of tablets are directly influenced with compression force, however, individual formulations were also influenced by the composition of the tablet. At the higher concentration of hypromellose decrease in the strength of tablets was detected while the disintegration time was prolonged. A similar effect on the tablet strength was observed when crospovidone was used. Extension of disintegration time was observed only for the combination of lower pre-compression/compression forces. At higher forces, however, relationship was not completely linear. The strength and disintegration time of tablets was significantly affected by the addition of sweeteners sucralose and/or sodium saccharin in both formulations investigated.

The results of this work allow to suggest a suitable formula for orodispersible tablets for administration of the active ingredient (VF) with the optimum strength and required disintegration time.

The study was supported by student grant SVV 260 183 and Zentiva, k.s.

ETHICS IN PHARMACEUTICAL COMPANIES

NOVÁKOVÁ, S.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

According to Study on Corruption in the Healthcare Sector one third of people think that taking of bribes and abuse of positions of power for personal gain are widespread among people working in the public health sector. The most serious situation is in the certification and procurement of medical equipment and in the autorisation and procure-

ment of pharmaceuticals.[1] Problems of ethics are connected with pharmaceutical industry inherently. Therefore number of pharmaceutical companies try to enhance their reputation by various of ethical tools and activities. Nevertheless having the working business ethics program is very complex, difficult and neverending process. Ethics are part of all activities of business and if the ethics program is efficient it helps company increase its profit and meet its goals. Sophisticated ethics program includes ethical tools such as a code of ethics, an ethical audit, a corporate social responsibility, an education in ethics, an organisation structures, a stakeholder analysis, a whistleblowing and many more. The aim of this work is to describe these tools, a process of creating an ethics program and find out the advance of ethics program in pharmaceutical companies operating in the Czech Republic. For this purpose we used the questionnaire sent by post to the pharmaceutical companies.

According to the answers collected for our study, all of them consider ethics to be an important part of the business. Most of them think the level of business ethics in pharmacy has developed, even though a lot of these companies do not use full range of tools of business ethics.

The study was supported by the Charles University (Project SVV 260 187).

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PRESENCE OF DRUGS INTERACTIONS IN CZECH POPULATION

PRŮŠA, P.,[1] PROKEŠ, M.,[2] SUCHOPÁR, J.[2]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Infopharm, a.s., Prague, Czech Republic
e-mail: prusap@faf.cuni.cz

Interaction between drugs is an important problem of our nowadays´ society. The risk of new interaction between drugs is raised with every one added medication. It is not only a matter of the amount of drugs people use. The number of doctors that cure a person and write prescriptions for them is also very important factor in this case. In these cases when there are lot of doctors prescribing medications for a person we could avoid interactions between drugs by using a system where doctors curing one person know about each other and know who prescribes what kind of medication. There are a lot of studies interested in these problems abroad. Unfortunately there are very few studies taking care of this theme so far in our country. My work is aimed to describe prevalence of these interactions and their importance within the population of Czech Republic and point out the need to be aware of its danger to the public.

The study was supported by Association of Innovative Pharmaceutical Industries.

THE PERMEABILITY AND MICROSTRUCTURE OF MODEL STRATUM CORNEUM LIPID MEMBRANES CONTAINING NON-HYDROXYLATED AND (R)- AND (S)-A-HYDROXYLATED CERAMIDES

ŠILAROVÁ, M.,[1] KOVÁČIK, A.,[1] OPÁLKA, L.,[1] PULLMANNOVÁ, P.,[1] ZBYTOVSKÁ, J.,[1,2] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Czech Republic
[2] Institute of Chemical Technology, Prague, Czech Republic
e-mail: silarom@faf.cuni.cz

Ceramides (Cer) are essential components in the uppermost layer of the skin, called *stratum corneum* (SC). In the SC, Cer with cholesterol (Chol) and free fatty acids (FFA) are in equimolar ratio. Cer molecules are amphiphilic structures with a small polar head and two hydrophobic chains (Fig. 1). Cer contain sphingoid bases, which are amino alcohols sphingosine (S), phytosphingosine (P), dihydrosphingosine (dS) or 6-hydroxysphingosine (H). These sphingoid bases are *N*-acylated by non-hydroxylated (N), α-hydroxylated (A) or ω-hydroxylated fatty acid, mostly by lignoceric (C24) acid.[1,2]

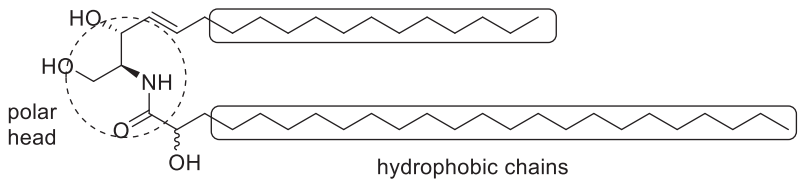

Fig. 1. Structure of Cer AS: the dashed circle shows small polar head and the rectangles show hydrophobic chains.

The goal of this work was to study the permeability and microstructure of the model membranes containing non-hydroxylated Cer including the commercially unavailable Cer NH, and further to study the effects of additional α-hydroxyl group in Cer including their stereochemistry at Cer α-C. Therefore, we prepared model membranes containing Cer/FFA ($C_{16–24}$)/Chol and small amount of CholS (5%wt) where Cer were either non-hydroxylated (NS, NdS, NP, and NH) or α-hydroxylated Cer (2′*R*) and (2′*S*)-diastereomers (AS, AdS, and AP). We investigated four permeability markers: electrical impedance, water loss through the membrane and flux of the theophylline and indomethacin. The microstructure and miscibility of Cer with other lipids were analyzed by infrared spectroscopy and X-ray powder diffraction.

The results confirmed that every type of Cer has unique properties and every change in their structure (type of sphingoid base, α-hydroxylation and stereochemistry) leads to differences in barrier function of model lipid membranes.

The study was supported by Charles University (SVV: 260 183 and GAUK 1868214).

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YIELD STRESS OF SEMISOLID PHARMACEUTICAL PREPARATIONS

ŠMÍDOVÁ, M., ŠNEJDROVÁ, E.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: smidovm8@faf.cuni.cz

Semisolid preparations (gels, ointments, creams) do not flow until the applied stress exceeds a certain critical stress, known as the yield stress. When the semisolid preparation is sheared at very low shear rates, in the range between 0.01–0.1 s^{-1} and below a critical strain the system is subjected to work hardening. This is characteristic of solid-like behaviour. At a shear rate in the range of 0.8 of the critical shear rate the reinforcing structure will start to break down. This is the point where the instantaneous viscosity reaches a maximum. This maximum corresponds to a critical value of shear stress – the yield stress. The chart shows the dependence of viscosity on the shear stress. The yield stress of this sample is determined from the viscosity maximum (Fig. 1).

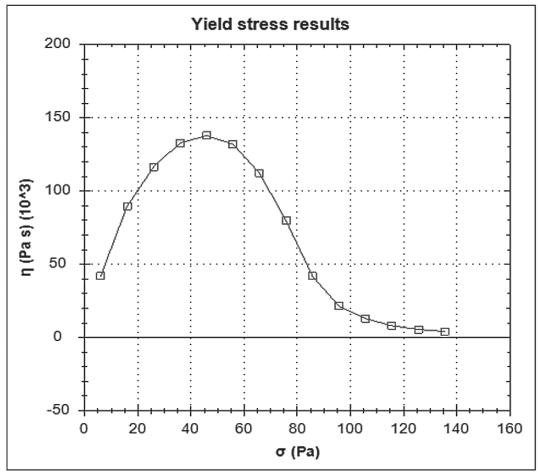


Fig. 1. Viscosity data for Paraffin soft white.

The aim of this work was to use yield stress analysis as simple but more standard method of characterisation of consistency of ointments and gels in comparison to penetrometry. Yield stress by stress ramp of Paraffin soft white, Carbomer gel, Cellulose derivatives gels etc. were measured on Malvern Kinexus rheometer using a CP4°/40 cone geometry, at

25.0 °C, and shear stress range from 0.1 to 300 Pa. Values of yield stress and viscosity on yield stress were expressed and compared with previously determined parameters of Power Law Model and values of consistency measured by penetrometry.

The study is supported by SVV 260183.

ANALYSIS OF SELF-MEDICATION WITH ANTIBIOTICS IN KOSOVO

VESELI, A.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: veselia@faf.cuni.cz

Evidently, the irrational and overuse of antibiotics continues to be a significant problem in Kosovo despite the increased risks of antibiotic resistance and adverse drug reactions. The main aim of this study was to analyze the knowledge, attitude and practice towards self-medication with antibiotics among the population in Kosovo.

The study was conducted in a community pharmacy in Prishtina where a total of 300 patients participated. Data was collected through using a validated, self-administered questionnaire which was developed in English at the department of Social and Clinical Pharmacy at Charles University, Faculty of Pharmacy in Hradec Králové. This questionnaire was spread to two groups of patients: To every patient that visited the pharmacy and to patients who specifically wanted to purchase antibiotics.

The prevalence of non-prescription use was high. In the first group 76.8% of the patients reported using non-prescribed antibiotics while in the second group 47.6% of the patients did not present a prescription at the time of the purchase. Utilization of an old prescription was the most common source of non-prescribed antibiotic use. In both groups the most common reasons for antibiotic consumption were urinary inflammations, cough and influenza which were followed by gastrointestinal and gynecological inflammations. In the first group 73% of the patients stored antibiotics at home while in the second group 60.3% of the patients did the same. On the other hand the majority of the patients were not aware of antibiotic resistant bacteria and the fact that antibiotics can kill off normal flora as well.

Results showed that unfortunately self-medication with antibiotics is common in Kosovo, indicating that there is a need for educational campaigns which will help the public understand the proper antibiotic use and diminish the inappropriate consumption of antibiotics.

The study was supported by SVV 260 187.

SECTION OF BIOLOGICAL SCIENCES

ALKALOIDS OF SELECTED *GALANTHUS, LEUCOJUM* AND *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

BREITEROVÁ, K.,[1] SIATKA, T.,[2] NOVÁK, Z.,[3] HRABINOVÁ, M.,[4] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech republic
[4] Centre of Advanced Studies, Faculty of Military Health Sciences in Hradec Králové, University of Defence in Brno, Czech Republic
e-mail: breiterk@faf.cuni.cz

More than 50% cases of dementia are nowadays caused byAlzheimer's disease (AD). AD is a progressive neurodegenerative disease and it causes gradual memory loss, disorientation and behavioral disorders which affect patient's social and occupational life. AD is characteristic by loss of neurons in some regions of brain – for example hippocampus and cortex. Ethiopathogenesis of this disease is not completely known – that is why the treatment is still just symptomatic. Formation of β-amyloid deposits in brain tissue plays an important role – it is a protein which creates extracellular plagues around neurites and causes their degeneration and death. Intracellular tangles are made up of the changed τ-protein. These tangles also cause death of the neuronal cell. The degeneration of neurons is supported by reactive oxygen radicals too. The another problem is a glutamatergic system disorder. This set of excitatory amino acids is important for correct long-term memory formation. Patients with AD suffer from glutamatergic system overactivation which leads to the formation of neuronal perturbation, excitotoxicity and apoptosis of neuronal cells. In patients with AD the acetylcholine (ACh) production is damaged. ACh is a neurotransmitter and its lack participates in the development of AD. ACh is physiologically decomposed by enzyme acetylcholinesterase (AChE). The second enzyme taking part in ACh degradation is a butyrylcholinesterase (BuChE). In severe forms of AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with normal condition, while the concentration of BuChE increases. That's why the new inhibitors with dual enzymatic activity against AChE and also BuChE are sought.

Galanthus, Leucojum and *Narcissus* species belong to *Amaryllidaceae* family. Plants of this family produce wide range of specific chemical substances called *Amaryllidaceae* alkaloids. These alkaloids have various biological effects like anti-inflammatory, antivirotic, antineoplastic, antiparasitic, antimycotic and they are also able to inhibit erythrocytic AChE (HuAChE) and serum BuChE (HuBuChE).

Alkaloidal extracts of seven selected species and cultivars were analysed by GC/MS and alkaloids were identified from their mass spectra, retention times and retention indexes. Summary extracts were tested *in vitro* for their ability to inhibit HuAChE and HuBuChE using Ellman's method. Interesting inhibitory activities were shown by alkaloidal extracts

of *Galanthus woronowii* ($IC_{50,HuAChE}$ = 8.65 ± 1.20 μg/mL), *Galanthus elwesii* ($IC_{50,HuAChE}$ = 10.29 ± 1.00 μg/mL), *Narcissus* cv. QUIRINUS ($IC_{50,HuAChE}$ = 17.72 ± 2.41 μg/mL) and *Narcissus* cv. VIRGINIA SUNRISE ($IC_{50,HuAChE}$ = 10.72 ± 0.83 μg/mL; $IC_{50,HuBuChE}$ = 29.62 ± 3.47 μg/mL) when galanthamine was used as a standard ($IC_{50,HuAChE}$ = 1.71 ± 0.007 μM; $IC_{50,HuBuChE}$ = 42.30 ± 1.30 μM).

Using preparative TLC (To : Et_2NH, 95 : 5) one alkaloid was isolated from alkaloidal extract of *Narcissus* cv. PROFESSOR EINSTEIN. The isolated compound was identified as a homolycorine and tested for its inhibitory activity against HuAChE (IC_{50} = 63.7 ± 4.3 μM), HuBuChE (IC_{50} = 151.0 ± 15.2 μM) and prolyloligopeptidase ($IC_{50,POP}$ = 173 ± 40.6 μM). Galanthamine and Z-Pro-prolinal ($IC_{50,POP}$ = 3.27 · 10^{-3} ± 0.02 · 10^{-3} nM) were used as positive standards.

The study was supported by SVV 260 184.

IN VITRO CULTURES OF MEDICINAL PLANTS XVII.

CINKOVÁ, L., TŮMOVÁ, L.

Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: halfarol@faf.cuni.cz

Milk thistle*, Silybum marianum* L. Gaertn., is a source of flavonoid taxifolin and flavonolignans – silymarin complex (silybin, silydianin, silycristin and isosilybin). Due to the the main active component of silymarin – silybin, milk thistle is used as hepatoprotectivum and antioxidant, in skin-health, and in the therapy of some kinds of cancer. New therapeutic potentials of *Silybum marianum* are still discovered.

Milk thistle is usually obtained by field cultivation. Alternative way for getting the active components, is the use of *in vitro* cultures. But the production of secondary metabolites by the *in vitro* cultures is low in comparison with plant. One of the possibilites how to increase this produciton is the method of elicitation.

In this study, ethephon as the elicitor, in the concentrations of 500 μmol/l, 400 μmol/l, 200 μmol/l, 100 μmol/l and 50 μmol/l was used with the aim to increase secondary metabolite production in suspension and callus cultures. The effect of ethephon was compared to its inhibitor (120μM $AgNO_3$). The levels of flavonolignans and taxifolin were measured by the method of HPLC. The samples were taken 24, 48, 72, 96 and 168 hours after the ethephon application and inhibitor treatment. The nutrient medium of suspension culture was also tested for the possibity of secondary metabolites releasing into medium.

The highest content of flavonoid taxifolin was found in the suspension culture medium after 48 h treatment with ethephon in conc. of 400 μmol/l. The level of taxifolin was increased by 197-fold to 1,97 mg/100 ml, compared to control sample.

The statistically significant production of taxifolin in the callus culture was reached after 96 hours of treatment with ethephon in conc. of 50 μmol/l (0.11 mg/g DW).

The statistically significant production of silybin A was reached in the nutrient medium 72 h after application of 400μM ethephon (0.51 mg/100 ml).

The statistically significant positive effect of $AgNO_3$ as inhibitor was found in the case of taxifolin in the medium, 168 hours after application of 400μM ethephon. Inhibitor increased taxifolin content by 58-fold to 0.58 mg/100 ml.

The statistically significant negative effect of inhibitor $AgNO_3$ was on silybin A content in medium, 168 hours after application of 400μM ethephon. Inhibitor completely decreased the effect of ethephon.

The study was supported by the Charles University, SVV 260 186.

DEVELOPMENT OF NEMATODES RESISTANCE TO ALBENDAZOLE

DĚDKOVÁ, T., LECOVÁ, L., MATOUŠKOVÁ, P., PRCHAL, L., SKÁLOVÁ, L.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: dedkovat@faf.cuni.cz

Nematodoses, including haemonchosis, disease caused by *Haemonchus contortus,* are responsible for substantial losses in livestock farming. *Haemonchus contortus* inhabits the abomasum of small ruminants and causes anemia and gastritis. Currently available anthelmintics used to treat haemonchosis are ineffective in many breeds because the increasing incidence of multiresistant strains of *Haemonchus* worldwide. Therefore, the research of the mechanism of drug resistance of these parasites is very actual and important. The aim of my work was to study whether short-term contact of eggs or adults of *Haemonchus* with anthelmintics can affect the expression of certain enzymes and can lead to development of resistant individuals. The study had two parts.

In the first part, the influence of anhelmintic drug albendazol and its main metabolite albendazol sulfoxide on *Haemonchus* eggs isolated from the feces of infected sheep was tested. In the second part, expression changes of the reference and selected genes in adult males and females of *Haemonchus* from two strains with different sensitivity to anthelmintics were studied. One group of adults of both genders and strains were exposed to culture medium with 10μM albendazole for 12 and 24 hours. ABZ-untreated group (controls) were exposed to culture medium without drugs for 12 and 24 hours. The genes for UGTs (UDP-glucosyl trasferases) were monitored, because in a previous study, higher UGTs activity and increased ability to deactivate albendazole via conjugation with glucose was found in the drug-resistant strain than in sensitive strain.

In my work, I tested the expression of UGT7, 12 and 13, but I didn't find statistically significant differences in their expression between strains. The contact of *Haemonchus* with albendazole also did not affect expression of these enzymes. For that reason, tested enzymes probably don't contribute to increased metabolism of albendazol in a resistant strain. Due to a large amount of UGTs (more than 40) in *Haemonchus*, it's possible that deactivation of albendazole is catalyzed by other UGTs. Study of UGTs is a part of an on-

going project which deals with mechanisms of helminths resistance and which promises other more interesting results.

The study was supported by SVV 260186.

EFFECT OF SULFORAPHANE ON BIOTRANSFORMATION ENZYMES IN RAT

DYMÁKOVÁ, A., SZOTÁKOVÁ, B.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: dymakoan@faf.cuni.cz

Sulforaphane (SF) is a diet-based isothiocyanate, which is occurring in *Brassicaceae* (cruciferous vegetables) for example in broccoli or cabbage. In vegetables it is in the form of glucoside – glucoraphanin. By the following cutting or chewing, it is hydrolysed into the corresponding isothiocyanate SF either by the plant thioglucosidase myrosinase or by bacterial thioglucosidases in the colon. Because of its lipophilicity and molecular size, SF is likely to passively diffuse into the enterocytes. Myrosinase is inactivated by higher temperature. So when we want SF to be absorbed from GIT, the best way to do so is eating raw vegetables or making juice from it. SF has several beneficial effects on human health, e.g. anticancer, antioxidant or neuroprotective effects.

The aim of our study was to evaluate the effect of SF on the activity of selected biotransformation enzymes. The effect of SF was studied on rat liver subcellular fractions and primary culture of rat hepatocytes. Hepatocytes were incubated with SF, with β-naphthoflavone (β-NF) and with SF and β-NF together, all for 24 hours. The activities of conjugating enzymes, reduction enzymes and cytochromes P450 were studied. In subcellular fractions was studied only an inhibitory effect of SF, but in hepatocytes the induction of abovementioned enzymes was studied as well.

Our results show, that SF in concentrations 10μM, 20μM, 50μM and 100μM weakly inhibits glutathione S-transferase (GST) in cytosol. But in contrast, in hepatocytes was observed induction of GST by 10μM SF itself or in combination with β-NF. Sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) were not detected in subcellular fractions**.** No effect of SF on UGT activity was proven in hepatocytes too.

In hepatocytes, SF induces aldo-keto reductase (AKR1C) alone or with β-NF. The same results were obtained with carbonyl reductase (CBR) and on top of that, there is a great synergism of SF and β-NF. They induce CBR together more than alone SF. We proved that SF induces NAD(P)H:quinone oxidoreductase 1 (NQO1) in rat hepatocytes and the synergism of SF and β-NF combination was also observed. In rat liver cytosol SF slightly inhibits NQO1.

The effect of SF on cytochromes P450 (CYPs) was studied as well. In subcellular fractions no effect of SF on CYP1A1 and CYP1A2 activities was proven. In hepatocytes we confirmed the inhibitory effect of SF on CYP1A1, but only when CYP1A1 was induced by β-NF at first. The activity of CYP3A was not found in hepatocytes, in subcellular fractions SF inhibits CYP3A.

The obtained results show that SF affects several biotransformation enzymes in rat. Therefore, sulforaphane appears to be a promising compound with induction effect on detoxifying enzymes.

This study was supported by the Charles University, SVV 260 186.

THE ENTRANCE OF INTRACELLULAR PATHOGEN *FRANCISELLA TULARENSIS* INTO B CELLS

HADÁMKOVÁ, B.,[1] KONEČNÁ, K.,[1] KROCOVÁ, Z.,[2] PLZÁKOVÁ, L.[2]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Molecular pathology and Biology, Faculty of Military Health Science, Hradec Králové, University of Defence, Brno, Czech Republic
e-mail: b.hadamkova@gmail.com

The main aim of the study was the analysis of entrance of intracellular pathogen *Francisella tularensis* into B lymphocyte.

Francisella tulerensis is intracellular gram-negative bacterium and it was chosen for its high virulence. It can be easily abused as a biological weapon.

The interaction with B cells is important, because a studies shown *F. tularensis* is able to induce an apopthosis in them. In the first part of the work we wanted to find out the influence of blocking receptors for entrance into cell on infection caused by *F. tularensis*.

It was detected by transmission electron microscopy and flow cytometry. We observed blocation on receptors CR1, CR2, CR3, CR4 a BCR. Next part of this was investigate effect of opsonization by antibody and complement system on the same infection. Their inhibitors were used for detection signal pathaways.

In the second part of this research we observed the cell fate – colocalization of bactery with endo-lysozomal markers. For the first part of work we used murine B lymphocytic cells line A20 and peritoneal B cells BALB/c. The colocalization was observed on the A20 cells only.

The study was supported by SVV 260 185.

EFFECT OF AMYGDALIN ACTIVATED WITH B-D-GLUCOSIDASE ON HELA, MCF-7 AND PC-3 CANCER CELLS PROLIFERATION.

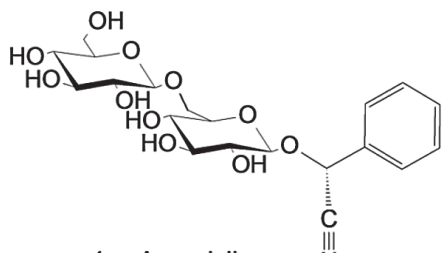
JANATOVÁ, M.,[1] JAHODÁŘ, L.,[1] MOLPECERES, J. [2]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Charles University, Czech Republic
[2] Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Alcalá University, Madrid, Spain
e-mail: Janatom1@faf.cuni.cz

Stone fruits from tribe Amygdaleae of Rosaceae family are known for their antioxidant activity and amount of nutrients and vitamins. Their seeds are connected with content of cyanogenic glycoside amygdalin and its possible effect on inhibition of cancer cells growing.

The anti-proliferative activity brought by stand-alone amygdalin (**1**) and amygdalin activated with β-D-glucosidase from almonds was evaluated in HeLa (cervical), MCF-7 (breast) and PC-3 (prostatic) human cancer cell lines. The MTT viability assay showed that all samples inhibited growth of all three cell lines in dose and time dependent manners. IC_{50} values on the proliferation of the three cell lines for 24 h were more than 15 mg/mL for stand-alone amygdalin and less than 7 mg/mL for amygdalin combined with β-D-glucosidase.

In vitro degradation study of amygdalin with β-D-glucosidase was examined by rp-HPLC to characterize enzymatic hydrolysis rate. Experiments showed that amygdalin could be decomposed to benzaldehyde during the first 1.5 h. Optimum reaction conditions were determined as follows: 37 °C, phosphate buffer system (pH 7.4), the ratio of amygdalin/enzyme 1 : 0.12. The results indicate that amygdalin in combination with β-D-glucosidase significantly inhibit *in vitro* growth of the carcinoma cells.



1 Amygdalin

The study was supported by the Charles University, SVV 260 184.

SYNTHESIS OF TETRAZOLE DERIVATIVES WITH HIGH ANTIMYCOBACTERIAL ACTIVITY AND THEIR INITIAL *IN VITRO* TOXICITY ASSESSMENT

KAVKOVÁ, V.,[1,2] NĚMEČEK, J.,[2] ROH, J.,[2] MACHÁČEK, M.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: kavkovav@faf.cuni.cz

Tuberculosis is persistently considered to be one of the most dangerous and widespread infectious disease with estimated 1.5 million annual deaths worldwide (one third of entire population is infected). It is very specific illness with unique pathogenesis, progression and

complicated treatment. The most frequent etiological agents of this disease are strains of *Mycobacterium tuberculosis*. Nowadays we can diagnose and cure this disease with success. On the other hand, it is still causing fatal issues in developing countries and is often found in immunosuppressed patients and people co-infected with HIV virus. Resistance to antituberculosis drugs has been rising constantly – the emergence of extensively drug resistance tuberculosis (XDR) has worsen the situation even more. From this reason a pharmaceutical laboratories all over the world are trying to develop new effective drugs that would provide faster and more effective treatment of this illness and prevent the forming and spreading of resistant strains.

The aim of this study was to synthetize three novel derivatives and subsequently to provide basic *in vitro* evaluation of their cytotoxicity (together with other substances from this series).

Cytotoxicity experiments were performed on 3T3 mouse nonmalignant fibroblast cell line. Cellular viability was assessed using Neutral Red uptake assay in 96-well plates. Epifluorescence microscopy was used for obtaining information of cellular and sub-cellular morphology changes using CellMask Green (cytoplasmic membrane), Hoechst 33342 (nucleus) and MitoTracker Red FM (mitochondria) fluorescent probes. Additionally changes in mitochondrial inner membrane potential were monitored using JC-1 fluorescent probe.

Results of this study showed absence of inherent toxicity of the most of studied compounds up to their solubility limit in waterbased media (up to 100 μM). Antimicrobial activities (MICs) of these substances are in ≈ 0.03–1 μM concentrations – this in combination with the low cytotoxicity render these substances as very promising antituberculosis agents.

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SCREENING FOR CYTOTOXIC ACTIVITY OF *AMARYLLIDACEAE* ALKALOIDS

KAVKOVÁ, Z.,[1] HOŠŤÁLKOVÁ, A.,[2] DOSKOČIL, I.,[3] KUNEŠ, J.,[4] CAHLÍKOVÁ, L.[2]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] ADINACO Research Group, Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
[4] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: kavkovaz@faf.cuni.cz

Oncological diseases are one of the leading causes of death in the developed countries and the increase of its prevalence seems to be inevitable. According to World Health Organization's International Agency for Research on Cancer (IARC) in Loyn, France, the incidence of cancer is expected to increase by more than 75% by the year 2030 in

developed countries. In most cases oncological patients die due to resistance of cancer to therapy, metastasis and dissemination of cancer cells into vital organs. The standard treatment covers surgical intervention, radiotherapy and/or chemotherapy.

Additionally convential anticancer treatments damage healthy tissue, resulting in a variety of side effects. Therefore, substantial efforts are being invested into identifying and developing compounds that would be able selectively target tumor cells while not damage healthy cells.

The search for new lead anticancer compounds is a crucial element of modern natural products research. Among various natural sources that have been investigated for constituents with potential use in cancer treatment, plants of the *Amaryllidaceae* family have been particularly promising and fruitful.

To date, about 50 of these alkaloids were tested against different cell lines. From these pilot studies we can conclude that the most active substances fall into free structural types: namely lycorin, crinine and pancratistatin. Most of these active compounds were studied for IC_{50} values on diverse mammalian cells howewer mechanism of action remains to be determined.

In curent study we screened and determined IC_{50} *in vitro* growth inhibitory activity (using the MTT colorimetric assay) of 15 *Amaryllidaceae* alkaloids at concentrations up to 100μM in to cancer cell lines Caco-2 (human epithelial colorectal adenocarcinoma cells) and HT-29 (human colon adenocarcinoma cells) using the MTT colorimetric assay. A human normal intestine cell line (FHS-74int) was used as a control fot the overall toxicity. All tested alkaloids have been previously isolated in our laboratory from three plant species *Zephyrantes robusta*, *Chlidantus fragrans* and *Nerine bowdenii*.

Among the tested compounds lycorine, haemanthamine and haemanthidine exhibited the most potent cytotoxic potential against both tested cell lines, with IC_{50} values of 0.99–3.28 μM for Caco-2 and IC_{50} 0.59–1.72 μM for HT-29. Lycorine and haemanthamine showed only moderate toxicity against normal cells (15 μM < IC_{50} < 30 μM) in comparison to used standard vinorelbine, which is significant toxic to used normal cells (IC_{50} 3.98 ± 0.26 μM). Other tested alkaloids showed moderate (10 μM < IC_{50} < 25 μM), weak (25 μM < IC_{50} < 100 μM) or no (IC_{50} > 100 μM) cytotoxic potential against all tested cell lines.

Further step of the current study is the preparation of semisynthetic analogues by changing different parts of the structure of the most active compound haemanthamine. This compound is isolated in our laboratory in sufficient amount (10 g) allowing such structure-activity relationship (SAR) study. The first series of haemanthamine analogues have been already synthetized. The prepared analogues are assayed for their cytotoxic activity.

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CIRCADIAN RHYTHM SLEEP DISORDER

KOLÁČKOVÁ, P.,[1] SEMECKÝ, V.,[1] SMOLÍK, P.[2,3]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Psychiatric Clinic, Centrum of Disorders of Sleep and Biorhythm, University Hospital in Hradec Králové, Czech Republic
[3] Department of Psychiatric Clinic, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
e-mail: kolackpa@faf.cuni.cz

Current lifestyle with more stress factors helps to impair physiological programming of circadian rhythm, because of that the number of patients with sleep disorders is growing.

Study was conducted at the Centrum of Disorders of Sleep and Biorhythm in University hospital in Hradec Králové, Czech Republic in co-operation with doc. MUDr. Petr Smolík. A total of 51 patients were enrolled in the study, each of them was controlled minimally once every 3 months. The two main groups are insomnia and circadian rhythm sleep disorder patients.

Method of study was the personal contact with patients, together filling in questionnaires and follow up work with medical history and consultation with psychiatrist from sleep laboratory.

Except standard sleep distribution, we distinguish patients with advanced sleep phase syndrome – ASPS (larks) or delayed sleep phase syndrome – DSPS (owls). Patients with ASPS aren't limited in social and economical life as much as patients with DSPS. Main problems of DSPS are demonstrated on two, to point out complexity of therapy in patients with DSPS.

The cases are focused on physiological programming and individualization of therapy, especially using right dosage of drug at the right time. Therapy is also based on complex change of patient's lifestyle, which is beneficial for enhancing patient's compliance and adherence not only to pharmacological part, but also to psychological part of therapy. Incorrectly administered dosage of drugs, usually associated with drug abuse, might not help patient, in one given case study it made patient's condition worse.

This project was supported by the Charles University, SVV 260 185.

THE EFFECT OF EPIGALLOCATECHIN GALLATE ON THE INDUCTION AND REPAIR OF THE DNA DAMAGE INDUCED BY HYDROGEN PEROXIDE IN HUMAN ADENOCARCINOMA CELLS A549

KUDELOVÁ, T.,[1,2] ŠTĚTINA, R.,[1] SZOTÁKOVÁ, B.[2]

[1] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences in Hradec Králové, University of Defence, Brno, Czech Republic
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

During their life, human cells are exposed to oxidative stress. The cell damage caused by reactive oxygen species (ROS) has been recognised as a major cause of cell ageing and the subsequent mutagenesis. The aim of our work was to identify the protective effects of epigallocatechin gallate (EGCG) on DNA in human lung cells A549 under oxidative stress generated by hydrogen peroxide (H_2O_2).

The A549 cells were treated with EGCG at several concentrations for one hour and subsequently exposed to hydrogen peroxide at different concentrations. In this way, the protective effect of EGCG against H_2O_2-induced damage was studied. Analogically, the DNA repair process was followed with A549 cells first exposed to hydrogen peroxide at several concentrations and subsequently incubated with EGCG at different concentrations for two reparation periods – 15 and 30 minutes. The impaired oxidised bases were detected by enzymes, endonuclease III (Endo III) and formamidopyrimidine-DNA-glycosylase (Fpg). The oxidative damage to DNA was assessed quantitatively using the Comet Assay method.

In the first case, the protective effect of EGCG against hydrogen peroxide induced DNA damage was confirmed. The A549 cells were treated with EGCG at concentrations from 12.5 up to 50 μg/ml, for one hour and then exposed to H_2O_2 at concentrations 0, 10, 25, 100 μM for 5 minutes. EGCG at concentrations, 25 and 50μg/ml has protective effect on DNA damage caused by oxidative stress compared to the control (no EGCG pre-treatment).

In the second case, A549 cells were exposed to H_2O_2 at concentrations of 0, 50, 100, 200 μM for 5 minutes, and afterwards they were treated with EGCG at concentrations of 12.5 and 25 μg/ml during two reparation periods: 15 and 30 minutes. The oxidised bases were detected using enzymes Endo III and Fpg. EGCG was found improving the reparation, however, the mechanism underlying this phenomenon has not been identified so far.

The study was supported by the Charles University, SVV 260 186.

VERIFICATION OF THE EFFICIENCY OF THE HERBAL BLEND DEFINED PD007 IN ACCELERATION ETHANOL METABOLISM AND DECREASING ITS TOXIC EFFECTS ON HUMAN ORGANISM

MACHAROVÁ, K.[1,2], STŘÍTECKÁ, H.,[1] BEDNARČÍK, P.,[1] FAJFROVÁ, J.[1]

[1] Department of Military Internal Medicine and Military Hygiene, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[2] Pharmaceutical Faculty in Hradec Králové, Charles University, Czech Republic
e-mail: macharok@faf.cuni.cz

This pilot evaluation assesses effects of using specific herbal blend, expecting to relieve the symptoms of organism intoxication with ethanol and accelerate ethanol metabolism.[1,4,5]

The aim of this study is to evaluate effects of the administration of standardized herbal blend tested on selected subjective and objective parameters of alcohol intoxication and monitoring the time course of alcohol levels changes in blood with or without tested herbal blend.

The herbal blend consists of *Hovenia Dulcis*, *Pueraria Lobata*, *Panax Quinquefolius*.

There are studies confirming the efficiency of individual plants in therapy of alcohol intoxication. Testing was performed on mice.[1,2,4,5,7] Scientists found that the main substance, dihydromyricetin, blocks receptors in the brain responsible for transmission of aminobutyric acids (GABA). Ethanol isn't bound on neurons and remains in the bloodstream, where it can be transferred to livers for further degradation.[1] As referred in the study: Mice remained sober in spite of excessive level of alcohol concentration in blood.[1,2,4,5] DHM accelerates liver ability to degrade both alcohol, and the toxic metabolite acetaldehyde.[1,2,3,4]

Substances contained in *Kudzu* root extract inhibits or modify the effects of alcohol dehydrogenase. It was also confirmed that isoflavonoids, found in root of *Kudzu*, increase concentration of serotonin and dopamine in the CNS, which leads to a gradual reduction of necessity to alcohol consumption in any form.[7,8,9,10]

The tested subjects use the herbal blend, which is in the form of a dry powder. Tested subject drinks a defined dose of ethanol with given alcohol concentration. The amount of the dose is calculated referring to body weight using specific mathematical formula. The aim is to achieve peak blood levels of alcohol in the range of 0.5–1 per mille. The level measuring is carried out by breathalyser. The part of monitoring the effect of alcohol on organism is performing the response test. The test shows changes in ability to focus on performance and in changes of fine motor coordination. Alcohol breath test is carried out every thirty minutes until the alcohol level is below 0.1 per mille. In the first test scheme the subjects are administered alcohol only. The values obtained will serve as a control measurement in further examinations of ethanol degradation. In the second test scheme the subject first uses the tested herbal blend and after thirty minutes drinks a specified dose of alcohol (the same dose as in scheme one). In the third test scheme monitored subject drinks alcohol (the same dose as in tests one and two) and thirty minutes from administering of alcohol the tested herbal blend is applied. According to the results of the tests number 2 and 3 the most successful scheme is selected and it will be performed with doubled dose of the herbal blend.

Data analysis evaluated referring to the duration of decreasing alcohol level below 0.1 per mille in relation to sex, body constitution and results of the response test.

The study would be important for diagnostic or therapeutic methods for medical science.[6]

The results can't be assessed at this very moment, but it is believed that one dose of herbal blend should be taken before alcohol consumption and another dose after finishing of the consumption even before going to bed to achieve maximal efficiency of the blend. If the tests will confirm the assumptions about the positive effect of the blend on reducing the symptoms of intoxication and acceleration of ethanol metabolism stated in literature than the results will serve as initiation of a broader study. The main objective of upcoming study would be obtaining objective data for possible use of the blend as a complementary therapy.[1,6,8,10]

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CHARACTERIZATION OF *LRRK2*-MUTANT IPSC-DERIVED ASTROCYTES

MADĚRYČOVÁ, Z.,[1,2] LEHTONEN, S.,[2] VOPRŠALOVÁ, M.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Neurobiology, Faculty of Health Sciences in Kuopio, University of Eastern Finland, Finland
e-mail: maderycz@faf.cuni.cz

Somatic cells derived from induced pluripotent stem cells (iPSC) are becoming a model tool of future that allows to study *in vivo* processes under *in vitro* conditions. The protocol for generation of iPSC-derived astrocytes was published only recently. Thanks to this fact, it is possible to study a complicated and not fully understood role of astrocytes under physiological and pathological conditions.

In our pilot study, iPSC-derived astrocytes in the primary research of Parkinson‘s disease (PD) were used for the first time ever. We focused on comparison of the gene expression profiles of iPSC-derived astrocytes obtained from a healthy individual and a patient with genetically conditioned PD. The G2019S mutation of the *leucine rich-repeat kinase 2* (*LRRK2*) gene was purposely studied. Presence of the *LRRK2* mutation also occurs in some patients with sporadic form of PD and therefore studying this mutation seems to be also beneficial for understanding mechanisms of PD in general.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR), immunocytochemistry (ICC) and western blotting (WB) were employed for an objective analysis

of both studied astrocyte lines. These methods analyzed morphological features of iPSC-derived astrocytes, astrocyte-specific markers, disease-associated markers, neuroprotective and pro-inflammatory markers, sensors of organelle functions as well as some enzymes.

Results of our work imply significant changes in astrocyte morphology and gene expression profiles that might be critical in pathology of PD.

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INFLUENCE OF TNF-α ON HENAC SUBUNITS EXPRESSION

MARTAN, D.,[1,2] LEMMENS-GRUBER, R.,[1], SHABBIR, W.,[1] AUFY, M.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Vienna, University of Vienna, Austria
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: martand@faf.cuni.cz

The human epithelial sodium channel (hENaC) or the amiloride-sensitive channel, is a type of ion channel which has the ability to control salt and water homeostasis. Therefore it is one of the main driving forces for the reabsorption of water through the alveolar epithelium. A dysfunction of this channel, respectively of this control mechanism, leads to a very severe disease – pulmonary edema and several other pathological conditions.

Previous studies tested a drug named AP301. AP301 is a cyclic protein comprising the human tumour necrosis factor-like domain sequence. This drug was recently developed as a potential treatment of pulmonary edema. The principle is that it activates hENaC by increasing the open probability. It was also shown that AP301 transiently increases the expression of hENaC subunits in mammalian cells.

In this study we used the western blot method to test the influence of tumour necrosis factor α (TNF-α) on hENaC subunits expression and we compared these results with the results from the studies with AP301.

We found that TNF-α transiently significantly increased the expression of δ subunit and it had a potential to increase the expression of α subunit. On the other hand, the expression of β- and γ-hENaC was not significantly increased.

Taken together, these results are analogical to those which were found in the studies with AP301.

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INTERACTION OF ANTIRETROVIRAL DRUG ABACAVIR WITH DRUG EFFLUX ATP-BINDING CASSETTE (ABC) TRANSPORTERS

MARTINEC, O., ČERVENÝ, L., NEUMANOVÁ, Z., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: martinon@faf.cuni.cz

Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) that is frequently used in combination antiretroviral therapy of HIV infection. Pharmacokinetics of many antiretroviral drugs is often affected by the activity of ATP-binding cassette (ABC) transporters. Drug-drug interactions on ABC transporters should be, therefore, always borne in mind as they may complicate therapy. To guarantee effective and safe abacavir-based therapy it is inevitable to have complex knowledge on abacavir interactions with ABC transporters. The aim of our work was to study interaction of abacavir with human drug efflux ABC transporters ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein), ABCC2 and ABCC5 (multidrug resistance-associated protein 2 and 5) using *in vitro* method of MDCKII cell monolayer in setup of bi-directional study and concentration equilibrium. Abacavir was tested at a low non-saturating concentration of 198 nM. Using both experimental setups we observed abacavir active transport across MDCKII-ABCB1 and MDCKII-ABCG2 reaching significantly higher transport ratio after six hours > 2 in bidirectional study and > 2 in concentration equilibrium studies when compared with transport across the parent MDCKII cells. Application of higher concentrations of abacavir (50 μM and/or 100 μM) caused partial saturation of the ABCB1- but not ABCG2-mediated transport. Additionally, it was demonstrated that dual ABCB1/ABCG2 inhibitor GF120918 completely abolished transcellular transport across both MDCKII-ABCB1 and MDCKII-ABCG2 monolayers. We can therefore conclude that abacavir is a substrate of ABCB1 and ABCG2, but not ABCC2 and ABCC5 and it can be hypothesized that ABCB1 and ABCG2 may affect its pharmacokinetics at the level of absorption, distribution and elimination. Additional studies are required to confirm relevancy of abacavir drug-drug interactions on these transporters *in vivo*.

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PHARMACOLOGICAL EVALUATION OF POTENTIAL ALZHEIMER'S DISEASE DRUGS

MATOUŠKOVÁ, L.,[1] ŠEPSOVÁ, V.,[2] VOPRŠALOVÁ, M.,[1] JUN, D.,[2] SOUKUP, O.[2,3]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences,
Hradec Králové, University of Defence, Brno, Czech Republic
[3] University Hospital Hradec Králové, Charles University, Czech Republic
e-mail: matousl9@faf.cuni.cz

Alzheimer's disease (AD) is a neurodegenerative disorder affecting primarily ageing population. It is characterized by aggregates of amyloid plaque, neuofibrillary tangles of tau proteins and by loss of cholinergic neurons in the basal forebrain and hippocampus.[1] Cause of AD is still unknown and only symptomatic treatment is available thanks to acetylcholinesterase inhibitors (AChEI) and memantine. M_1 muscarinic positive allosteric modulators (PAMs) represent another variant of treatment that can improve cholinergic transmission. Thanks to their selectivity, they are able to decrease side effects.

The aim of the study was to measure novel compounds' abilities to inhibit AChE and BChE and simultaneously act as M_1 PAM. Enzymes inhibition was measured spectrophotometrically (according to Ellman's method) using 96 microwell plates and IC_{50} values were determined. The CHO cell line stably expressing the M_1 subtype mAChR was used for fluorescent measurement of compounds interaction with mAChR. Fluo-4 NW was used as fluorescent indicator, oxotremorine as an orthosteric agonist and BQCA (benzyl quinolone carboxylic acid) as a positive allosteric modulator[2]. Statistical analysis of results was performed in GraphPad Prism6.

Unfortunately, none of the tested compounds acted as a PAM/allosteric agonist. All novel compounds acted as M_1 inhibitors. Moreover, AChE and BChE inhibition was comparable with standards.

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COPPER CHELATING AND REDUCING EFFECTS OF QUERCETIN METABOLITES

MISAŘ, J.,[1] KARLÍČKOVÁ, J.,[1] ŘÍHA, M.,[2] KŘÍŽ, J.,[2] MLADĚNKA, P.[2]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: misarjak@faf.cuni.cz

Copper is an essential microelement in particular due to its ability to easily convert between both redox forms: oxidized (Cu^{2+}) and reduced (Cu^{+}).[1] Flavonoids are common components of the human diet and they may positively influence human health.[2] They are converted into smaller phenolic acids during digestion by bacteria in the colon. Although properties of flavonoids have been well studied, the same is not true for their metabolites – phenolic acids.[3]

In this *in vitro* study, 10 phenolic acids, which are known metabolites of commonly tested flavonoid quercetin, were analyzed for their copper chelating activity and copper reducing activity at 4 pathophysiologically relevant pHs. Simple spectrophotometric methods based on hematoxylin and bathocuproinedisulfonic acid disodium salt were used for the assessment chelation and reduction of copper ions.[4]

As expected, metabolites possessing a dihydroxygroup in an *ortho* position were able to chelate cupric ions, however their chelation activity disappeared when challenged with a more powerful copper chelator. The degree of cupric reduction differed among tested compounds. All *o*-dihydroxycompounds were the most active and achieved 100% of cupric ion reduction in low compound to copper ratio.

In conclusion, based on this study, it appears that metabolites of quercetin can influence the kinetics of copper in human.

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GENERATION OF DNA CONSTRUCTS FOR THE STUDY OF GENE REGULATION *VIA* NUCLEAR RECEPTORS

MORAVČÍK, Š.,[1] TECHLOVSKÁ, Š.,[2] HYRŠOVÁ, L.,[1] PÁVEK, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Molecular Genetics, Academy of Sciences, Prague 4, Czech Republic
e-mail: moravcis@faf.cuni.cz

Nuclear receptors PXR, HNF4α and CAR are transcription factors that control expression of major xenobiotic-metabolizing enzymes and transporters.

The gene reporter assay is widely used method in the study of gene regulation through nuclear receptors. The method is based on a gene reporter DNA construct with target gene promoter sequence upstream of a firefly luciferase or green fluorescence protein (GFP).

In my project I introduced GENEART^{TM}Site-Directed Mutagenesis and DNA plasmid ligation techniques to generate the following DNA constructs:

(i) the organic cation transporter-1 (OCT1) luciferase reporter constructs with mutated HNF4α response elements for the study of OCT1 regulation;

(ii) gene reporter (both luciferase and GFP) constructs for cytochrome P450 CYP3A4 gene, a target gene regulated by PXR and CAR;
(iii) generation of Constitutive androstane receptor (CAR) chimera expression construct with extra alanine residue in CAR ligand binding domain and enhancer green fluorescence protein (EGFP) for the study of CAR-mediated regulation of CYP3A4 gene and CAR activation

I generated three OCT1 luciferase gene reporter constructs with mutated HNF4α and USF1 binding sites in OCT1 promoter. Further gene reporter experiments with the constructs in HepG2 cells helped to reveal the significant roles of the factors in OCT1 regulation.

Next, we generated two CYP3A4 gene reporter constructs with XREM and proximal regulatory regions of CYP34 gene and with both firefly and GFR reporter genes. Functional cellular experiments confirmed correct construction of the plasmids.

Finally, we generated chimera CAR expression construct with inserted alanine residue and EGFP, which confers low constitutive activity and no false translocation to nucleus. In fluorescent microscopy experiments, we confirm nuclear localization of CAR+A/EGFP protein after treatment with CAR ligand in HepG2 cells and activation of CYP3A4 luciferase gene reporter construct.

I can conclude that generated DNA constructs are functional and are valuable tools for further study of gene regulation.

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CYTOMEGALOVIRUS INFECTION WITH HCMV STRAIN AND ITS RELATIONSHIP TO THE IMMUNOSUPPRESSION

NOVÁ, A.,[1,2] KUBELKOVÁ, K.[1]

[1] Department of Molecular Pathology and Biology, Faculty of Military Health Sciences in Hradec Králové, University of Defence, Brno, Czech Republic
[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: novaan@faf.cuni.cz

The main goal of our study is a contribution to the study of *in vitro* interaction of human cytomegalovirus, belonging to the family *Herpes viridae* with selected immunosuppressed host cells.

During the academic year 2014/2015, we were focused on the infection of human lung fibroblasts MRC-5 with human cytomegalovirus strain VR-1590. During the study, basic laboratory techniques have been used. Among these methods, we were employed to work with cell cultures and viral isolates where the concentration of cytomegalovirus has been quantified using plaque-based assay. Moreover, ionizing radiation was applied to ensure the condition of immunosuppressed host. Subsequently, selected signalling pathways of host cells have been examined in relation to the radiation and/or infection using PathScan antibody technology.

In this study, the cytomegalovirus infection model using immunosuppressed host cells has been introduced and quite well optimized. We have also investigated the target signaling pathways of host cells using specific antibody-determined technology. The relationship between immunosuppressed host cells and viral infection has been studied on the basis of changes of selected transduction pathways signals of these cells. PathScan technology will be further optimized in the context of the study of other selected signaling pathway signals in the future.

This study was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense, Czech Republic.

UHPLC-MS/MS ABSOLUTE QUANTIFICATION OF CYTOCHROME P450 ENZYMES IN C3A, CACO2 MODIFIED CELL LINES AND IN HUMAN LIVER MICROSOMES

REJŠKOVÁ, L.,[1] AURIOLA, S.,[2] SZOTÁKOVÁ, B.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Eastern Finland in Kuopio, Finland
e-mail: rejskovl@faf.cuni.cz

Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism. They can be responsible for the failure of treatment, adverse and toxic effects or drug–drug interactions. Knowledge of expression levels and their susceptibility to be either induced or inhibited would be the basic tool for personalized therapy. Therefore, *in vitro* and *in vivo* experiments of CYP mediated metabolism is an essential part of the drug development and clinical research.

In vitro studies can be done with primary cells or cell lines. Cell lines are phenotypic stable and immortal but their CYPs levels are low. From this point of view, modified C3A and CACO2 cell lines with constitutive androstane receptor (CAR) and pregnane X receptor (PXR) might be used for these experiments. CYP enzymes should be expressed continuously in these modified cell lines.

With regard to pharmacokinetic and pharmacological importance, the expression levels of metabolizing enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A5 and CYP3A4 were studied in my diploma thesis.

Absolute quantifications of CYP enzymes were carried out by UHPLC in line coupled with tandem mass spectrometry working in scheduled MRM mode. Data assessment was conducted by Skyline 2.6 software.

CYP enzymes were not detected in CACO2 and C3A modified cell lines. However, these enzymes were found in human liver microsomes. Average values were ranging from 0.6 pmol/mg to 21.5 pmol/mg of microsomal protein. The lowest detected amounts of CYP protein were 0.006–0.210 pmol/mg of microsomal protein in a hundred times diluted human liver sample. These findings point out that CYPs protein levels in modified C3A and CACO2 cell lines were apparently below the limit of detection.

Results show that up-regulation of CYP enzymes in modified cell lines CACO2 and C3A does not reach CYPs levels in human liver microsomes. Further studies have to be conducted in order to optimize cultivation conditions, presence of co-regulators and ligands to get modified cell lines with measurable CYP levels.

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RELIABLE REFERENCE GENE SELECTION FOR QUANTITATIVE REAL TIME PCR IN *HAEMONCHUS CONTORTUS*

RŮŽIČKOVÁ, M., LECOVÁ, L., SKÁLOVÁ, L., MATOUŠKOVÁ, P.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: ruzicmi1@faf.cuni.cz

Parasite anthelmintic resistance is a great problem of these days. Prophylaxis, treatment and consequences of parasitic infections represents an important economic burden on livestock production worldwide. Mechanisms of drug resistance are still not fully understood. Molecular biology methods, *e.g.* gene expression studies, could contribute to understanding of these mechanisms and thus help in resistance management. Use of suitable reference genes is essential for an accurate normalisation of gene expression levels.

Haemonchus contortus is a parasitic nematode of small ruminants, whose multi-resistance to anthelmintics means global problem. The genome and transcriptome have been published recently, allowing extensive gene expression research to be conducted. Suitable reference genes for different strains of *H. contortus* have not been validated yet.

The aim of this work was to identify and validate reliable reference genes for gene expression studies in adult *H. contortus*. 11 candidate genes were chosen for further assessment of their expression stability in males and females of two genetically divergent *H. contortus* strains: drug-susceptible (ISE) and multi-drug-resistant (WR). The candidate genes were selected based on their common use as endogenous controls, supplemented by genes identified bioinformatically based on stable expression in RNA-seq data.

Total RNA was extracted from ten adult *H. contortus* males or females and reverse transcribed to cDNA. An identical reaction without reverse transcriptase was carried out simultaneously. The resulting cDNA was diluted 1 to 50 and used for quantitative real-time PCR assay (qPCR). iQ5 Real Time PCR Detection System (Bio-Rad, USA) with SYBR green I detection was used for qPCR analyses. Specificity and efficiency of designed primer sets were checked using standard dilutions, efficiency for all primers was between 91–104%.

Expression stability was evaluated by computer programs BestKeeper, geNorm, NormFinder and the comparative ΔΔCt method. Different calculation methods caused slightly different ranking order of genes obtained from each program. However, all methods found ama, farb, gapdh, ncbp and sodc to be the five most stable genes.

By this study we demonstrated, that the combination of commonly used gapdh gene and at least one of the other best ranked genes would be appropriate for gene expression studies in *H. contortus* adults.

The study was supported by the Charles University (Research project SVV 260 186).

CHARACTERIZATION OF HUMAN WARFARIN REDUCTASE

SOKOLOVÁ, S., MALÁTKOVÁ, P., ŠKARKA, A., WSÓL, V.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: sokolosi@faf.cuni.cz

Warfarin is widely used anticoagulant drug. Considering the narrow therapeutic window of warfarin, it is important to fully understand its metabolism in human body. Oxidative, reductive and conjugation reactions are involved in warfarin metabolism. However, the reductive metabolism of warfarin has not been studied in details until now.

The reduced metabolite of warfarin, i.e. warfarinalcohol, is produced by the conversion of the carbonyl group of the side chain. It is known that human liver cytosolic and microsomal fractions exhibit warfarin reductase activity but the specific enzymes catalysing the reduction of warfarin are not known yet.

The aim of this study was to identify the enzyme(s) participating in reduction of warfarin and to describe enzyme kinetics. Human liver cytosolic and microsomal fractions and recombinant enzymes AKR1A1, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C3, AKR1C4, CBR1 and CBR3 were incubated with warfarin at various concentrations. The produced warfarinalcohol was quantified by UHPLC and the specific activities of enzymes and subcellular fractions were determined.

The warfarin reductase activity was confirmed in cytosolic and microsomal fractions. The reduction of warfarin was higher in the liver cytosol than liver microsomes. From the enzymes tested, AKR1C3 and CBR1 were found as the main enzymes participated in the production of warfarinalcohol. Other enzymes showed only low or no activity.

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HUMAN MEMBRANE-BOUND ENZYMES AS TARGETS OF ORACIN-IMMOBILIZED AFFINITY CARRIER

ŠTÝBNAROVÁ, R.,[1] ANDRÝS, R.,[1] LENČO, J.,[1,2] ZEMANOVÁ, L.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Institute of Molecular Pathology, Faculty of Military Health Sciences in Hradec Králové, University of Defence, Brno, Czech Republic
e-mail: stybnara@faf.cuni.cz

Carbonyl reducing enzymes play important role in the metabolism of various eobiotic (e.g. steroids, prostaglandins) and xenobiotic (e.g. doxorubicin, daunorubicin, oracin, NNK, haloperidol) compounds. Due to their substrate specificity, they also play a role in development of some diseases like hormone-dependent cancers and metabolic syndrome. While cytosolic carbonyl reducing enzymes are well characterised the knowledge about membrane-bound types is quite poor because their study is demanding.

Actually, until today there are only three described microsomal carbonyl reducing enzymes participating in the metabolism of xenobiotic compounds (11β-HSD1, DHRS7 and DHRS3)[1,2] of which only the 11β-HSD1 is well characterized. However, based on the research of anticancer drug oracin reduction stereospecificity, there were predicted other microsomal carbonyl reducing enzymes involved in its metabolism and inactivation[3].

In order to isolate these "unknown" enzymes the in-house developed affinity carrier with immobilized ligand oracin[4] was introduced into the purification protocol of human liver membrane-bound enzymes[3]. Proteins having affinity towards oracin were successfully captured by our affinity carrier and subsequently gently eluted by 100 mM glycine buffer, pH 10.5. Using mass spectrometry enzyme 17β-HSD6 was identified. Despite its metabolism of eobiotics (e.g. retinol, testosterone and estradiol) was already described there are still no published information about its role in the metabolism of xenobiotics. Thus its isolation and identification as a potential target of drug oracin could significantly extend our knowledge about its role in biotransformation.

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EXPRESSION OF DHRS8 AND DHRS12 ENZYMES IN HUMAN TISSUES

SVOBODOVÁ, M.,[1] LUNDOVÁ, T.,[1] ŠAFR, M.,[2] NOVOTNÁ, E.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Legal Medicine, Faculty of Medicine and University Hospital in Hradec Králové, Charles University, Czech Republic
e-mail: svobom13@faf.cuni.cz

Dehydrogenase/reductase (SDR family) member 8 (DHRS8, SDR16C2) and dehydrogenase/reductase (SDR family) member 12 (DHRS12, SDR40C1) are human microsomal enzymes belonging to the superfamily of short-chain dehydrogenases/reductases (SDR). This superfamily represents one of the largest protein groups. SDR enzymes participate in the metabolism of various xenobiotic and endogenous compounds and are involved in physiological and pathological processes[1,2]. However, there are still many enzymes which are only poorly characterised.

To this date, the expression on mRNA level and catalytic activity toward 5α-androstane-3α,17β-diol are the only available information about DHRS8[3,4]. Moreover, there is still no published information (apart from the prediction) regarding DHRS12.

The aim of this study was to examine the protein expression of DHRS8 and DHRS12 enzymes in various human tissues. The tissue samples were collected from five middle aged male subjects after the sudden death without apparent disease. Proteins of interest were detected using western blotting and specific antibodies. Recombinant form of searched proteins (DHRS12, DHRS8) expressed in sf9 insect cells was used as a control.

According to our results, DHRS8 is widely expressed in many tissues with the highest level in the liver and adrenal glands. On the other hand, the expression of DHRS12 was detected only in the brain. Our data could help to estimate the role of these enzymes in human body.

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STUDY OF EFFECTS OF COMMONLY USED ANTIRETROVIRALS ON ABCG2-MEDIATED TRANSPORT OF ABACAVIR ACROSS CELL MONOLAYER

ŤUPOVÁ, L., ČEČKOVÁ, M., ŠTAUD, F., ČERVENÝ, L.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: tupoval@faf.cuni.cz

Nucleoside reverse transcriptase inhibitor (NRTI) abacavir is a drug commonly used in combination antiretroviral therapy of HIV infection. It has been previously observed in our laboratory that drug efflux ATP-binding cassette (ABC) transporter, breast cancer

resistance protein (ABCG2), recognizes abacavir as a substrate. ABCG2 thus can be an important factor affecting its absorption, distribution, and elimination. As many antiretrovirals are substrates or inhibitors of ABCG2 the aim of our study was to investigate their drug–drug interactions with abacavir on the ABCG2 transporter. For this purpose we used well established *in vitro* model of monolayer formed by MDCKII cells stably expressing ABCG2 in setup of concentration equilibrium. First we analyzed effect of Ko134 (2 μM), model inhibitor of ABCG2, that completely abolished ABCG2-mediated active transport of abacavir across MDCKII monolayer and abacavir was demonstrated to have saturation transcellular kinetics. Subsequently, we tested several concentrations (ranging from 0.1 μM to 100 μM) of eight antiretrovirals (zidovudine, didanosine, lopinavir, atazanavir, ritonavir, stavudine, nevirapine, and rilpivirine) originating from three distinct drug groups (NRTI, non-NRTI, and protease inhibitors). We observed significant inhibition in all cases tested. Protease inhibitors lopinavir (20 μM), ritonavir and atazanavir (both at concentration of 100 μM) and non-NRTI rilpivirine (0.1 μM – 20 μM) showed the highest potency to inhibit ABCG2-mediated transport reaching abacavir was translocated across the MDCKII-ABCG2 monolayer by passive mechanism only. It can be concluded that co-administration of the tested antiretrovirals with abacavir might change pharmacokinetics of abacavir. Our data thus broaden knowledge on abacavir drug-drug interaction, however, it will need to be verified *in vivo*.

The study was supported by SVV/2015/260-185.

CHANGES OF MPV DURING END-STAGE RENAL FAILURE: A LINK BETWEEN PLATELET SIZE, INFLAMMATION AND MAIN CAUSES OF CHRONIC KIDNEY DISEASE

VAŇKOVÁ, B.,[1] MALÝ, J.,[1] DUSILOVÁ-SULKOVÁ, S.,[3] PAVLÍKOVÁ, Z.,[1] KUDLÁČKOVÁ, Z.[2]

[1] 4th Department of Internal Medicine-Hematology, Faculty Hospital in Hradec Králové, Czech Republic
[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3] Centre of Hemodialysis, Faculty Hospital in Hradec Králové, Czech Republic
e-mail: vankovb@faf.cuni.cz

Mean platelet volume, a simple indicator of platelet size, is automatically calculated by blood analysers. Higher MPV refers to larger platelets, which are more reactive. These thrombocytes are assumed to have the greatest role in development of haemostatic disorders and the most of other cardiovascular diseases (CVD), which are the main cause of death in patients with end-stage renal failure[1]. The chronic renal failure (CRF) also supports a small permanent inflammation in the body[2] which can be measured by C-reactive protein. The aim of the study was to find out if there is a relation between MPV and CRP in patients undergoing a continuous renal replacement therapy (CRRP). We also focused on etiopathology of renal failure. The main causes of CRF all over the world are chronic glomerulonephritis, diabetic nephropathy, hypertension, polycystosis of kidneys and chronic

interstitial nephritis[3]. We compared MPV values from three of these groups and tried to establish the correlation between pathogenesis of the disease and platelet activation.

A total of 102 patients who received the CRRP between November 2014 and February 2015 were taken into this retrospective study. The collected data included basic information such as gender, age, fundamental cause of renal failure and the length of dialysis program, if the transplantation of kidney has been realized and if the patient suffered from any cardiovascular disorders or the diabetes mellitus. Then MPV, platelet count and CRP taken from every first week in these 4 months and finally some technical information such as the method of dialysis (hemofiltration, hemodialysis or hemodiafiltration and the regular regime of dialysis.

The sex ratio (M/F) of all patients was 11 : 6, the average age was 66.2 (23–91) years. 45.1% of patients had already suffered from diabetes mellitus, 39.2% had some cardiovascular disease (ischemic heart disease, heart insufficiency, apoplexy etc.).

The mean MPV was 10.44 ± 0.96 which is closed to the upper limit of physiologic values (10.5 fl). The mean platelet count was 219×10^9/l and the mean CRP was 9.56 ± 10.70 mg/l. As predicted, the MPV values slightly diminished with increasing CRP (so with the higher level of inflammation), but the value of reliability of linear regression was very low ($R^2 = 0.0013$) that is why we could not consider it as a valuable confirmation of our theory.

The main causes of chronic renal failure were diabetic glomerulosclerosis (in 30 patients, 1st group), chronic interstitial nephritis (11, 2nd group), chronic glomerulonephritis (9, 3rd group) and other (62). The average value of MPV was the highest in the 1st group (10.79 fl) and the lowest in the 2nd group (10.57 fl).

We did not detect a significant relation between MPV and CRP, even if the trendline showed a negative correlation. The problem could be in interactions with other diseases the patients suffered, as CVD, hypertension and DM, which could influenced our values. The MPV was higher in patients with diabetic glomerulosclerosis. The other two groups could have lower MPV values due to an inflammatory etiology of the ailment.

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SOLUBILISATION, PURIFICATION AND RECONSTITUTION OF HUMAN 17-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 3 (HSD17B3)

ZAHRADNÍKOVÁ, T., ŠKARKA, A., ZEMANOVÁ, L.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: zahradt@faf.cuni.cz

Short-chain dehydrogenases/reductases (SDR) superfamily is a large group of NADP(H)/NAD(H)-dependent oxidoreductases. Human SDR enzymes are classified into 47 families, including cytosolic and membrane-bound ones. The objective of this study is a human membrane-bound enzyme 17-beta-hydroxysteroid dehydrogenase type 3 (HSD17B3), which participates in the biosynthesis of steroidal hormones and mainly catalysis the conversion of androstenedione to testosterone. The main goals of this study are to find a suitable detergent for successful solubilisation, purify the enzyme and prepare a reconstitution system for studying of the pure HSD17B3 behavior in the membrane.

Microsomes containing overexpressed HSD17B3 were isolated from Sf9 insect cells (*Spodoptera frugiperda)*. The first step is the solubilisation process, which involves detergent screening. Six detergents were tested, each in final concentration of 0.1%, 0.5% and 1.0% (w/v): ASB 14-4, C12E8, DDM, CHAPS, Igepal CA-630, Triton X-100. The detergent ASB 14-4 in concentration 0.5% (w/v) has been indentified to be the best one for the HSD17B3 solubilisation.

The next step is enzyme purification, using the His10-tag located on C-terminus of the HSD17B3 and Ni-metal affinity chromatography (Ni-IMAC). This method enabled to obtain the pure protein in the concentration 248.92 μg/ml and specific activity 5.16 nmol/mg/60 min based on the reduction of androstendione to testosterone.

The last step was successful incorporation of the *HSD17B3* into custom prepared liposomes, whose phospholipid composition was based on the membrane of the human liver endoplasmic reticulum.

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EFFECT OF SESQUITERPENES ON BIOTRANSFORMATION ENZYMES IN TISSUE SLICES

ZÁRYBNICKÝ, T., LNĚNIČKOVÁ, K., SZOTÁKOVÁ, B.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: zarybnto@faf.cuni.cz

High-precision tissue slicers (e.g. Krumdieck tissue slicer[1]) allow the rapid production of equally sized tissue slices of less than 250 μm thickness. Tissue slices are viable explants of tissue, cultivated *ex vivo*, with a reproducible and defined thickness and they serve as a multipurpose *in vitro* model. It contains all cell types of the tissue in their natural environment. The thin slices realistically and reliably represent the *in vivo* situation and have been used to study the metabolism, transport and biotransformation of xenobiotics, as well as for toxicological studies and others.

The essential factors, for the viability and function of cells inside the slices, are incubation conditions and slice thickness. These limit sufficient oxygen supply to the inner cell layers and exchange of nutrients and metabolites.

The aim of the study was to evaluate influence of several sesquiterpenes, secondary metabolites mainly produced by higher plants, as possible modifiers of biotransformation enzymes. Sesquiterpenes α-humulene, β-caryophyllene and β-caryophyllene oxide were chosen for this purpose. Above mentioned sesquiterpenes showed a potential as inducers of the detoxifying enzyme glutathione S-transferase in forestomach, liver and small bowel mucosa of A/J mice[2].

In our project we used rat liver slices that were 8 mm in diameter and 200 μm thin. They were cut on the Krumdieck tissue slicer in ice-cold and oxygenated Krebs-Heseleit buffer set up to pH 7.4. After cutting, the tissue slices were incubated for 3, 6 and 24 hours in medium containing sesquiterpene and in carbogen atmosphere (95% O_2/5% CO_2). After, the tissue was homogenized. The results were compared to control homogenates from slices incubated in clear medium. Advantage of the method lies in producing multiple tissue samples that can be incubated with variable compounds in different concentrations and reducing the number of animals necessary for experiment.

So far, we managed to optimize the method and raise the viability of the tissue slices, especially the viability after 24 hours. The first sesquiterpene studied was α-humulene. However, the results did not show any induction of glutathione S-transferase in comparison to control. We also tried to assess the effect of α-humulene on sulfotransferase and quinone oxidoreductase 1, but the measured activities were at detection limit. Further studies have to be conducted in order to evaluate effect of β-caryophyllene and β-caryophyllene oxide on biotransformation enzymes.

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INTERACTION OF ANTIRETROVIRAL DRUGS ETRAVIRINE AND RILPIVIRINE WITH ABC DRUG EFFLUX TRANSPORTERS *IN VITRO*

ZELINSCAIA, V., ŘEZNÍČEK, J., ŠTAUD, F., ČEČKOVÁ, M.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: Zelinscv@faf.cuni.cz

Etravirine and rilpivirine, are relatively new antiretroviral drugs that belong to the second-generation non-nucleoside reverse transcriptase inhibitors used in combination therapy (cART) of HIV positive patients. ABC (ATP-binding cassette) transporters are extensively expressed in normal tissues (e.g. liver, kidney, intestine, blood-brain barrier, placenta), where they are able to affect pharmacokinetic behavior of various drugs. ABCB1 (P-glycoprotein), ABCG2 (BCRP) and ABCC2 (MRP2) represent the most com-

mon drug transporters, on which drug-drug interactions (DDI) can occur. Nevertheless, the current knowledge on interactions of etravirine and rilpivirine with the transporters and their potential to create transporter-mediated DDI is only limited so far. In this study we therefore aimed to investigate inhibitory potency of etravirine and rilpivirine towards ABCB1, ABCG2 and ABCC2 drug efflux transporters employing *in vitro* accumulation/efflux studies with relevant fluorescent substrates.

The accumulation assays with Hoechst 33342 and Rhodamine 123 on MCDKII-ABCB1 cells have demonstrated that rilpivirine is a potent ABCB1 inhibitor able to bind H- as well as R-site of the transporter, while etravirine does not inhibit ABCB1 at all. In MDCKII-ABCG2 cells, both antiretrovirals revealed significant inhibitory potency to ABCG2. Nevertheless, using the efflux experiments with calcein-AM in MDCKII-ABCC2 cells neither etravirine nor rilpivirine caused inhibition of MRP2.

Since our data clearly showed that rilpivirine and etravirine are ABCB1 and/or ABCG2 inhibitors, we have additionally employed transport assays to evaluate possible DDI of these drugs with tenofovir disoproxil fumarate (TDF), another antiretroviral drug used in cART and being confirmed as ABCB1 and ABCG2 substrate. Our transport experiments on MDCKII-ABCB1 and MDCKII-ABCG2 cell monolayers demonstrate that both antiretrovirals significantly affect TDF permeability across the cellular monolayer due to inhibition of the ABC drug efflux transporters. These results suggest that etravirine and rilpivirine possess a great potential for drug efflux transporters-mediated DDI, which could have an impact on antiretroviral dosage scheme during cART in clinical practice.

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PROCEEDING PAPERS

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TEXTBOOKS

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DEGREES

Doctoral Dissertation Thesis (Ph.D.), Faculty of Pharmacy in Hradec Králové (CZ), Charles University in Prague (CZ), 2013

1. Mgr. AHMADIMOGHADDAM , DAVOUD: Organic Cation Transporter 3 (OCT3/SLC22A3) and Multidrug and Toxin Extrusion 1.(MATE 1/SLC47A1) Protein in the Placenta: Expression, Localisation and Function.
2. PharmDr. BARTAS, ROBERT: Retrospektivní analýza spotřeby hypolipidemik (Retrospective Analysis utilization of Lipid Modifying Agents).
3. PharmDr. BÍLEK, TOMÁŠ: Využití GC a SPME v analýze přírodních látek (The Using of GC and S of GC and SPME in the Analysis of Natural Products).

4. Mgr. BLAŽKOVÁ, ŠÁRKA: Management osteoporózy na úrovni primární péče v ČR (Osteoporosis management in primary care in the Czech Republic).
5. Mgr. BRABCOVÁ, IVANA: Využití moderních chromatografických přístupů a úprav vzorků v analýze biologicky aktivních látel (The Use of Modern Chromatographic Approaches and Sample Preparation in the Analysis of Biologically Active Substances).
6. Mgr. DOLEŽEL, JAN: Deriváty thiazolu jako potenciální léčiva (Derivatives of thiazole as potential drugs).
7. Mgr. DOSEDĚL, MARTIN: Analýza vybraných rizik farmakoterapie (Analysis of Selected Risks of Pharmacotherapy).
8. Mgr. DRASTÍKOVÁ, MARKÉTA: Využití separačních metod v klinickém výzkumu (Using of Separation Methods for Clinical Research).
9. Mgr. FIKROVÁ, PETRA: Hodnocení poškození a reparace DNA u pacientů s nemalobuněčným plicním karcinomem vzhledem k farmakoterapii deriváty platiny (Evalution of DNA Damage and Repair in patients with Non-small Cell Lung Cancer Due to Drug Therapy with Platinum Derivatives).
10. Mgr. FILIPSKÝ, TOMÁŠ: Výzkum látek s potenciálem chelatovat železo a jejich případné využití v terapii akutního infarktu myokardu (Screening of Iron-chelating Substances and their Potential Use in the Therapy of Acute Myocardial Infarction).
11. RNDr. HANUŠOVÁ, VERONIKA: Možnosti zvýšení účinnosti vybraných cytostatik (Possibilities to Increase the Effectiveness of Selected Cytostatics).
12. Mgr. HONEGR, JAN: Vývoj metod pro zvýšení citlivosti detekce v kapilární zónové elektroforéze polyfenolických přírodních látek s využitím online elektromigračních prekoncentračních technik (Development of Methods for Sensitivity Enhancement in Capillary Zone Electrophoresis of Natural Polyphenolic Compounds with Use of Online Electromigration Preconcentration Techniques).
13. Mgr. HROMÁDKOVÁ, LUCIE: Analýza vybraných lékových problémů u některých revmatických nemocí (Analysis of the Selected Drug Problems in some Rheumatic Diseases).
14. Mgr. JIRKOVSKÁ, ANNA: Kardiotoxicita protinádorových léčiv: Studium molekulárních mechanizmů a možnosti farmakologické kardioprotekce (Cardiotoxicity of Antineoplastic Drugs: Study of the Molecular Mechanisms and the Possibilities of Pharmacological Cardioprotection).
15. Mgr. KALAFUT, PETER: Možnosti uplatnenia nových stacionárnych fáz v analýze liečiv (The possibilities of utilization of alternative stationary phases in pharmaceutical Analysis).
16. Mgr. KOZIC, JÁN: Syntéza a design potenciálně antimikrobiálně aktivních sloučenin (Synthesis and Design of Potentially Antibacterial Active Compounds).
17. Mgr. MALÝ, JOSEF: Analýza možnosti aplikovat klinickou farmacii do farmaceutické péče (Analysis of Possibility of Applying Clinical Pharmacy to Pharmaceutical Care).
18. PharmDr. MELICHAROVÁ, LUDMILA: Analýza biologického chování receptorově specifických radiofarmak *in vitro* (Analysis of the Biological Behavior of Receptor-Specific Radiopharmaceuticals *in vitro*).
19. Mgr. NOVÁ, ALICE: Interakce léčiv s nukleárními receptory při regulaci biotransformačních enzymů a lékových transportérů (Drug Interactions with Nuclear Receptors in the Regulation of Drug Metabolizing Enzymes and Drug Transporters).
20. PharmDr. PILAŘOVÁ, PAVLA: Analýza biologicky aktivních látek kapalinovou chromatografií (Analysis of Biologicaly Active Substances Using Liquid Chromatography).
21. Mgr. ŘEHÁČKOVÁ, PETRA: Biomechanické vlastnosti kostí v experimentu a vliv homocysteinu na kostní zdraví (Biomechanical Properties of Bones in the Experiment and the Effect of Homocysteine in Bone Health).
22. MUDr. SMETANOVÁ, LIBUŠE: Transportní mechanismy xenobiotik (model Caco-2 buněčné monovrstvy) ve vztahu k biofarmaceutickému klasifikačnímu systému (BCS) (Transport Mechanisms of Xenobiotics (Caco-2 Cell Monolayer Model) in Relation to the Biopharmaceutics Classification Systém (BCS)).
23. Mgr. ŠIROKÁ, JITKA:Vývoj elektroforetických metod pro analýzu biologicky aktivních látek s využitím tvorby komplexních sloučenin (Development of Electrophoretic Methods for the Analysis of Biologically Active Compounds using Complex Formation).

1. PharmDr. BÁRTOVÁ, BARBORA: Farmakoterapie chronické nenádorové bolesti u geriatrických pacientů (The Pharmacotherapy of Chronic Non-malignant Pain among Geriatric Patients).
2. PharmDr. BAŽANTOVÁ, TEREZA: Vývoj a validace HPLC metody pro analýzu pilokarpinových lékopisných očních kapek (Development and Validation of HPLC Method for Analysis of Pharmacopoeial Eye Drops with Pilocarpine).
3. PharmDr. BELEŠOVÁ, MONIKA: Stanovení albendazolu a jeho metabolitů pro studium rezistence helmintů vůči benzimidazolovým anthelmintikům (Determination of Albendazole and Its Metabolites for Study of Anthelmintic Resistance).
4. PharmDr. BRIESTENSKÝ, DAVID: Identifikace a kvantifikace anthelmintik a jejich Metabolitů u tasemnic metodou LC/MS (Identification and quantification of anthelmintics and their metabolites in tape).
5. PharmDr. CIHLÁŘ, ZDENĚK: Viskoelasticita kostí – metodika měření (Viscoelasticity of bones – the methodology of measurement).
6. PharmDr. ČERNÁ, IVANA: Hodnocení individuálně připravovaných polotuhých přípravků (Reviews of Individually Prepared Semisolid Preparations).
7. PharmDr. ČTVRTEČKOVÁ, TEREZA: Zhodnocení výskytu zearalenonu v potravinách a krmivech na území ČR (The Evalution of Zearalenone in Food and feed in the Czech Republic).
8. PharmDr. DVOŘÁKOVÁ, MARTINA: Stanovení flavonoidů v doplňcích stravy s využitím micelární elektrokinetické chromatografie (Determination of flavonoids in food supplements using micellar electrokinetic capillary chromatography).
9. PharmDr. FIALOVÁ, BARBORA: Studium matrice vzorku pro SIA stanovení hliníku v reálných vzorcích (Study of Sample Matrix Effect for SIA Determination of Aluminium in Real Samples).
10. PharmDr. FRAŇKOVÁ, ALENA: Osmolalita parenterálních přípravků. Chlorid vápenatý (Osmolality of Parenteral Preparations. Calcium Chlorid).
11. PharmDr. FRNOCHOVÁ, LENKA: Vliv Sunitinibu na expresi ICAM-1 u normotenzních a hypertenzních potkanů (Sunitinib Effects on the Expression of ICAM-1 in Normotensive and Hypertensive Rats).
12. PharmDr. GOTTVALD, TOMÁŠ: Vývoj metody pro stanovení entekaviru v biologických materiálech s využitím UHPLC-MS/MS (Development of method for the determination of entecavir in biological materialsusing UHPLC-MS/MS).
13. RNDr. GRADOŠOVÁ, IVETA: Sledování kostního metabolismu ovlivněného vybranými léky (Monitoring of bone metabolism affected by selected drugs).
14. PharmDr. HAŠKOVÁ, VERONIKA: Degradace polyesterových nosičů ve vodném médiu (Degradation of Polyester Carriers in Aqueous Medium).
15. RNDr. HAVELEK, RADIM: Možnosti průtokové cytometrie v analýze reakce buněk na genotoxický stres (Possibilities of flow cytometry in analysis of cellular response to genotoxic stress).
16. PharmDr. HECZKOVÁ, JANA: Analogy acetylpyrazin-thiosemikarbazonů jako potenciální léčiva (Acetylpyrazin-thiosemicarbazone analogues as potential drugs).
17. RNDr. HLAVÁČKOVÁ, MARKÉTA: Vývoj HPLC metody pro stanovení betakarotenu v potravních doplňcích (HPLC method development for betacarotene determination in nutraceuticals).
18. PharmDr. HNILIČKOVÁ, PETRA: Postoje kuřáků a nekuřáků k rizikům pasivního kouření (Attitude of Smokers and Non-smokers Towards Risks of Passive Smoking).
19. PharmDr. HORÁKOVÁ, VERONIKA: Vliv diklofenaku na autotrofní organismy (Effect of Diclofenac on Autotrophic Organisms).
20. PharmDr. JÄGEROVÁ, KATEŘINA: Vývoj HPLC metody pro stanovení vybraných aktivních látek v potravních doplňcích (HPLC method development of active substances in nutraceuticals).
21. PharmDr. KAMENÍČKOVÁ, DANIELA: HPLC stanovení vybraných insekticidů v kosmetickém přípravku (HPLC determination of selected incestisides in cosmetic).
22. PharmDr. KÖNIGOVÁ, ELIŠKA: Optimalizace přípravy nanočástic z větvených polyesterů s terbinafinem (Optimisation of Preparation of Nanoparticles from Branched Polyesters with Terbinafine).
23. PharmDr. KOREC, DAVID: Studium radioaktivního značení dota-sargastrinu a stanovení stability značeného produktu (Study of Radiolabelling DOTA-Sargastrin and Determination the Stability of Labeled Product).

24. RNDr. KOŘÍNKOVÁ, MARTINA: Vliv quantum dots částic na průběh chemiluminiscence založené na oxidaci manganistanem (The influence of Quantum Dots Particles to Chemiluminiscence Based on Oxidation by Permanganate).
25. PharmDr. KOTALOVÁ, PETRA: Analýza lékových pochybení v preskripci identifikovaná farmaceutem při poskytování lékárenské péče (Analysis of Prescribing Errors Identificated by Pharmacist During Provision of Pharmaceutical Care).
26. PharmDr. KOTILOVÁ, LUCIE: Hodnocení složení mateřského mléka ve vztahu k nutrici kojící ženy (Evaluation of the Composition of Breast Milk in Relation to Nutritional Lactating women).
27. PharmDr. KRPELÍKOVÁ, IRENA: Srovnání reaktivační účinnosti nově připravených oximů: K250, K251 s již používanými oximy proti tabunu u laboratorního potkana (A Comparison of the Reactivating Efficacy of Newly Developed Oximes K250, K251 with Commonly used Oximes against Tabun in Rats).
28. PharmDr. KŘEPELOVÁ, JANA: Produkce fenylpropanoidů v rostlinné explantátové kultuře (Production of phenylpropanoids in the plant explantat culture).
29. PharmDr. LEJSALOVÁ, ALŽBĚTA: Měření mechanických vlastností kůže po aplikaci přípravku proti vráskám (Measurement of the skin mechanical properties after application of the anti-aging product).
30. PharmDr. LUŽOVÁ, VERONIKA: Elektroforetické stanovení vybraných myorelaxancií s využitím bezkontaktní vodivostní detekce (Electrophoretic determination of selected muscle relaxants using contactless conductivity detection).
31. PharmDr. MACÁKOVÁ, PETRA: Cytotoxicita beauvericinu, citrininu, deoxynivalenolu a T-2 toxinu stanovena metodou *in* vitro v buňkách vero (Cytotoxicity of beauvericin, citrinin, deoxynivalenol and T-2 toxin by *in vitro* method using vero cells).
32. PharmDr. MACHOVÁ, IVA: Studium mechanismu účinku chinoxalinového derivátu VN-034 na respirační systém *in vitro* (*In vitro* Mechanism of Action of Quinoxaline Derivate VN-034 on the Respiratory Systém).
33. MAREK, JAN: Příprava a testování látek obsahujících kvartérní dusík (Synthesis and Evaluation of Quaternary Nitrogen Compounds).
34. PharmDr. MAŠATOVÁ, PAVLÍNA: Energetické hodnocení lisovacího procesu tablet z nového typu silicifikované mikrokrystalické celulosy (Energy Evaluation of Compression Process of Tablets from the New Type of Silicified Microcrystaline Cellulose).
35. PharmDr. MERCOVÁ, MIROSLAVA: Optimalizace diagnostických metod oxidačního stresu (Optimization of Oxidative Stress Diagnostic Methods).
36. PharmDr. MLČOCH, MICHAL: Alkylaminoderiváty pyrazinamidu jako potenciální antituberkulotika (Alkylamino- Derivatives of Pyrazinamide as Potential Antituberculotic Drugs).
37. PharmDr. MOČÁRKOVÁ, ZDEŇKA: Analýza lékových problémů identifikovaných farmaceutem při dispenzační činnosti a vliv intervence farmaceuta směrem k předpisujícím lékařům (Analysis of drug related problems identificated by pharmacist during provision dispensation and the influence of pharmacistś intervention on prescribing doctors).
38. PharmDr. NAGYOVÁ, LUCIE: Metabolický syndrom – ovlivnění profilu rizik (Metabolic Syndrome – Changing Profile of Risks).
39. RNDr. NÁPRAVNÍKOVÁ, LENKA: Porovnání stanovení 25 (OH) D v lidském séru metodami UHPLC – MSIMS a CMIA Abbot Architekt (A Comparison of the Determination 25(OH) in the Human Serum by Methods UHPLC-MS/MS and….).
40. PharmDr. NETOLICKÁ, JANA: Hodnocení bioimpedančních parametrů u pacientů s bronchogenním karcinomem (Evaluation of the bioimpedance parameters in patients with bronchial carcinoma).
41. PharmDr. NOSKOVÁ, VERONIKA: Varianty emulzní metody přípravy nanočástic (Formulation of Polyester Nanoparticles using Variants of Emulsion Methods).
42. PharmDr. PAGÁČOVÁ, LUCIE: Vliv rodinné zátěže a prostředí na rozvoj alergických onemocnění za posledních 10 let v ČR (The Effect of Familial Burden and Enviroment on the Progress of Allergic Diseases in the last 10 Years in the Czech Republic).
43. RNDr. PAVELKOVÁ, PETRA: HPLC analýza isoxikamu v krvi s využitím fluorometrické detekce (HPLC analysis of isoxicam in blood with using fluorometric detection).
44. PharmDr. PETRŽELOVÁ, MARKÉTA: Studium lisovatelnosti prostého zrněného prášku (Effect of Formulation Factors on Simple Granules to Compacting Process Parameter).

45. PharmDr. PRAMUKOVÁ, ZUZANA: Porovnanie účinku látok (VN 008 a VN 014) zo skupiny derivátov chinazolínu na modeli izolovanej priedušnice (Comparison of the Effect of Substances (VN 008 and VN 014) from the Group of Quinazoline derivatives on the model of isolated trachea).
46. PharmDr. PRUCHOVÁ, ZUZANA: Modifikace glutathion-S-transferasy vybranými glykačními činidly (Modification of glutathione-S-transferase by selected glycating agents).
47. PharmDr. PTÁČNÍKOVÁ, JANA: Hodnocení vybraných spirometrických a dynamometrických parametrů u bronchogenního karcinomu (Evaluation of Spirometry and Dynamometric Parameters in Advanced Lung Cancer).
48. PharmDr. RACHAČOVÁ, LENKA: Viskoelasticita směsí plniv se stearanem hořečnatým (Viscoelasticity of Fillers with Magnesium Stearate).
49. PharmDr. RÖDER, JAN: Příprava modulátorů cholinesterás a cholinergních receptorů (Preparation of modulators of cholinesterases and cholinergic reeptors).
50. PharmDr. ROUŠAROVÁ, PAVLA: Vliv frekvence na velikost mechanických parametrů biologických vzorků (The influence of frequency on values of mechanical parameters of biological samples).
51. PharmDr. RUFFEROVÁ, LENKA: Studium pulzního průběhu degradace alifatickcých Oligoesterů (Pulse degradation proces of aliphatic oligoesters research).
52. RNDr. SEJKOROVÁ, ANDREA: Analýza citrusových flavonoidů ve farmaceutickcých přípravcích a potravních doplňcích metodou kapilární zónové elektroforézy (Analysis of citrus flavonoids in pharmaceuticals and food supplements by capillary zone electrophoresis).
53. PharmDr. SLAVĚTINSKÁ, LENKA: Deriváty pyrazinu jako potencionální antitubekulotika (Derivatives of pyrazine as potential antituberculotic drugs).
54. PharmDr. STAŇKOVÁ, VERONIKA: Katechiny: interakce s lidským sérovým albuminem a ovlivnění jeho glykace methylglyoxalem (Catechins: Interaction with Human Serum Albumin and Affecting its by methylglyoxal).
55. PharmDr. ŠINÁGLOVÁ, PAVLA: Studium nového směsného suchého pojiva na bázi laktosy a mikrokrystalické celulosy (Studyof a New Mixed Dry Binder based spray-dried lactose and Microcrystalline cellulose).
56. PharmDr. ŠÍNOVÁ, IVANA: Vliv Spiruliny platensis na expresi hemooxygenázy-1 u myší (Effect of *Spirulina Platensis* on Heme Oxygenase-1 Expresion in Mice).
57. PharmDr. ŠTĚPÁNOVÁ, HANA : Účinky nových derivátů chinazolinu na respirační systém (Effects of New Quinazoline Derivates on the Respiratory Systém).
58. PharmDr. TOBIAS MIRIAM, CHRISTINE: Kvantifikovaní viskoelastických vlastností aortální stěny prasete v závislosti na směru namahání, vzdálenosti od srdce a stáří dárců (Quantification of viscoelastic properties of porcine aortic walls in dependence on direction of strain, distance from heart and age of donors).
59. RNDr. TOMANOVÁ, RADANA: Role karbonylreduktas v biotransformaci léčiva Bupropionu (Carbonyl Reductases Role in the Biotransformation of Drug Bupropion).
60. PharmDr. VACULOVÁ, GABRIELA: Mikroskopické hodnocení květů z různých pěstovaných odrůd bezu cerného (Microscopy of *Elderberry* Flowers from Cultivated Varieties).
61. RNDr. VÁLOVÁ, OLGA: Dvoudimenzionální separace v nízkotlakém systému sekvenční injekční chromatografie (Two dimensional separations in Sequential Injection Chromatography low pressure systém).
62. PharmDr. VÍCHOVÁ, EVA: Využití DSC pro hodnocení kusových léčivých přípravků II (Use of DSC to Determinate Volume of Active Substances II).
63. PharmDr. ŽÁKOVÁ, MARIE: Studium účinků sunitinibu na cévní endotel u normotenzních a hypertenzních potkanů (Study of Sunitinib Effects on Vessel Endothelium in Normotensive and Hypertensive Rats).

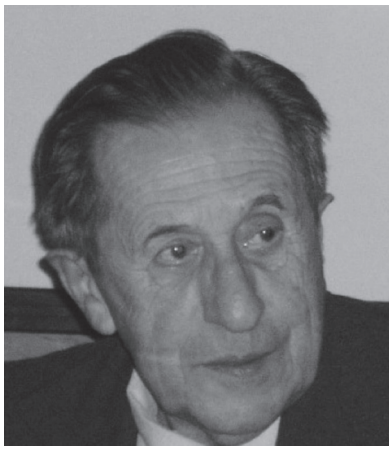
PATENTS

LIBRA, A., BUNČEK, M., MILETÍN, M., ZIMČÍK, P., NOVÁKOVÁ, V., KOPECKÝ, K.: Zhášeč Q-30 (Quencher Q-30). Hradec Králové: Generi Biotech s.r.o., 2013.

RABIŠKOVÁ, M., SPILKOVÁ, J., DVOŘÁČKOVÁ, K., LAMPRECHT, A.: Farmaceutická kompozice obsahující rutin určená pro přívod účinné látky do oblasti tlustého střeva (Pharmaceutical composition containing rutin intended for delivery of active substance to the colon area). Prague: Industrial Property Office, 2013. CZ 25453 U1.

SOCIAL HAPPENINGS

IN MEMORY OF Prof. RNDr. PhMr. VLADIMÍR JOKL, DrSc.



Prof. Vladimír Jokl was born in Hustopeče on 18th January 1926. During the World War II, he was in a forced labor. Then he graduated from the grammar school and performed two years' practice in a pharmacy. Afterward he studied pharmacy and science at the Faculty of Science of the Masaryk University in Brno and graduated in 1949 as a Master of Pharmacy (PhMr.). RNDr. degree was awarded to him in 1952 on the basis of the thesis entitled "Analytical Importance of 1,2-Aminooximes". At that time, he already worked as junior assistant and since 1954 as a senior assistant at the Department of Analytical Chemistry of the Faculty of Science of the Masaryk University. In 1961, he defended the dissertation "Behaviour of Complexes in Iontophoresis on Paper" and became a Candidate of Sciences (CSc. – equivalent to PhD degree).

When a new Faculty of Pharmacy was established in Hradec Králové, prof. Jokl became the head of the Department of Analytical Chemistry of this faculty and had to form it with new staff and equipment.

Based on his doctoral dissertation with the topic "The Questions of Theory and Application of Zone Electrophoresis of Complex Compounds" he obtained the scientific degree Doctor of Sciences (DrSc.) in 1974. Three years later, he was appointed full professor of analytical chemistry. In addition to his work at the Department of Analytical Chemistry he also participated in the management of the Faculty of Pharmacy in Bratislava and in Hradec Králové as a vice-dean.

Prof. Jokl passed away on 7th August 2015. To commemorate his personality and his importance for the development of both analytical chemistry and pharmacy we attach a personal recollection of one of his students and collaborators.

V. Opletalová

Dear Prof. Jokl,

I had the good fortune to collaborate with you for almost 50 years. I met you for the first time in 1959 during studies of analytical chemistry at the Faculty of Science of the Masaryk University in Brno where you had worked since the year 1950.

The second period of your pedagogical and research activities was associated with the establishment of the Faculty of Pharmacy in Bratislava (the only Faculty of Pharmacy in the then Czechoslovakia) where all pharmacy students and many teachers passed in 1960. There you were engaged in building the Department of Analytical Chemistry, you founded the Laboratory of Separation Methods and developed zone electrophoresis method for characterization of complex compounds.

After nearly 10 years we moved again in connection with the establishment and construction of a new Faculty of Pharmacy of Charles University in Hradec Králové. It was a period that required an extraordinary effort as it was necessary to start everything from the beginning and build the department with new teachers and new instrumentation.

Today I can responsibly say that under your leadership new academic and research workplace was created at a high level and very solid foundations. When you handed me over the management of the department at your retirement in 1989, I could exploit its potential and to continue the teaching and research activities.

You were an excellent teacher. Your work was always associated with the teaching of analytical chemistry, with a perfectly-led lectures and practical training, and education of a number of researchers. Your research activities were mainly focused on the development of electrophoretic separation methods and their application to study complexes and pharmaceuticals.

During your many years at the Department of Analytical Chemistry at the Faculty of Pharmacy in Bratislava and in Hradec Králové, I knew you as a very modest and open person always willing to advice, as an exemplary teacher and hardworking scientist who dedicated all knowledge and forces to analytical chemistry and education of a new generation of pharmacists.

I am proud to say that I was your pupil and collaborator.

Prof. RNDr. Rolf Karlíček, DrSc.

IN MEMORY OF RNDr. DUŠAN CHLAPEK, CSc.

RNDr. Dušan Chlapek, CSc. passed away on 24th January 2015 after a futile battle with serious illness. He started his professional pharmaceutical carrier at the Department of Organization and Management of Pharmacy (later Department of Social and Clinical Pharmacy) of the Charles University in Hradec Králové. He stayed faithful to teaching also after he left the faculty and became a head of the community pharmacy which served also for professional internships of the students. Now, we would like to commemorate his activities and thank him.

Dušan Chlapek was born on 3rd June 1952 in the maternity ward of the hospital in Ostrava-Zábřeh. Then he lived with his parents in Šenov where he also attended the local nine-year basic school. His father, PhMr. František Chlapek, became then the first chief pharmacist of the newly created city Havířov. Dušan successfully graduated from the grammar school in this town in 1970 and was admitted to the new Faculty of Pharmacy in Hradec Králové. After his graduation in 1975, he became a postgraduate researcher in the field Science on Health Care at the Department of Organization and Management of Pharmacy (supervisor prof. RNDr. PhMr. Jan Solich, CSc.).

After passing one-year military service, he continued his work. RNDr. degree was awarded to him on the basis of the rigorous exam and the defense of the rigorous thesis entitled "Some questions of professiography of the specialist workers in the pharmaceutical service" in 1979. Based on the agreement between the Faculty of Pharmacy and the District Institute of National Health, Dr. Chlapek passed from the Faculty to the Pharmacy Service (on 30th November 1983) where he became an operating pharmacist, was appointed the head of the Faculty Pharmacy, and in 1984 also the district pharmacist. In the same year, he defended his dissertation "A study of the factors influencing the education and career opportunities of pharmacists" and achieved the CSc. degree (equivalent to PhD degree).

During his stay at the Department of Organization and Management of Pharmacy, Dr. Chlapek was engaged also in pedagogical activities and spent 3 months at the Department of Social Pharmacy of the Pharmaceutical Faculty of Uppsala University (Sweden).

He got acquainted with problems of modern pharmacy, the role of drugs in the society as well as methods of education. His stay abroad resulted also in the improvement of English that he then used in contacts with international visitors at the Faculty of Pharmacy in Hradec Králové, and also during traineeship and excursions of foreign students in the Faculty Pharmacy.

His activities in the Czech Pharmaceutical Society must also been mentioned. He was the Science Secretary of the Section of Social Pharmacy and the main organizer of the successful 5th Social Pharmacy Workshop that was held in Prague in 1988. On that occasion, about 70 social pharmacist from fourteen Western and Eastern countries met for the first time behind the iron curtain. The response to the workshop was great, and the then Czechoslovakia was well represented. Many new connections with international schools of pharmacy and scientists were established due to the effort of Dr. Chlapek.

The Velvet Revolution of 1989 has brought many changes in plans for pharmaceutical education, for establishing new faculty pharmacies, and for developing concept of a pharmacist as a collaborator of the physician and an adviser of the patient. Nonetheless, Dr. Chlapek actively joined the activities the Czech Chamber of Pharmacists promoting its healthcare focus and mission. This can be documented by his work in Pharmacentrum Inc., by his engagement in the continuing education and the co-operation with the Faculty of Pharmacy which is a part of the prestigious Charles University.

Characteristic tranquility and good mood, cooperative efforts and good interpersonal relationships were typical for Dr. Chlapek throughout his professional and family life till his last moments.

May he be an example for other graduates from the Faculty of Pharmacy. We thank him for everything on behalf of all colleagues who have known him.

Honor his memory.

J. Solich, V. Opletalová

INSTRUCTIONS FOR AUTHORS FOR FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE

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

Manuscript should be divided into sections:

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

Names of Authors – first name and surname with the reference to institution's name (Times New Roman 12, center alignment) (JIŘÍ GASPARIČ[1], MILENA ČERMÁKOVÁ[2]). Write the names in lowercase letters and then format them using Font – All caps (Písmo – Všechna velká).

Names of Institutions – (Times New Roman 10, centre alignment) (1 Department of …, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic).

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

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

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

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

Figures must be submitted in the best quality and original size (not more than 12.5 × 18 cm) separately as a supplement. Indicate the placement of the figure in the text. Captions and notes are placed below (10, centre alignment).

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

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

Chemical structures should be drawn with a suitable drawing program.

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

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

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

UNIVERSITAS CAROLINA PRAGENSIS

FOLIA PHARMACEUTICA UNIVERSITATIS CAROLINAE XLVI

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Lectured: doc. RNDr. Věra Klimešová, CSc., doc. RNDr. Pavel Doležal, CSc.

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