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SECTION OF BIOLOGICAL SCIENCES

CYTOSTATIC EFFECT OF NEW PACLITAXEL DERIVATIVES IN SELECTED CANCER LINES

AMBROŽ, M., HANUŠOVÁ, V., SKÁLOVÁ, L.

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

Paclitaxel (PTX) is cytostatic drug used for therapy of breast, lung and ovarian cancer. It stabilizes microtubules and as a result, interferes with the normal breakdown of microtubules during cell division. Unfortunately it makes no difference between normal and malignant cells and affect all proliferating cells. To increase selectivity effect of PTX on cancer cells, conjugates of PTX with gonadotropin-hormon (GnRH) were prepared in Institute of Experimental Botany of Czech Academy of Sciences in Prague.

The aim of our study was to compare effect of PTX conjugates and PTX alone on cell viability and proliferation. For this purpose, cancer cell lines MDA-MB-231, CaCo2 and primary cultures of rat hepatocytes were used. MDA-MB-231 is human breast adenocarcinoma line with receptors for GnRH. CaCo-2 line is derived from epithelial colorectal adenocarcinoma cells. Caco-2 cells and hepatocytes have not GnRH receptors. Viability of cells was monitored using X-Celligence system for real-time cell analysis and assayed using two end-point methods (MTT, NRU).

Conjugate MP264 has been showed lower antiproliferative effect than PTX in cell lines CaCo2 and MDA-MB-231. Conjugates MP265 and MP394 have been showed comparable antiproliferative effect with PTX in cell lines CaCo2 and MDA-MB-231. Conjugate MP 264 has been showed lower cytotoxic effect in primary cell culture of rat hepatocytes.

The study was supported by Charles University, project SVV267 004.

EFFECT OF FLUBENDAZOLE ON PROLIFERATION OF COLORECTAL CARCINOMA CELL LINES *IN VITRO*

ČÁŃOVÁ, K[1]., SZOTÁKOVÁ, B[1]., KRÁLOVÁ, V.[2]

[1]Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Kralové,
Charles University in Prague, Czech Republic
[2]Department of Medical Biology and Genetics, Faculty of Medicine in Hradec Králové,
Charles University in Prague, Czech Republic
e-mail: canovak@faf.cuni.cz

Flubendazole is widely used anthelmintic drug belonging to benzimidazole group. The molecular mechanism of action of flubendazole is based on specific binding to tubulin, which results in disruption of microtubule structure and function and in the interference with the microtubule-mediated transport of secretory vesicles in absorptive tissues of helminths. The microtubule-disrupting properties of benzimidazole derivatives raised recently interest in these compounds as possible anti-cancer agents. Because considerable concentrations of flubendazole in intestinal cells after p.o. administration could be reached, we performed this study to investigate the antiproliferative potential of flubendazole in a panel of intestinal cancer cell lines.

Three colon cancer cell lines SW480, SW620 and NCM460 were treated with different concentrations of flubendazole (0.1–10 μM) for 24, 48 and 72 hours. Cell viability was assayed using Neutral red uptake test and WST-1 test. The effect of flubendazole on the cell cycle distribution was analysed with flow cytometry. Flubendazole induced accumulation of cells in G2/M phases of the cell cycle and significantly inhibited cell proliferation in concentration-dependent and time-dependent manner in comparison to the control samples.

In conclusion, anthelmintic benzimidazole drug flubendazole shows significant cytostatic effect in human intestinal cancer cell lines.

The study was supported by research program SVV267 004.

IDENTIFICATION OF DNA SEQUENCE BINDING C/EBP AND C/EBPB PROTEINS INVOLVED IN RANKL EXPRESSION

DUŠEK, L.,[1] KŘIVČIKOVÁ, L.,[1] MLAKAR, V.[2]

[1]Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
[2]Chair of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Slovenia
e-mail: duseklu@faf.cuni.cz

Bone thinning – osteoporosis – is an increasing problem of human health worldwide. Johnell and Kanis[1] had estimated that in 2000 occurred more than 9.0 million of osteoporotic fractures – that makes about 3 fractures from osteoporosis every second. Because decrease in bone mineral density (BMD) is associated with age and due the aging

of the world population, the issue of bone health and especially osteoporosis is expected to emerge on its urgency. Discovery of osteoprotegerin (OPG) / receptor activator of nuclear factor kappa-B (RANK) / RANK-ligand (RANKL) pathway in late 90′ gave scientist a fruitful target for future investigations about bone metabolism and revealed interesting interconnection with cardiovascular diseases, immunity and cancer survival and targeting.

The goal of the study was to identify transcription factors and DNA binding sites in –662 to –438 region that control RANLK expression.

For identification was used pGl3-F3 plasmid construct with restricted RANKL promotor, which had most significant decrease of RANKL expression when compared to other pGl3 constructs in transfected human osteosarcoma (HOS) cells CRL-1533™ (*Mlakar – unpublished data*). Scanning for transcription factors with Noris Medical Library revealed two binding places (A, B) in pGl3-F3. Effect of mutations on RANKL expression was measured with Dual Luciferase Assay after transfection of the plasmid to HOS cells. In order to identify transcription factor binding to mutated sites electrophoretic mobility shift assay (EMSA) were carried out using GATA1, Lyf and C/EBPβ antibodies.

Mutation at places A (–512) and B (–502) resulted in 43.3% and 19.0% restoration of RANKL promoter activity respectively, when comparing F2 and F3. The results show that both sites are binding the transcription repressors. EMSA showed binding of C/EBPβ but not GATA to site B and excluded the binding of Lyf to A site. Interestingly, competitive oligomers of site B were able to reduce the shift of the whole complex while the competitive oligomers to A resulted in reduction of only upper most shift. In order to control for specificity of binding site competitive oligomers with mutation were used. The results showed that the competitive oligomers were unable to bind biotinilated oligomers even though it was in 200-fold excess.

The research showed that mutation at place A is able to partially restore the expression of reporter gene indicating the functionality of the investigated site. No significant difference was noted when mutating site B suggesting that the site is not important for RANKL expression. However, when performing EMS assay to identify the transcription repressors, B site played a crucial role in forming the whole complex. The result indicated a sequential mechanism of complex formation where protein is able to bind site A only when site B is already occupied by partner protein, possibly forming hetero or homodimer of C/EBPβ or C/EBP protein. The results obtained with functional study of pGl3-F3 region and EMSA are therefore conflicting. This might be due to insufficient mutation B site of F3 region. It was shown that GATA1 and Lyf probably do not play any role in the binding of the two sites as no suppershift or disappearance of shift was noted. To resolve the dilemma we suggest antibodies against C/EBP should be used to confirm its binding and larger mutation in site B to be inserted.

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BIOLOGICAL EFFECTS OF α-TOMATINE ON SELECTED TUMOR CELL LINES *IN VITRO*

FALTUSOVÁ, B.,[1] RUDOLF, E.,[2] SZOTÁKOVÁ, B.[1]

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

Cancer is one of the most common diseases, so it is looking for appropriate treatment.

The topic of my thesis was to investigate the biological effects of α-tomatine on selected tumor lines.

α-tomatine is a steroidal alkaloid found in the green parts of tomatoes (Lycopersicon esculentum). α-tomatine consists of aglycon tomatidine and tetrasacharid β-lycotetraosa which contains two molecules of D-glucose and one molecule of D-galactose and D-xylose. α-tomatine has proven antifungal and antimicrobial properties. Experiments done on laboratory mice and rats have demonstrated anti-inflammatory. Recently, it was found that α-tomatine also exhibits anti-proliferative activity against tumor cell lines derived from prostate (PC-3), colon (HT-29), liver (HepG2), stomach (AGS), as well as breast cancer (MCF-7) and lung (A549).

As tumor cell lines we chose the line of the colon (SW 620) isolated from primary colon adenocarcinoma with metastases in the lymph nodes, cervical carcinoma line (HEP-2 USA) and human skin melanoma line (BOWES) which is considered very invasive carcinoma.

In this work we used various tests to assess cell proliferation and viability, such as WST-1 test, flow cytometry (cell cycle). We used time-lapse microscopy and fluorescence microscopy for comparison the morphology of cell and nuclei. Cytotoxicity of α-tomatine was obtained by WST-1. In the first phase of testing, we tested the general effect of different concentrations of α-tomatine in time 24–72 hours. Based on the obtained results (efficiency depends on the dose) we chose specific concentration, which we used in other experiments. Then we measured the effect of α-tomatine on different cell concentrations. Using flow cytometry, we measured cell cycle however cell cycles of individual lines did not significantly change after treatment of α-tomatine. Cell morphology was monitored by time-lapse microscopy, the morphology of nuclei by fluorescence microscopy. Cells were rounded and degradated, they lost surface adherence in all cell lines after treatment of 9 μM α-tomatine. Nuclei in cells exposed to 9 μM α-tomatine concentration were rapidly shrinking and changing its shape. In some cases, there were irregular chromatin condensation in the context of cellular necrosis, it was possible to detect pouring chromatin from cells into the medium. These phenomena were observed in all model lines, most in HEP-2 USA line.

According to our results we can say that α-tomatine has an effect on tumor lines of colon, cervix and melanoma *in vitro*.

ENERGY EXPENDITURE AND UTILISATION OF NUTRITIVE SUBSTRATES OF POLYTRAUMATIC AND SEPTIC PATIENTS

HLADOVÁ, E.,[1] ŠVRČKOVÁ, A.,[1] HRONEK, M., [1,2] KOVAŘÍK, M.,[1,2] HAVEL, E.,[3] ZADÁK, Z.[2]

[1]Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department of Research and Development, University Hospital in Hradec Králové, Czech Republic
[3]Surgery Department, ICU, University Hospital in Hradec Králové, Czech Republic
e-mail: hladove@faf.cuni.cz, svrckoal@faf cuni.cz

The aim of the study was to determine whether there are any differences in metabolism between a polytraumatic patient and patient with sepsis. We compared the Flow phase of the hypermetabolism during shock. Mainly, we compared the utilization of nutrients. The patients are given standard nutrition in a form of standard solutions (both enteral and parenteral). The two fundamental phases of shock metabolism are Ebb phase and Flow phase. The Flow phase follows the Ebb phase, and it is crucial for reparation of damages. Also, it is extremely important for resolving the trauma/infection. The initiation of the flow phase seems to be different in every patient; it is characterized by a higher demand for nutrients and increased metabolism. The condition of the organism before trauma or infection influences the intensity of reparations. Malnutrition can inhibit the transition from Ebb phase to Flow phase of the metabolism.

The results were acquired based on examinations of 20 patients at the Intensive Care Unit. We compared the results between the two separate groups – 14 polytraumatic patients and 6 patients with sepsis, or we can compare the two groups as a whole. At polytraumatic patiens energy expenditure was 1722 ± 359 kcal/day, utilisation of carbohydrates 78 g/day, lipids 96 g/day, proteins 144 g/day. At septic patients expenditure was 1624 ± 109 kcal/day, utilisation of carbohydrates 140 g/day, lipids 103 g/day, proteins 127 g/day. It was discovered that both groups of patient react to the given nutrition in a similar way. This knowledge will be used in clinical practice for determination of the nutritional support for patients at ICU.

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AGE-RELATED CHANGES IN ACTIVITY OF CARBONYL REDUCTASE

HLAVÁČOVÁ, J., SKÁLOVÁ, L.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Kralové, Charles University in Prague, Czech Republic
E-mail: hlavj8ar@faf.cuni.cz

Carbonyl reductase (CBR) is an ubiquitous cytosolic enzyme that plays an important role in the phase I metabolism of endogenous and as well exogenous substances. Many xenobiotics like anthracycline anticancer drugs belong among substrates of this enzyme.

CBR is proved to be involved in detoxification of organism and protection against oxidative stress.

It is known that the ability of organisms to metabolize xenobiotics is changing during the process of ageing. The decrease in cytochrome P450 activities in old humans and animals has been mentioned in several studies, but there is not much information about age-related changes in activity of other antioxidant enzymes. Therefore we have decided to study CBR activity in young and old both rats and humans to find out possible differences.

Firstly, the specific activities of CBR were measured in human and rat hepatic subcellular fractions using menadione as substrate. Secondly, a protein expression of CBR was determined by methods of SDS-PAGE electrophoresis and imunoblotting.

As a result, 2.6-fold higher specific activity of CBR has been assessed in senescent rats in comparison with young ones. It has also come out that inter-individual variability in activity of the enzyme increased significantly with rising age. Immunoblotting proved increased CBR expression in old rats in comparison to young ones.

On the other hand, no significant differences in activity of CBR have been observed in young and old humans.

The age-related increase of CBR activity in rats can change biotransformation of metabolized drugs. In actual fact, it may reduce their efficiency. At the same time more active CBR provides higher protection against toxic effect of both xenobiotics and eobiotics. This issue should be studied in more detail to analyze the consequences of the above-mentioned facts.

UNIVERSAL DETECTION METHOD FOR OF HIS-TAGGED RECOMBINANT PROTEINS

JAVORSKÁ, L., ŠKARYDOVÁ, L.

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

Recombinant proteins are proteins produced in genetically modified organism (e.g. *E. coli*, insect Sf9 cells) thanks to recombinant DNA technology that enable insert DNA sequence of target protein in expression cell. This technology allows preparation of actually any protein in any quantity without difficulties associated with demanding purification of native protein from tissues. Recombinant proteins are produced often in form of fusion proteins with suitable peptide tag (e.g. His tag, FLAG tag) that can help with their detection and enable simple one step affinity purification. Today, these proteins play irreplaceable role not only in research but also in industry and medicine.

The aim of this project was to introduce and optimize universal method for the detection of recombinant proteins labelled with His-tag (6x histidine) during preparation process and after purification. The model recombinant protein with His-tag was enzyme carbonylreductase 1 (CBR1) produced by expression system *Escherichia coli*. Besides purified CBR1 also samples obtained during expression process were utilized. SDS polyacrylamide electrophoresis for protein separation and immunochemical detection after

Western Blotting method with commercially available primary rabbit Anti-6x His tag® – ChlP Grade (abcam®) and secondary polyclonal swine anti-rabbit antibody conjugated with HRP (Dako) were used. Final vizualization was achieved by chemiluminiscent commercially available kit (Amersham™ ECL™ Prime Western Blotting Detection Reagent, GE Healthcare Life science). Optimization of antibody concentration by dot blot analysis leads to large saving of antibody. This method will be widely used to evaluation of production of diverse recombinant protein at the department.

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SCREENING OF NUTRITIONAL STATUS IN PATIENTS WITH CANCER

JENČO, J.,[1] NETRIOVÁ, J.,[2] KRAJČOVIČOVÁ, Z.,[3] MELUŠ, V.[3]

[1]Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
[2]Department of Clinical Biochemistry and Hematology, St. Michael Hospital inc. in Bratislava, Slovakia
[3]Department of Laboraty Medicine, Faculty of Healthcare, Alexander Dubček University in Trenčín, Slovakia
e-mail: jencoj@faf.cuni.cz

Nutrition and nutritional support plays significant role in todays modern medicine and cancer treatment. But still the most common complication, related with bad income and mortality apart from diagnose itself is kachexia, which in many cases turns into malnutrition. Malnutrition is a state of acute undernourishment, which reflects the worsening of therapy, shortened patient survival and increased mortality. Before malnutrition has occured, many changes can be tracked in biochemical parameters accompanied by changes in the psyche, loss of appetite and a series of accompanying symptoms. Knowledge of these factors can predict how the disease develops and by nutritional therapy to improve patient health, reduce mortality, or if disease is not curabile, to improve palliative care for them

We evaluated 40 patients admitted to the oncology department of the University Hospital in Trenčín. Nutritional profile of every patient was examined, and nutritional indices have been calculated at st. Michael's hospital in Bratislava. The aim of this work was to compare the nutritional status of these patients between sample take, between each group of diagnoses and find differences between nutritional status before and after chemoterapeutic intervention. All samples were then compared with reference values and statisticaly analyzed.

All of the biochemical parameters were taken at the beginning of hospitalisation, before chemotherapy and after chemoterapy. Statistic analyse prooved no significant changes in concetration between takes of albumin, prealbumin, haptoglobin and C-reactive protein. Lowered significancy was due to large intraindividual variability of analytes in patients which distorted the interpretation. Statisticaly significant difference was in total protein concentration ($p = 0.0431$) and α1acid glycoprotein concentration ($p = 0.0240$). There may be many reasons why they were lowered but loss of total protein concentration and α1acid glycoprotein are warning factors in development of malnutrition. At the edge of significancy ($p = 0.668$) were

observed differences between concetration of urea. Reason of urea concetration rise was in higher metabolisation of proteins due chemotherapeutic toxicity.

Analysis of nutritional indices PINI, PNI, CSI and NBI showed enormous variability and caused statistical insignificance.

When derived into groups of diagnosis to GIT and OTHER diagnosis there were no significant age variations ($p = 0.280$) in patients, due to larger age scale in patients with localisation of cancer in other places than gastrointestinal tract.

Between groups of diagnosis there was significant difference in concentration of total protein ($p = 0.014$) before and after chemoterapeutic intervention. According to spearman correlation coeficient we used for nutrition indices, we found that with the rise of malignant progression controlled with CSI index, PINI index also rises. PINI index correlates with the worse income and relapse of cancer.

Evaluation of reference values and values taken from our patients showed falling tendency and decrease in almost all parameters. Decrease below reference values is indication to nutritional support therapy or even enteral nutritional therapy.

Screening of nutritional status should be applied into clinical praxis to decrease amount of complications related with cancer treatment. Today, modern medicine aims in "personalized medicine" which takes patient individual metabolic profile, so our research enriches knowledge in that area. Nutritional profile should be apropriate optioned according to needs of patient. This will make the progress of nutritional therapy more effective, which is easy non-invasive addon of classic cancer treatment. Nowdays it is undoubtedly clear, that in case of oncological patients, nutrition is relevant factor with impact on therapy and which may be considered in many key procedures.

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BIOLOGICAL ACTIVITY OF PLANT METABOLITES XXXIII. ALKALOIDS OF *CORYDALIS CAVA* (L.) SCHWEIGG. & KÖRTE AND THEIR EFFECT ON ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE

KALUZSNÁ, B.,[1] CHLEBEK, J.,[1] OPLETAL, L.,[1] NOVÁK, Z.[2]

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

The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) is very important for the treatment of Alzheimer's disease (AD). The loss of cholinergic transmission is one of the main reasons why the development of dementia occurs. The aim of AChE inhibition is to maintain and increase levels of acetylcholine (ACh) in the brain and to slow down the development of dementia. Inhibition of AChE is the most important for early and middle stages of AD. However, in the later stages of AD is the use of

cholinesterase inhibitors questionable. There is a reduction in the total amount of ACh and a lack of choline acetyltransferase (ChAT), which is responsible for the synthesis of ACh and the deficit of AChE. It is interesting that the levels of BuChE, which also contributes to the degradation of ACh, increase progressively and significantly in AD[1].

Consequently, there is a need for new inhibitors with dual enzymatic activity, stronger action and less side effects.

Corydalis cava (L.) Schweigg. et Koerte is known as a poisonous plant from *Fumariaceae* family. However, it contains a number of interesting alkaloids with many therapeutic effects, including inhibition of cholinesterase, antioxidant, antimicrobial and cytotoxic effect.

Diethylether fraction from tubers *Corydalis cava* (L.) Schweigg. et Koerte was prepared by column chromatography and three substances were isolated and purified. They were identified by GC/MS, ^{1}H and ^{13}C NMR as tetrahydropalmatine, domestine and canadine. They were also tested for inhibition of human erythrocyte AChE and plasma BuChE[2].

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ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY COMPOUNDS FROM *FUMARIA OFFICINALIS* (FUMARIACEAE)

KASSEMOVÁ, D.,[1] CHLEBEK, J.,[1] MACÁKOVÁ, K.,[1] KUNEŠ, J.,[2] CAHLÍKOVÁ, L.[1]

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

Alzheimer's disease (AD) is the most frequently neurodegenerative disorder of central nervous system among elderly, affecting 25% of population over 80 years of age. In patients with AD, brain regions associated with higher mental functions (cortex and hippocampus) are affected by extracellular deposits of β-amyloid plaques, intracellular deposits of neurofibrillary tangles and there is also a progressive lost of neurons in the basal forebrain. Deficiency of cholinergic functions and decreased levels of acetylcholine in the cortex is responsible for memory impairments. Acetylcholinesterase (AChE) rapidly hydrolyzes the neurotrasmitter acetylcholine (Ach) and thus, AChE plays a critical role in termination of nerve impulse trasmission. In late AD stages, the role of AChE declines as a result of decrease levels of AChE and hence, butyrylcholinesterase (BuChE) represents the dominant cholinesterase, which hydrolyzes Ach in the brain. Thus, inhibition both AChE and BuChe has become a promissing aproach in research of new drugs for treatment

of AD. Alkaloid extracts has showed AChE inhibitory activity from family Fumariaceae in the screening of natural AChE inhibitors.

A summary alkaloid extract of *Fumaria officinalis* (Fumariaceae) showed a significant inhibitory cholinesterase activity towards human erythrocytic acetylcholinesterase (HuAChE) and serum butyrylcholinesterase (HuBuChE) with $IC_{50HuAChE}$ 39.23 μg/mL and $IC_{50HuBuChE}$ 40.32 μg/mL. The ether extract of tertiary alkaloids was fractionated into 11 fractions (A_1–A_{11}) in alumina chromatographic column using step gradient elution with petrol, $CHCl_3$ and ethanol. Four alkaloids fumaricine, protopine, bicuculine and cryptopine were isolated from fraction A_5 by preparative TLC and subsequent crystallization. Their structures of isolated alkaloids were elucidated by spectroscopic techniques and compared with literature data. Isolated alkaloids were tested for HuAChE and HuBuChE inhibitory activity and established IC_{50} values for each isolated alkaloid were compared with inhibitory standards (galanthamine, huperzine A and eserine).

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RATING OF CHOLINESTERASE ACTIVITIES IN THE CZECH POPULATION

MADĚRYČOVÁ, Z.,[1,2] TŮMOVÁ, M., ŘEHÁČEK,V.,[3] ŽĎÁROVÁ, KARASOVÁ, J.[1]

[1]Department of Public Health, Faculty of Military Health Science in Hradec Králové, University of Defence Brno, Czech Republic
[2]Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[3]Transfusion Department, University Hospital, Hradec Králové, Czech Republic
e-mail: maderycz@faf.cuni.cz

Acetylcholinesterase is well-known enzyme located in membrane of neuronal junction which plays important role in neurotransmission. Dimmer form of acetylcholinesterase is also located in membrane of erythrocytes. This form is in the first contact with its inhibitors such as pesticides or warfare gases. Acetylcholinesterase inhibition rate indicates magnitude of intoxication. A 15 to 25 percent acetylcholinesterase depression means that slight poisoning has taken place. A 25 to 35 percent drop signals moderate poisoning and a 35 to 50 percent decline in the cholinesterase readings indicates severe poisoning[1]. This division was established without a date of default activity of acetylcholinesterase in healthy population. The main aim of our study was to determine acetylcholinesterase activity according to age, sex and smoking. Activity of acetylcholinesterase was determined in population of healthy blood donors (n = 400). Blood sample of healthy persons was achieved in cooperation with Transfusion department, University Hospital, Hradec Králové. Samples were divided into 4 groups according to the age (until 25, more than 25 until 35, more than 35 until 45, more than 45) and these samples were subsequently statistically evaluated according to sex and smoking. Measuring was based on modification on Ellman method for determination of cholinesterase in blood[2]. Achieved results should be helpful for solving further problems in area of toxicology. Comprehensive results will be presented oraly during conference.

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CHOLINESTERASE INHIBITORY ACTIVITY OF ALKALOIDS FROM *FUMARIA OFFICINALIS* L. (FUMARIACEAE)

MALÝ, L.,[1] OPLETAL, L.,[1] MACÁKOVÁ, K.,[1] LOČÁREK, M.,[1] NOVÁK, Z.,[2] CHLEBEK, J.[1]

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

Alzheimer's disease (AD) is the chronically progressive neurodegenerative disorder of central nervous system causing dementia, which is characterized by difficulty in remembering events and a loss of cognitive functions. The current approved therapy includes anticholinesterase inhibitors and the NMDA blockers. Cholinesterase inhibition is the most used therapeutic treatment for the symptoms of AD, although spectrum of used anticholinesterase compounds is relatively limited. Alkaloids from Fumariaceae family showed *in vitro* AChE inhibitory activity[1], which should be further examined for a potential application in therapy of AD.

Fumaria officinalis herbs were extracted by ethanol. The obtained mixture of tertiary alkaloids was fractionated in alumina chromatography column using step gradient elution with petrol, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of protopine, cryptopine, fumaricine, parfumidine and fumariline. Alkaloids were identified by GC/MS, ^{1}H and ^{13}C NMR analyses. Isolated compounds were tested for inhibition activity towards human erythrocyte acetylcholinesterase and serum butyrylcholinesterase. IC_{50} values of these alkaloids were compared to IC_{50} values of standards galanthamine, huperzine A and eserine.

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USE OF FRESHLY ISOLATED RENAL CELLS TO STUDY RENAL UPTAKE OF SELECTED ANTIVIRALS

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

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

Administration of antiviral drugs may be in some case associated with severe nephrotoxicity. This effect can be based on active renal transport and accumulation of the drug in the renal cells. Several experimental *in vitro* methods are available to study the mechanisms responsible for drug uptake in the kidney including renal cell lines or renal slices. The aim of this study was to evaluate usefulness of isolated rat renal cells to determine which renal transporters may be involved in transport of selected antivirals into renal cells. The freshly isolated rat renal cells were prepared by two-phase collagenase perfusion. The renal uptake of two radiolabeled antivirals, tenofovir and adefovir, was evaluated to confirm preservation of functions of the renal cellular preparation. To evaluate contribution of active and passive transport mechanisms to the renal uptake, incubation at normal and low temperature was evaluated. To confirm previous information on renal transport, we also determined which groups of transporters contributed to the renal accumulation of the studied antivirals. For this purpose, we use compounds acting as specific inhibitors of the appropriate transporters. The results demonstrated that adefovir and tenofovir were transported into the renal rat cells mostly by active transport mechanisms. The most potent inhibitor of the renal uptake was OAT inhibitor probenecid. Inhibitors of OCTs and CNTs had a significantly lower effect on the renal accumulation of the compounds. Both antivirals had similar transport characteristics. In conclusion, the found results are in accordance with the published data on transmembrane transport mechanisms in adefovir and tenofovir. This fact documents validity of the used method for accumulation study in drugs. In addition, the results confirm the suggested significance of OATs for renal uptake of the studied antivirals.

The study was supported by European social fund and the state budget of the Czech Republic Project No. CZ.1.07/2.3.00/30.002 and by Charles University in Prague (Project SVV 265003).

CATHETER DIRECTED THROMBOLYSIS USING ALTEPLASE – THERAPY OF DEEP VEIN THROMBOSIS IN WOMEN

PROKŠOVÁ, M.

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Department of Cardiovascular Medicine I. University Hospital in Hradec Králové, Czech Republic
e-mail: proksovm@faf.cuni

Thrombolysis is a process of breaking down a venous thrombus. In this study we administred Alteplase – a throbolytic drug – directly to the vein using a catheter. Alteplase

is a tissue plasminogen activator and it activates the transformation of plasminogen to plasmin and than plasmin breaks down blood clots and helps to renew the blood floow in the affected vessel.

Study was conducted in the Department of Cardiovascular Medicine I. of University Hospital in Hradec Králové, Czech Republic. After the thrombolysis pacient are monitored at the Angiology clinic at the University Hospital in Hradec Králové.

In this study the 67 women were treated regardless of their age. The aim of the study was to observe how long thrombolysis takes, which leg was affected and whether there were present any undesirable side effects of fibrinolysis (bleeding, haematoma, …). If there was bleeding present we divided it into two different groups – minor bleeding and major bleeding. Than we observed risk factors (e.g. hormonal replacement therapy or hormonal contraception, injury with immobility or others). During the fibrinolysis the levels of platelets and fibrinogen were also checked.

Catheter directed thrombolysis is highly effective method. There is a risk of bleeding therefore thrombolysis is indicated in patients with low risk of bleeding and in patients with severe manifestations of deep vein thrombosis. The two most important risk factors in women were hormonal contraception together with smoking.

PRECISION-CUT KIDNEY SLICES AS A METHOD FOR PHARMACOLOGICAL STUDIES

PŠENICA, P., OLEJÁROVÁ, A., KOCINCOVÁ, J., MANDÍKOVÁ, J., TREJTNAR, F.

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

Some of antiviral drugs are known to exhibit nephrotoxicity due to active accumulation in the renal tissue. Therefore, *in vitro* methods aimed at investigation of the renal mechanisms responsible for the renal uptake of antivirals could be very useful. Primary aim of this study was to introduce an *in vitro* method for experimental studies on renal drug transport based on precision-cut kidney slices. Advantages of precision-cut kidney slices are possibility of selection of the target renal section, preserved tissue morphology and rapidity of experimental evaluation. Secondary aim of the study was to demonstrate validity of the method for the study of transport mechanisms in selected antiviral drugs.

Krumdieck® tissue slicer was used as the tool for preparation of fresh rat kidney slices. The slices were incubated under standard conditions in an incubator. The viability of the kidney slices was assessed using two fluorometric tests. As the next step, we evaluated several methods how to dissolve the prepared slices. A radiolabeled antiviral, tenofovir, with known accumulation in the kidney was used as the experimental compound. The studied antiviral was incubated with several inhibitors of various drug transporters such as probenecid (OAT inhibitor), TEA, MPP^+ (OCT inhibitors), and uridine (NT inhibitor) to confirm previous results declared in literature.

The adequate viability of the slices was kept up to 15–30 min of incubation. Probenecid showed a very high potency to inhibit tenofovir accumulation (86%). The other used

inhibitors were significantly less effective in lowering of antiviral renal uptake at comparable concentrations. In conclusion, the precision-cut slices appear to be a valid tool to perform short-term *in vitro* studies on drug transport mechanisms. Further, the used method provided similar results on tenofovir uptake in the renal cells as previously published studies. These facts prove not only its credibility but also correctness. A disadvantage seems to be limited viability of the slices under the used conditions.

The study was supported by European social fund and the state budget of the Czech Republic Project No. CZ.1.07/2.3.00/30.002.

COMBINATION TREATMENT OF A TUMOUR CELL CULTURE WITH miRNA MIMETICS AND ONCOGENE-TARGETED siRNAs

ŠABOUKOVÁ, M.,[1] BAUMAN,V.,[2] WINKLER, J.[2]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department of Medicinal Chemistry, University of Vienna, Austria
e-mail: saboukom@faf.cuni.cz

During the last two decades, the attention of many scientists has been attracted by a newly discovered group of regulatory molecules – RNA interference. These RNA molecules are capable of very efficient gene silencing. The main pathways of this mechanism are used by two major types of small noncoding RNAs – microRNA (miRNA) and small interfering RNA (siRNA). These RNAs are a direct product of genes and are able to bind to mRNA molecules and influence their activity. Due to this knowledge, scientist started examining the effect of RNAi in cancer cells and tried to find a way how RNAi can be applied in therapy.

The main topic of my work is the potential of miRNA replacement to harm cancer cell proliferation. In cancer cells, miRNA levels are downregulated or upregulated, depending on the nature of cancer and miRNA. Restoring levels of miRNAs can lead to elimination of cancer cell by apoptosis or stop cell in cell cycle arrest.

miRNA replacement was used first to examine the function of a single miRNA in HeLa cancer cells. The change in the expression level of miRNA treated cells is verified by reverse transcriptase quantitative PCR and the proliferation inhibition is examined using proliferation assays. The most potent miRNAs inducing significant proliferation inhibition were combined together and analyzed. In the second part of my work, miRNAs were combined with oncogene targeted siRNAs and transfected to HeLa cells, in order to seek for possible synergistic effect.

COMPARISON OF BODY COMPOSITION BETWEEN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND WITHOUT RESPIRATORY IMPAIRMENT

SEHNALOVÁ, L.,[1] MALINOVÁ, B.,[1] PAVLÍČKOVÁ AIMOVÁ, P.,[1,2] HRONEK, M.,[1,2] KOVAŘIK, M.[1,2]

[1]Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department of Research and Development, University Hospital and Faculty of Medicine, Hradec Králové, Czech Rrepublic
e-mail: kovarikm@faf.cuni.cz

Chronic obstructive pulmonary disease (COPD) is one of the top leading causes of death and its morbidity and mortality worldwide is still increasing. It is a lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and is not fully reversible. Besides the respiratory symptoms there are often developed alterations in metabolism and body composition in COPD patients. Clinically important are mainly malnutrition and skeletal muscle protein loss. Especially if the respiratory muscles are affected, the lung function is negatively influenced.

The main aim of this study was to compare the body composition between 41 patients with COPD and 9 patients of control group without respiratory impairment and comparable anthropometric characteristics (age, body height and weight). We determined the composition of main body compartments by means of bioelectrical impedance analysis. We also calculated the body mass (BMI), lean tissue mass (LTI) and fat mass (FMI) indexes and compared them with reference values. Results are presented as mean ± standard deviation.

We described the body composition of control group as follows: the mean amount of lean tissue was 48 ± 9% of body weight, total fat 38 ± 6% and visceral fat 12 ± 5%. Mean values of BMI, LTI and FMI were 27.4 ± 4.0; 13.0 ± 2.4 kg/m^2 and 14.2 ± 3.8 kg/m^2, respectively. Although no patient of control group was scored as underweight according to BMI value, 2 patients have lower values of LTI than reference range.

In conclusion, although we confirm signs of skeletal muscle loss in some patients, we found no significant differences in main parameters of body composition between control group and COPD patients.

The study was supported by PRVOUK P40 and UNCE 204026/2012.

7-METHOXYTACRINE-TACRINE HETERODIMERS: SYNTHESIS, BIOLOGICAL EVALUATION, MOLECULAR MODELING STUDIES AND TOXICITY ASSESSMENT OF NOVEL AGENTS FOR ALZHEIMER'S DISEASE TREATMENT

BABKOVÁ, K.,[1] KORABEČNÝ, J.,[2,3] MUSÍLEK, K.,[2,3] OPLETALOVÁ, V.,[1] KUČA, K.[2,3]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]University Hospital Hradec Králové, Czech Republic
[3]Department of Toxicology, Faculty of Military Health Sciences, Hradec Králové, Czech Republic
e-mail: babkovak@faf.cuni.cz

The first acetylcholinesterase (AChE, E.C. 3.1.1.7) inhibitor approved for Alzheimer's disease treatment by the US FDA was tacrine, licensed in 1993. However, its severe side-effects manifested mainly by hepatotoxicity and cholinergic effects upon the gastrointestinal tract limited its usage. Recent contributions to the development of tacrine related agents disclosed 7-methoxytacrine to be less toxic, indeed, with equal pharmacological profile. In the process of searching for highly potent tacrine analogues, heterodimers embodying both moieties were synthesized and tested for their ability to inhibit AChE and butyrylcholinesterase (BChE, E.C. 3.1.1.8).[1] Molecular modeling studies showed that analogue **23** binds to both, the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of AChE (Fig. 1). Moreover, these hybrids also proved inhibition abilities towards β-amyloid (Aβ) aggregation in micromolar ranges as well as capability of Aβ depolymerization. Together with low values of acute toxicity, these findings underline two analogues (**19** and **23**) as very interesting candidates for further studies associated with their possible use in the treatment of AD that will be discussed in our contribution.

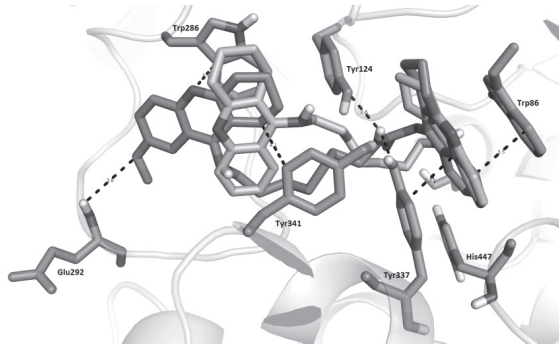

Fig. 1. Comparison of the binding mode of bis(7)-tacrine (magenta carbon atoms) and highlighted 7-MEOTA-tacrine derivative 23 (green carbon atoms). Selected residues interacting with 23 are rendered with yellow carbon atoms. Non-polar hydrogens are omitted to improve figure clarity. The figure was created with PyMol.

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The study was supported by the specific research (SV/FVZ201201), 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 Long Term Development plan – 1011 and by Ministry of Education, Youth and Sport (SVV-267-001).

SYNTHESIS OF NEW DERIVATES OF COMBRETASTATINS

BRŮŽA, Z., KRATOCHVÍL, J., POUR, M.

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

Combretastatins are natural compounds originated from South African tree *Combretum caffrum*. Their analogues (Fig. 1) are currently being investigated for their cytostatic activity. However, high lipophilicity of the compounds is a problem that remains unsolved.

The main goal of this study was to synthesize new derivatives of combretastatins containing 3,4-diphenyl-2,5-dihydrofuran-2-one pattern (Fig. 2). The resultant lactones were modified by hydroxymethylation. The introduction of hydrophilic substituent should give us access to analogues with better physical properties, and allow further derivatization.

The preparation involves comprises bromination of acetophenone derivatives, nucleophilic substitution with carboxylate followed by cyclisation and hydroxymethylation. The products are being evaluated for antibacterial, antifungal and cytotoxic activity.

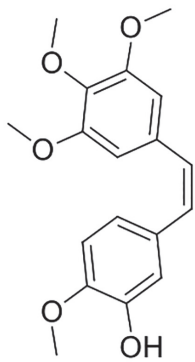


Fig.1

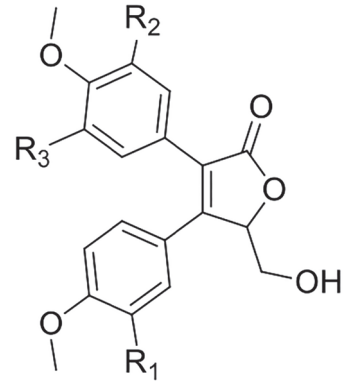


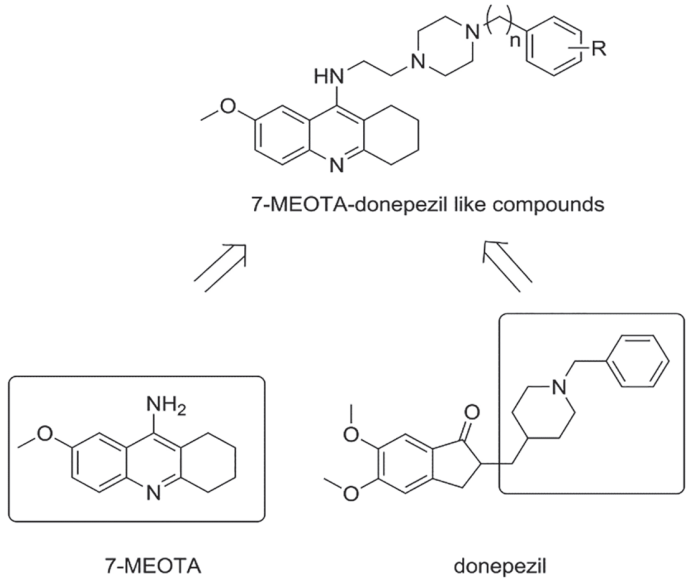
Fig.2

SYNTHESIS AND *IN SILICO* STUDIES IN THE SERIES OF NOVEL 7-METHOXYTACRINE-DONEPEZIL LIKE COMPOUNDS

ČÁBELOVÁ, P.,[1] KORÁBEČNÝ, J.,[2,3] MUSÍLEK, K.,[2,3] KUČA, K.,[2,3] OPLETALOVÁ, V.[1]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department of Toxicology, Faculty of Military Health Sciences, Hradec Králové, Czech Republic
[3]University Hospital Hradec Králové, Czech Republic
E-mail: cabelop@faf.cuni.cz

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder of the brain characterized clinically by loss of memory, deterioration of activities of daily living and cognition. The pathological features of AD include neuritic plaques composed of amyloid-β peptide fibrils, neurofibrillary tangles of hyperphosphorylated tau, and neurotransmitter deficits. The aim of the study was to design and synthesize 7-methoxytacrine-donepezil-like compounds as potential inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). New compounds consist of 7-methoxytacrine the less toxic derivative of tacrine and benzylpiperazine as donepezil-like moiety.[1] To determine the potential interest of new derivatives *in vitro* AChE and BChE inhibitory activities of the new molecules were assessed according to the method of Ellman et al. and compared with tacrine and 7-methoxytacrine.[2] All compounds exhibit ability in μM and sub-μM ranges of IC_{50} values. To investigate possible binding of the most potent compounds molecular docking studies on human AChE were carried out. All studied compounds interact simultaneously with active and peripheral sites. Dual binding site character for AChE and their physical/chemical properties together with excellent inhibition properties of AChE/BChE make them interesting for other testing in AD area.



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The study was supported by the specific research (SV/FVZ201201), 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 Long Term Development plan – 1011 and by Ministry of Education, Youth and Sport (SVV-267-001).

SIMULTANEOUS DETERMINATION OF DEXPANTHENOL, PHENOXYETHANOL AND PRESERVATIVES (METHYL-, ETHYL-, PROPYL-, BUTYL- AND ISOBUTYLPARABEN) IN PHARMACEUTICAL PREPARATION BY VALIDATED HPLC METHOD

DITTRICHOVÁ, K.,[1] GALLA, L.,[2] MOKRÝ, M.[1]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]QA, QC Department, Dr. Müller Pharma s.r.o., Hradec Králové, Czech Republic
e-mail: dittk8ar@faf.cuni.cz, katerina.dittrichova@gmail.com

Dexpanthenol is commonly used as supportive treatment of mucus affects (bronchitis, tracheitis, laryngitis, pharyngitis, stomatitis, etc.) and also externally supports epithelization of ulcers, burn and scratch wounds. Dexpanthenol is usually formulated in multi-vitamin preparations, parenterals and some local cosmetic preparations.[1]

Parabens (4-hydrobenzoic acid esters) are widely used as antimicrobial preservatives in pharmaceutical products and local cosmetics. Recently, preservatives (especially parabens) received keen attention because of their possible side-effects on humans. As a result, a fast, simple and accurate method of analysis is necessary.[2]

A liquid chromatographic method was developed for simultaneous determination of dexpanthenol, phenoxyethanol and preservatives (methyl-, ethyl-, propyl-, butyl- and isobutylparaben). Separation of these compounds was performed on column Discovery C_{18} (5 µm, 150 mm × 4.6 mm I.D.) by isocratic elution with a potassium dihydrogen phosphate buffer (0.01 mol l^{-1}, pH adjusted to 2.5 with a phosphoric acid 85%) and an acetonitrile (67: 33, v/v) at the flow rate 1.00 ml min^{-1}. The injection volume 5 µl was used. The diode array detector operated at two wavelengths (210 nm for dexpanthenol and 254 nm for phenoxyethanol and preservatives). Three basic system suitability parameters were evaluated: the number of the theoretical plates of all compounds was greater than 6000 units, determined symmetry factors of all peaks were in interval from 1.1 to 1.3, and calculated values of resolution were greater than 1.5. Accuracy, precision, linearity, specificity and selectivity tests were satisfactorily performed. The method showed good recovery: from 98.00% to 101.00% for all compounds. From the linearity experiment, the correlation coefficient was at least 0.99900 for all compounds. LOD and LOQ parameters

were obtained. The method was successfully applied for determination of dexpanthenol, phenoxyethanol and preservatives in gel based on acrylamide.

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SYNTHESIS OF NOVEL CARDIOPROTECTANTS AND METABOLITES OF POTENT ANTICANCER DRUG – BP4ET

EISNER, T., ŠŮS, J., ROH, J., VÁVROVÁ, K., HRABÁLEK , A.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
e-mail: eisnertom7@gmail.com

Anthracyclines (ANT) such as doxorubicin, daunorubicin, or epirubicin rank among the most effective anticancer drugs. However, their major side effect is chronic cardiotoxicity leading to irreversible cardiac damage and congestive heart failure. It is assumed that this side effect is caused by reactive oxygen species, whose formation is catalyzed by the complexes of anthracyclines with iron ions. The only clinically used drug preventing ANT cardiotoxicity is dexrazoxane (DXZ, Fig. 1).

In this work we deal with the synthesis of novel DXZ analogues, because the structure-activity relationship studies have not been performed yet. Recently, the first analogue named ES-5 (Fig. 1) was synthesized and its cardioprotective effect evaluated both *in vitro* and *in vivo*.

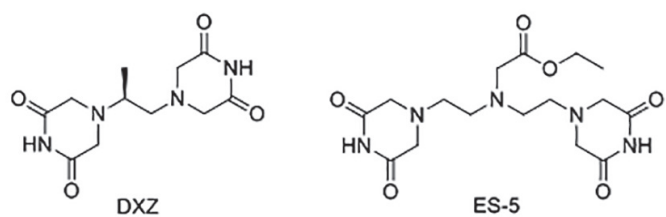


Fig. 1. Structures of DXZ and ES-5

Furthermore, thiosemicarbazone Bp4eT (2-benzoylpyridine-4-ethyl-3-thiosemicarbazone, Figure 2), a potent anticancer agent and its metabolites were synthesized. These compounds were used as standards in metabolic and pharmacokinetic studies. The chelating and anticancer properties of these metabolites will also be evaluated.

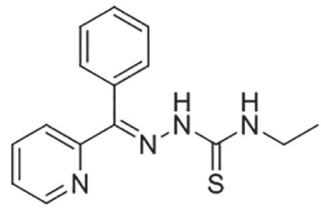


Fig. 2. Structure of iron chelator Bp4eT

This project was supported by the Charles University in Prague (Project UNCE 33/2012 and SVV 267 001).

SYNTHETIC ROUTES TO 2-PHENYLBENZOTHIAZOLES WITH POTENTIAL APPLICATION IN CANCER THERAPY PET IMAGING

FRYDRYCH, J.,[1] WESTWELL, A. D.,[2] OPLETALOVÁ, V.[1]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, Wales, CF10 3NB, UK
e-mail: frydryj1@faf.cuni.cz

There are three main tasks reported in this article. At the first we report an improved procedure for synthesis of biologically relevant 2-(phenyl)benzothiazoles with various substitution on phenyl ring. Reported 2(phenyl)benzothiazoles were synthesised by heating equimolar amounts of 2-aminothiophenol disulfides with appropriate benzaldehydes with p-toluensulfonic acid in the presence of polymer-bound triphenylphosphine using mixture of toluene and DMF as a solvent. Main features of reported method include simple product isolation (removal polymer- bound by-product by filtration through Celite® layer), avoidance of column chromatography, rapid synthesis and good yields of correspondent benzothiazole. The second goal of this article is solution phase synthesis of 2-(phenyl)benzothiazoles bearing different substituent on both the benzothiazole and phenyl ring. We tried to synthesise different 2-(pheny)lbenzothiazoles by heating equimolar amount of substituted 2-aminobenzothiazoles with relevant benzaldehydes in high-boiling solvents using sodium metabisulfite as mild oxidant. The results of this method were unconvincing. We got several traces of desired compound with 6-methyl or 6-methoxy substituted 2-aminobenzothiazoles but in other cases we could not isolate our desired compounds. The third task was the synthesis of precursors of 18F radio labelled 6-Fluoro-2(2,3-dimethoxyphenyl) bezothiazole and 5-Fluoro-2(2,3-dimethoxyphenyl)bezothiazole (GW 610) as potential PET agent for Alzheimer's disease diagnosis and developing potential method for their radiolabeling. Particularly on the interest was the synthesis of 18F fluorinated GW 610 because of its extraordinary anticancer activity *in vitro* and *in vivo* reported in recent years. We successfully synthesised 2-(2,3-dimethoxyphenyl)6-nitrobenzothiazole via Jacobson cyclization. We subsequently performed direct aromatic nucleophile substitution of the

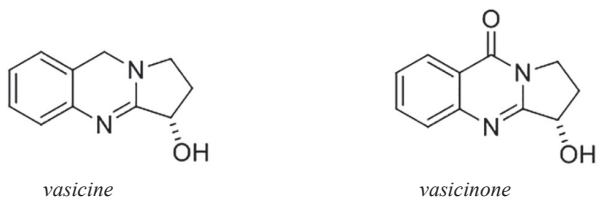
nitro precursor using F- in presence of Kryptofix 2.2.2® using both DMSO and DMF as a solvent to establish condition for future radiolabelling. Furthermore we synthesised via Jacobson cyclization followed by palladium-catalyzed stannylation two candidates for 18F-F labelling, namely 2-(2,3-dimethoxyphenyl)6-tributylstannylbenzothiazole and 2-(2,3-dimethoxyphenyl)5-tributylstannylbenzothiazole (GW610). Both organotin compounds can be used both for direct 18F fluorination using 18F-F and for more favourable preparation even more reactive diaryiodonium salt, suitable precursors for 18F- / Kryptofix 2.2.2® labelling.

SYNTHESIS OF POTENTIAL ORGANOCATALYSTS BASED ON QUINAZOLINE ALKALOIDS

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

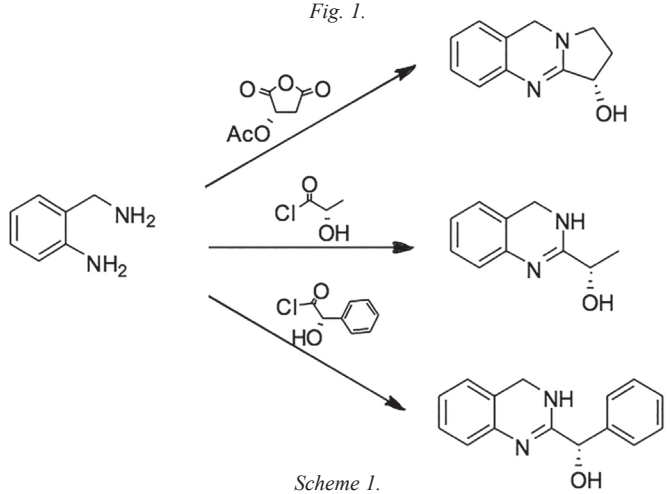
Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
e-mail: goreckil@faf.cuni.cz

A series of substances derived from vasicine-type alkaloids (Fig. 1) was synthesized. Three methods using different types of α-hydroxy carboxylic acids (malic, lactic, mandelic) were optimized (Scheme 1). These derivatives are currently being tested for their potential organocatalytic activity.



vasicine *vasicinone*

Fig. 1.



Scheme 1.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. P207/10/2048) and Charles University Research (SVV-265-001).

DETERMINATION OF ENTECAVIR IN RAT URINE BY SOLID PHASE EXTRACTION AND UHPLC-MS/MS

GOTTVALD, T.,[1] VLČKOVÁ, H.,[1] TREJTNAR, F.,[2] MANDÍKOVÁ, J.,[2] SOLICH, P.,[1] NOVÁKOVÁ, L.[1]

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

Entecavir is a synthetic guanine nucleoside that plays important role in treatment of chronical hepatitis B virus infection. This work was focused on development of highly sensitive method to determine entecavir concentrations in rat urine. Quantitative determination of entecavir was carried out by ultra-high performance liquid chromatography-tandem mass spectrometry using positive ion electrospray mode. Triple quadrupole was set-up to selected reaction monitoring (SRM) mode. Entecavir $C_2{}^{13}N^{15}$, stable isotopically labeled internal standard, was used for quantitation.

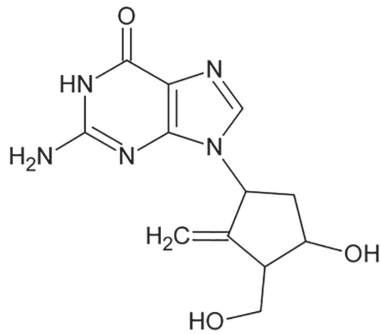


Fig. 1. The structure of entecavir.

Hydrophilic interaction chromatography seems to be a suitable technique for retention and separation of polar compounds present in analyzed sample. One of the main goals was to confirm this fact by comparison with commonly used reverse phase chromatography mode (RP-UHPLC) on BEH C18 stationary phase. Conditions for RP-UHPLC were optimized as follows: mobile phase composed of acetonitrile/0.01% formic acid (4:96). The HILIC conditions on BEH Amide stationary phase were optimized using isocratic elution with mobile phase composed of acetonitrile/5mM ammonium acetate pH 4.0 (75:25). HILIC method provided much better results in terms of linearity and repeatability.

Due to high polarity of entecavir Oasis HLB cartridge was chosen for solid phase extraction that was suitable preparation technique for the treatment of rat urine sample.

Advantageously entecavir was eluted by 75% acetonitrile in water, which was the same composition as HILIC mobile phase. Therefore, evaporation step could be omitted.

Several strategies were used to overcome matrix effect, such as UHPLC separation, HILIC chromatography, SPE sample pre-treatment, stable isotopically labeled internal standard and dilution step. Therefore, influence of matrix effects, which were determined by post-column infusion and post-extraction addition method, was negligible in this method. The following validation parameters demonstrated the suitability of this method for the determination of entecavir in rat urine – accuracy (< 5% error), recovery (87–109 %), precision (< 3% RSD), selectivity and sensitivity (LOQ = 100 pg/ml).

The study was supported by research projects of Charles University in Prague UNCE 204026/2012 and SVV/267007.

INFLUENCE OF PROTECTING GROUPS ON CATALYTIC CYCLIZATION OF 1,5-ENYNES

CHARVÁT, M., MATOUŠOVÁ, E., KUNEŠ, J., POUR, M.

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

Our resarch group focused on cyclization reactions of 1,5-enynes. This work deals with the influence of nitrogen protecting groups on the reaction course and on subsequent cyclizations (Scheme 1). Derivatives of sulfonic and carboxylic acid were used as the protecting groups (Fig. 1).

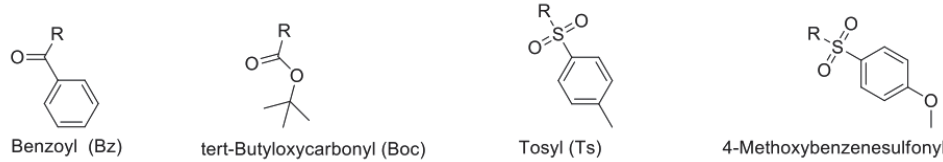
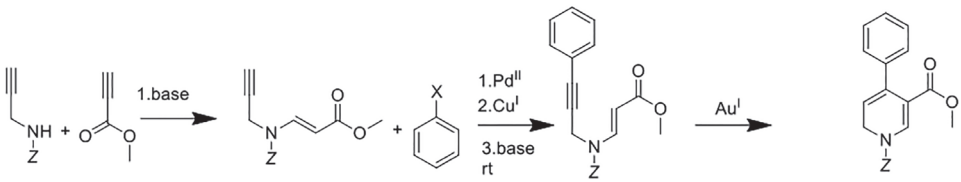


Fig. 1.



Scheme 1.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. P207/10/2048) and Charles University Research (SVV 265-001).

IDENTIFICATION OF PHENOLIC COMPOUNDS IN LEAVES OF *FRAGARIA VESCA L. U*SING UHPLC-MS/MS

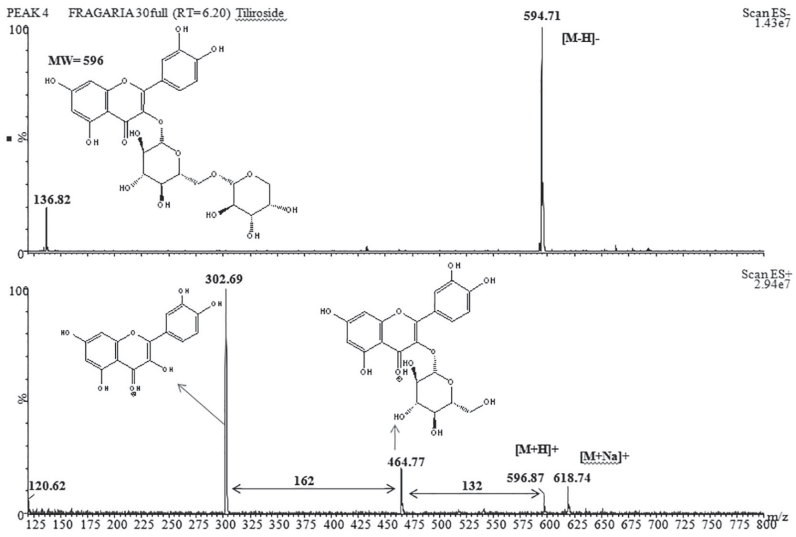
KMEŤOVÁ, I.,[1] SPILKOVÁ, J.,[2] SOLICH, P.,[1] NOVÁKOVÁ, L.[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
[2]Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
e-mail: kmeti6aa@faf.cuni.cz

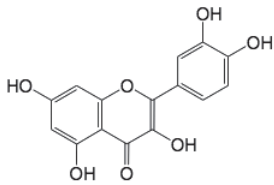
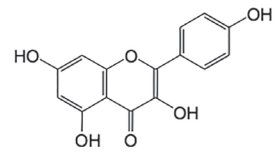
The aim of this study was identification of phenolic compounds in dry lyophilized water extract of leaves of wild strawberry (*Fragaria vesca L.*) using ultra-high performance liquid chromatography coupled with electrospray tandem mass spectrometry (UHPLC-ESI-MS/MS) system, advantageous in identification of complex plant samples.

Phenolic compounds represent a group of very complex naturally occurring molecules in plants, possessing a wide variety of biological and physiological functions and acting as chemotaxonomic marker substances. This work was focused on flavonoids and related substances.

For separation, UHPLC was used instead of more common HPLC due to its much higher separation efficiency that is crucial for successful separation of complex samples. Moreover, UHPLC enabled reduction of analysis time. Determination of structures of phenolics was based on evaluation and comparison of MS and MS/MS spectra in both negative and positive ESI ion modes. Full scan spectra, product ion spectra, neutral loss scans and reconstructed ion chromatograms of selected masses were used. The latter was very important in order to identify low abundant compounds. Neutral loss scan was especially valuable for the identification of sugars in the structure of phenolic compounds. For some structures also reference standards were available, therefore the identification was performed using MS data and retention times as well.



UHPLC-MS/MS analysis of dry strawberry extract led to tentative characterization of variety of phenolic compounds including catechin, flavonoids peltatoside and tiliroside, aglycons of kaempherol and quercetin and their derivatives. The position of sugar moiety on flavonoid skeleton was not yet elucidated, except for the compounds for which reference standards were available.

Summary of flavonoids in dry strawberry leaves extract	
quercetrin	kaempherol
	
pentoside	glucoside
ribo-glucoside (peltatoside)	ribo-glucoside
glucosid (isoquercitrin)	feruoyl-pentoside
glucuronide	glucuronide
	rhamno-glucoside
	coumaroyl-glucoside (tiliroside)

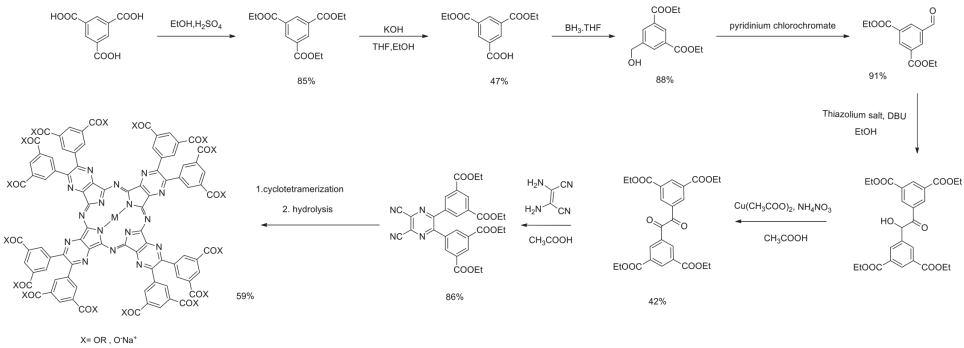
This study was supported by research projects of Charles University in Prague by UNCE 204026/2012 and SVV 267007.

SYNTHESIS OF AZAPHTHALOCYANINE CONTAINING ANIONIC GROUPS

KOLLÁR, J., ZIMČÍK, P.

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

Photodynamic therapy (PDT) is type of therapy that can be used in the treatment of cancer. It consists of 3 essential components: photosensitizer (PS), light, and oxygen. None of these is individually toxic but together they initiate a photochemical reaction that culminates in the generation of a highly reactive product termed singlet oxygen ($^1O_2$). Research in Azaphthalocyanine group focuses on synthesis, photophysical and photochemical behaviour of phthalocyanines and azaphthalocyanines (AzaPc) in respect to the application in PDT for a long time. Aim of this work is a synthesis of new PS from the group of water-soluble AzaPc with future potential to be used in PDT. The synthesized compound should contain peripheral carboxylic groups



that can be turned into anionic by formation of salt. Presence of anionic groups is expected to lead to inhibition of undesired aggregation of the AzaPc in water medium. The starting compound for synthesis was trimesic acid that was esterified by ethanol (see Scheme). The triester was partially hydrolysed to mono carboxylic acid. Subsequently, the free carboxyl group was selectively reduced to hydroxyl and followed by oxidation to aldehyde. Benzoin condensation of this aldehyde gave acyloine that was oxidized to diketon. Substituted pyrazine-2,3-dikarbonitrile, a precursor for AzaPc, was obtained by condensation of diaminomaleonitirile with this vicinal diketon. Cyclotetramerizationusing magnesium butoxide as initiator gave AzaPc substituted with sixteen alkylcarboxy groups. An attempt was also made to hydrolyze the ester to AzaPc with free carboxylic groups.

The work was supported by SVV 267 001 and by Czech Science Foundation (13-27761S).

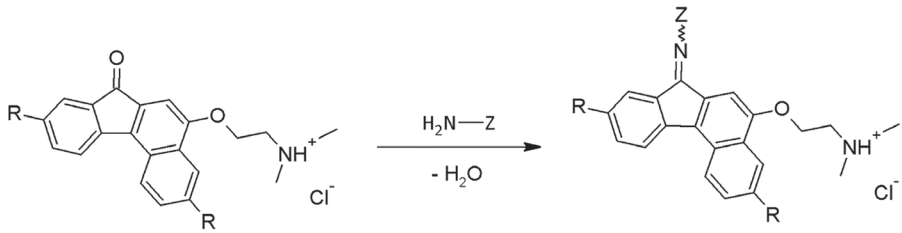
SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW DERIVATIVES OF DIMEFLURONE AND BENFLURONE

KOLENIČ, M.,[1] KRATOCHVÍL, J.,[1] POUR, M.,[1] TOMŠÍK, P.,[2] SUCHÁ, L.[2]

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

Dimeflurone and benflurone are planar polyaromatic compounds with a carbonyl group on the central five-membered cycle. Water solubility is enabled by a tertiary ammonium salt group on the side chain. Although both of them are effective against proliferating carcinomas, they are also toxic and undergo fast metabolic deactivation.

The oxo function, the main target of tumor cell reductase, is essential for therapeutic effect. Therefore we focused on as yet unreported structural modifications keeping the double bond intact. For this purpose, nucleophilic addition-elimination was the easiest procedure to consider.



R = OMe or H

To this end, we prepared eight derivatives of dimeflurone and benflurone with hydroxylamine-hydrochloride, *O*-methylhydroxylamine-hydrochloride, hydrazine-hydrate and thiosemicarbazide. Their cytostatic activities were tested on mice with inoculated breast cancer.

SYNTHESIS OF CARDIOPROTECTIVE IRON CHELATORS DERIVED FROM DEXRAZOXANE

KRATOCHVÍL, M., EISNER, T., ROH, J., VÁVROVÁ, K., HRABÁLEK, A.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
e-mail: kratocm4@faf.cuni.cz

Anthracycline antineoplastic antibiotics such as doxorubicin or daunorubicin are important anticancer drugs. These highly active chemotherapeutic drugs are also associated with acute cardiotoxic effects and a dose-related cardiomyopathy due to their effect on heart muscle cells. This cardiomyopathy is characterized by left ventricular enlargement and global systolic dysfunction, usually with associated mild to moderate mitral insufficiency. It is assumed that the major side effect is caused by reactive oxygen species, whose formation is catalyzed by the complexes of anthracyclines with iron. The only drug used worldwide is Dexrazoxane (DXZ). DXZ is metabolized to substance ADR-925 *in vivo*, which chelates the iron ions. DXZ also acts as a topoisomerase II inhibitor. Interestingly, the cardioprotective effects of DXZ were discovered accidentally and only few structure-activity relationship studies have been carried out yet.

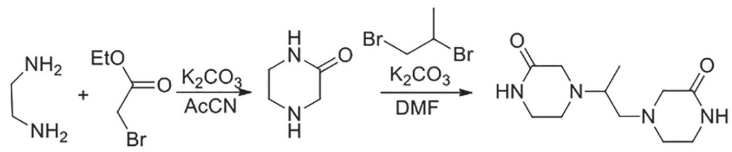


Fig. 1. Synthesis of MK-15

In this work, preparation of DXZ amide analogue named MK-15 is described (Fig. 1). First, large-scale synthesis of piperazin-2-one from ethylenediamine with ethyl bromoacetate was carried out and the reaction conditions were optimized. In the second

step, two molecules of piperazin-2-one were connected with 1,2-propylene linker using 1,2-dibromopropane. The reaction conditions were optimized and the product was prepared and purified in several batches. Cardioprotective effects of MK-15 were evaluated *in vitro* on isolated rat neonatal cardiomyocytes and *in vivo* on model of chronic anthracycline cardiotoxicity. These results can help us to understand the mechanism of action of DXZ and potentially clarify the role of chelation in DXZ-mediated cardioprotection.

This project was supported by the Charles University in Prague (Project UNCE 33/2012 and SVV 267 001).

ACETYLCHOLINESTERASE INHIBITORS USED IN THE TREATMENT OF ALZHEIMER'S DISEASE: BASIC PHARMACOKINETIC PARAMETERS

KREJČIOVÁ, M.,[1] HROCH, M.,[2] ZEMEK, F.,[1] KORABEČNÝ, J.,[1]
KUČA, K.,[1] ŽDÁROVÁ, KARASOVÁ, J.[1]

[1]Department of Public Health and Department of Toxicology, Faculty of Health Sciences, University of Defence, Hradec Králové, Czech Republic
[2]Department of Biochemistry, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic
e-mail: Marketa.Krejciova@gmail.com

Alzheimer's disease (AD) is the most common type of dementia in human population. In case of AD the memory, reasoning, mood and communication abilities are significantly influenced. Cognitive disorders are connected with a lower cholinergic activity. acetylcholinesterase inhibitors such as Donepezil, Rivastigmin and Galantamin have beneficial effects on the typical AD symptoms. They are still considered as a cornerstone treatment, although there are many new strategies in AD therapy investigated. The late stage of AD should be treated with Memantin; an NMDA receptor inhibitor.

This study deals with pharmacokinetic of two acetylcholinesterase inhibitors, tacrine and its derivative 7-methoxytacrine. The main aim of this study was to determine real inhibitors' concentrations in plasma and brain tissue in different time intervals after i. m. application. Both cholinesterase inhibitors were applied in equimolar doses to rats (male; tribe Wistar, 240 ± 20 g). After sample preparation, by liquid-liquid extraction, the concentration levels were measured by using High Performance Liquid Chromatography (HPLC) in combination with fluorescence detector.

Our results showed that the concentration levels of the inhibitors were time dependent. 7-methoxytacrine reached the maximum concentration 84.80 ± 36.66 ng/ml in plasma and 14.17 ± 2.56 ng/ml in brain tissue both in the 15th minute. The maximum of tacrine concentration in rat plasma was reached in about 15 minutes giving 37.56 ± 9.01 ng/ml. In the brain tissue the maximally tacrine concentration 18.86 ± 2.45 ng/ml was in the 30th minute. 7-methoxytacrine shoved higher plasma concentration but tacrine has better permeation through biological barriers.

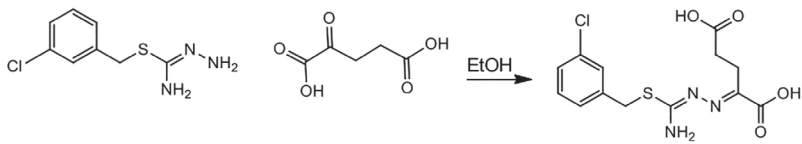
The study was supported by the project of Grant Agency of Czech Republic, No. P303/11/1907 and by the Ministry of Defence (Czech Republic).

^{15}N NMR SPECTROSCOPY IN STRUCTURAL ANALYSIS

LESCHINGEROVÁ, A.,[1] NOVOTNÁ, E.,[2] VOKŘÁL, I.,[3] NOVÁK, Z.,[1] KUNEŠ, J.[1]

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

This project deals with the usage of NMR spectroscopy in structural analysis of unknown product obtained according to the scheme:



^{1}H and ^{13}C NMR experiments showed that the actual structure of the reaction product was different than was expected.

Advanced multidimensional NMR experiments (COSY, ^{1}H-^{13}C gHSQC, ^{1}H-^{13}C gHMBC, ^{1}H-^{15}N gHMBC) were necessary to reveal the structure of prepared compound.

These experiments as well as mass spectrometry revealed intramolecular cyclization.

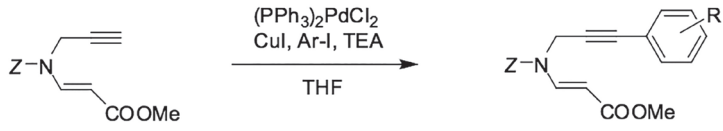
This project was supported by grants SVV UK 267 001.

GOLD CATALYZED CYCLIZATIONS IN THE SYNTHESIS OF SUBSTITUTED PYRIDINE DERIVATIVES

MATOUŠ, P., MIKUŠEK, J., MATOUŠOVÁ, E., POUR, M.

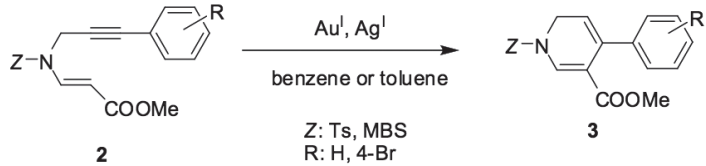
Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
e-mail: matousp1@faf.cuni.cz

p-Toluenesulphonyl (Ts) or p-methoxybenzenesulphonyl (MBS) protected propargylamine reacts with methyl propiolate to form enyne **1** that undergoes Sonogashira coupling. To this end, phenyl and 4-bromophenyl derivatives (**2**) were prepared.



1 Z: Ts, MBS; R: H, 4-Br **2**

Substituted enynes (**2**) form cyclic derivatives (**3**) in the presence of gold and silver catalysts.



2 **3**

Currently, various types of cyclization conditions are being tested.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. P207/10/2048) and Charles University Research (SVV-265-001).

TACRINE-FERULIC ACID HETERODIMERS FOR ALZHEIMER'S DISEASE TREATMENT: DESIGN AND SYNTHESIS OF NOVEL ACETYLCHOLINESTERASE INHIBITORS

PEŠEK, T.,[1] KORABEČNÝ. J.,[2,3] MUSÍLEK, K.,[2,3,4] OPLETALOVÁ, V.,[1] KUČA, K.[2,3,4]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]University of Defence, Faculty of Military Health Sciences, Department of Toxicology, 500 01 Hradec Králové, Czech Republic
[3]Center for Biomedical Research, University Hospital in Hradec Králové, Czech Republic
[4]Department of Chemistry, Faculty of Science, University of Hradec Králové, Czech Republic
e-mail: peset7aa@faf.cuni.cz

Tacrine was the first drug approved by FDA for the treatment of Alzheimer's disease (AD). However, its use was restricted in function of side effects observed in some patients. Further investigation on the structural basis by which tacrine inhibits cholinesterases activity brought new perspectives for the design of more potent analogues with fewer side effects resulting in so-called multi-targeted directed ligands (MTLDs). These MTDLs affects more biological processes (e.g. BACE-1 inhibition, Aβ aggregation induced by acetylcholinesterase, antioxidant activity) resulting in agents with better pharmacological profile. Within our contribution there will be presented design and synthesis of novel dual binding site heterodimers based on 7-methoxytacrine and 6-chlorotacrine. Both tacrine moieties are connected with ferulic acid, well known compound with antioxidant properties.

References

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2. Chen, Y. et al.: Tacrine-Ferulic Acid-Nitric Oxide (NO) Donor Trihybrids as Potent, Multifunctional Acetyl- and Butyrylcholinesterase Inhibitors. J. Med. Chem., 55, 2012, 4309–4321.

This study was supported by the specific research (SV/FVZ201201), 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 Long Term Development plan – 1011 and by Ministry of Education, Youth and Sport (SVV-267-001).

OPTIMIZATION OF SAMPLE PREPARATION STEP FOR UHPLC-MS/MS ANALYSIS OF ATORVASTATIN, ROSUVASTATIN AND THEIR METABOLITES

PILAŘOVÁ, V., VLČKOVÁ, H., SVOBODA, P., NOVÁKOVÁ, L.

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

Statins are very important group of drugs which are used to treat hypercholesterolemia and to prevent cardiovascular diseases. They are HMG-CoA reductase inhibitors. HMG-CoA reductase is the key enzyme that catalyzes the synthesis of cholesterol in human body. Statins exist in two forms – acid and lactone form. Acid form is the effective form, while lactone form is transformed to open hydroxy-acid in human body. Both statins – atorvastatin and rosuvastatin – belong to the most frequently used statines because of their biological half-live and other pharmacological properties.

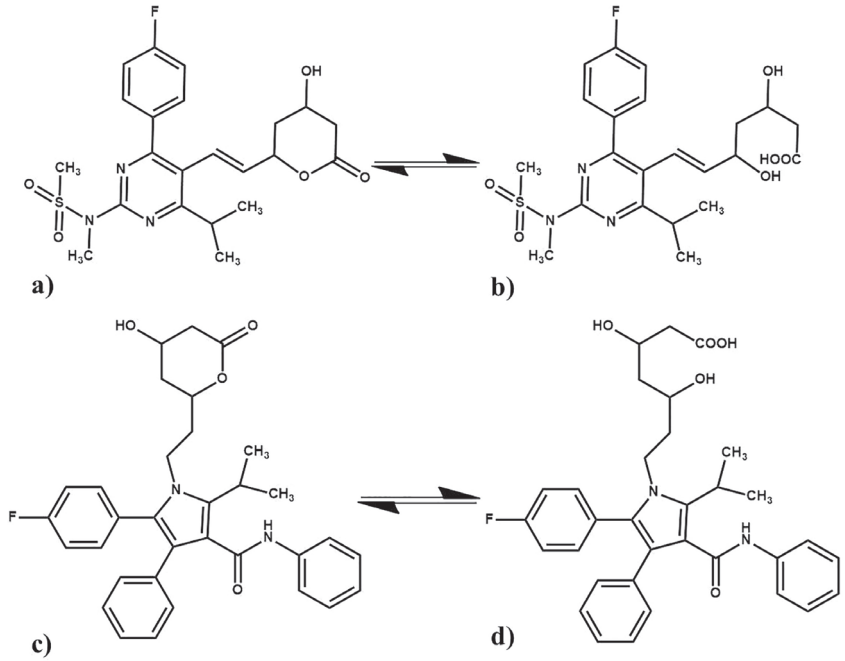


Fig. 1. Non-effective lactone form a) atorvastatin lactone c) rosuvastatin lactone; Effective open hydroxy-acid form b) atorvastatin d) rosuvastatin.

A simple, sensitive and rapid ultra high performance liquid chromatography – tandem mass spectometry (UHPLC-MS/MS) method was developed and validated for quantification of atorvastatin, rosuvastatin and their metabolites in human serum using atorvastatin-d_5 and rosuvastatin-d_6 as internal standards. MEPS (microextraction by packed sorbent) method was used for sample preparation of biological material.

C18 was used as an extraction sorbent. Washing and elution reagents consisting of acetonitrile and ammonium acetate in various ratios were optimized. The mixture of acetonitrile and 0.1 M ammonium acetate pH 4.5 in a ratio of 95:5 was chosen as an elution agent. A mixture of acetonitrile and 0.01M ammonium acetate pH 4.5 in ratio of 5:95 was chosen as washing agent. The method was applied to the biological material (human serum).

The analytes were separated using the Acquity BEH C18 column (50 × 2.1 mm, 1.7 μm, Waters) with gradient mobile phase (mixture of ammonium acetate and acetonitrile) and detected by electrospray (ESI) tandem mass spectrometry.

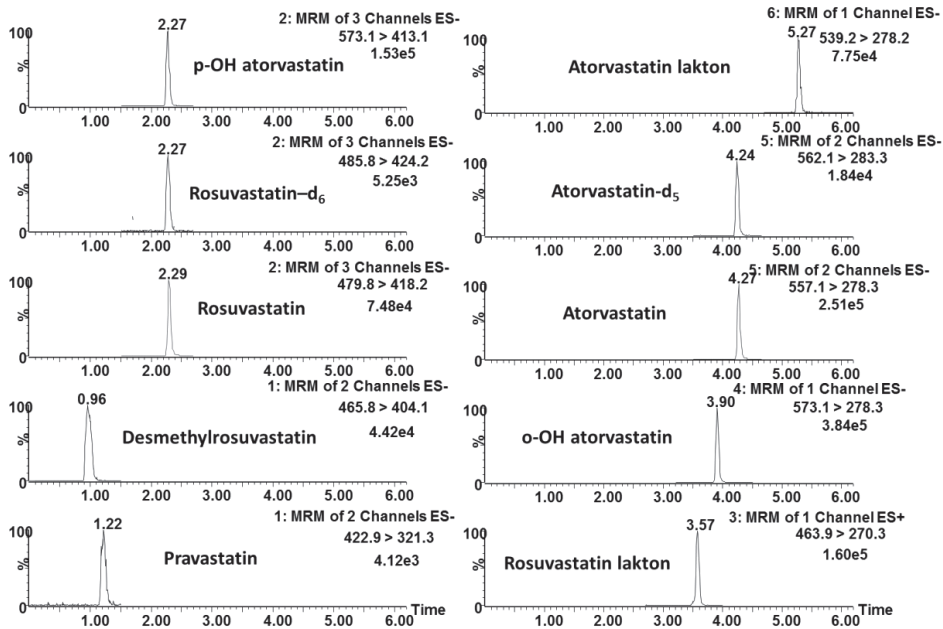


Fig. 2. SRM for standard analytes – mixture of atorvastatin, atorvastatin lactone, p-hydroxyatorvastatin, o-hydroxyatorvastatin, atorvastatin-d5, rosuvastatin, rosuvastatin lactone, rosuvastatin-d6, N-desmethylrosuvastatin, pravastatin. Dissolved in ACN and 0.5 mM ammonium acetate pH 4.0 (30:70), Concentration of analytes was 5 × 10 -7 mol/L. HPLC conditions: stationary phase Acquity BEH C18 (50 × 2.1 mm, 1.7 μm, Waters); mobile phase ACN and 0.5 mM ammonium acetate pH 4.0; pH 4.0; flow rate 0.3 ml/min; injection 5 μl; gradient elution.

Finally the method was validated. The linearity, accuracy, precision, selectivity of the method were verified. The limit of detection and quantification and matrix effects were verified too. The method was linear with correlation coefficients in the range from 0.9982 to 0.9998. Limit of detection (LOD) ranged from 0.25 to 1.0 nmol / l and limit of quantification (LOQ) from 0.5 to 2.5 nmol / l. Precision was less than 15%, recovery, which indicates the accuracy, was in the range of 70 and 125% for all of analytes.

The study was supported by SVV 267 007 and UNCE 204026/2012.

SYNTHESIS OF METABOLITES OF THIOSEMICARBAZONE ANTICANCER AGENTS

PRŮŠA, P., ROH, J., VÁVROVÁ, K., HRABÁLEK, A.

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

Iron chelators represent a promising group of potent anticancer agents. These compounds inhibit ribonucleotide reductase, a key enzyme in DNA synthesis, via uptake of iron (Fe) from its active site. This inhibition deteriorates DNA replication and suppresses cancer cell growth. Furthermore, complexes of these compounds with Fe can enter the redox cycle leading to the release of intracellular reactive oxygen species, e.g., hydroxyl radicals. These radicals damage especially DNA and cell membranes. Recently, thiosemicarbazone iron chelators derived from 2-benzoylpyridine (BpT) and di-2-pyridylketone (DpT) have been studied for their high and selective antiproliferative activity (Fig. 1).

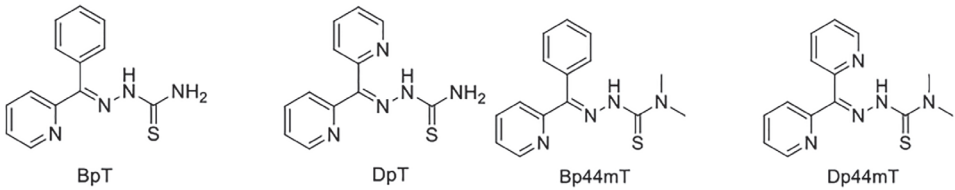


Fig. 1. Structures of thiosemicarbazone iron-chelators

The most effective substances from this group with proven *in vivo* anticancer potency are 2-benzoylpyridine-4,4-dimethyl-3-thiosemicarbazone (Bp44mT, Fig. 1) and di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT, Fig. 1).

In this study, we designed and synthesized semicarbazone analogues of these substances, which will be used as standards in metabolic and pharmacokinetic studies of their parent drugs. Moreover, the chelating as well as antiproliferative activities of selected metabolites will also be evaluated.

This study was supported by the Charles University in Prague (Project UNCE 33/2012 and SVV 267 001).

COMPARISON OF PHOTOPHYSICAL PROPERTIES OF DIFFERENT TYPES OF AZAPHTHALOCYANINES

REIMEROVÁ, P., NOVÁKOVÁ, V.

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

Phthalocyanines and their aza analogues azaphthalocyanines are organic dyes with planar macrocyclic structure. They can be used in many ways – for example as sensors, dyes or sensitizers in photodynamic therapy.

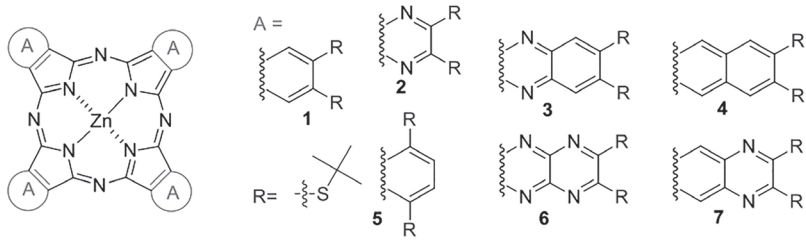
The aim of this study was to compare photophysical (absorption, fluorescence) and photochemical properties (singlet oxygen production) and solubility of several molecules **1**–**7** that differ in the type of macrocyclic core (see Figure below). Compounds were designed to have the same peripheral substituents (alkylsulfanyls) and the central metal (zinc) in order to avoid the influence of these parts on the dye properties.

The strength of absorption expressed as an extinction coefficient increased with the bathochromic shift of the Q-band. The position of the Q band depended on the number of conjugated double bonds and on the position of the peripheral substituents. Isosteric exchange of benzene rings to pyrazine rings shifted Q band to shorter wavelength.

Quantum yields of singlet oxygen (Φ_Δ) and quantum yields of fluorescence (Φ_F) were determined in pyridine and THF by comparative method with zinc phthalocyanine as a reference. Φ_Δ ranged between 0.49 and 0.92, while Φ_F were significantly lower 0.06–0.40.

Solubility of the compounds in the series **1**–**7** was compared in toluene and ranged between 0.15–112.80 mg/mL. Further, solubility of **1** and its corresponding aza-analogue **2** was determined also in other solvents (toluene, benzene, THF, dioxane, acetone, pyridine, DMF) showing on better solubility of aza-analogue **2**.

The work will continue by measuring further properties such as photobleaching, dissociation constants (aggregation) and acidity of azomethine nitrogens.



The work has been supported by grant SVV 267 004.

ANALYSIS OF LIPIDS IN EPIDERMIS WITH REDUCED FILAGGRIN EXPRESSION

SOCHOROVÁ, M.,[1] ŠKOLOVÁ, B.,[1] KUCHLER, S.,[2] VÁVROVÁ, K.[1]

[1]Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department for Pharmacy, Ludwig-Maximilian University in Munich, Germany
e-mail: sochormi@faf.cuni.cz

Filaggrin (FLG) is crucial for correct development of epidermis and for its function as skin barrier. Mutation in the FLG gene and loss of his function is associated with diseases such as ichtyosis vulgaris and atopic dermatitis. The aim of the study was to evaluate the impact of FLG knock-down on the composition of intercellular lipids in the stratum corneum (SC).

We used *in vitro* skin constructs with reduced (FLG–) and normal gene expression (FLG+) for the analysis. It enables to observe separately influence of FLG on composition and organization of SC lipids.

FLG is a protein which is degraded to metabolites which cause an acidification of SC. pH is important for optimal activity of enzymes which are pivotal for formation of ceramides from their precursors. Therefore we assumed lower ceramide content in skin barrier when FLG is reduced. Our assumption was supported with findings in patients with atopic dermatitis. However our HPTLC analysis showed that the amount of lipids is similar in both FLG– and FLG+ skin construct. The only statistically significant difference was the nearly twofold higher content of free fatty acids in FLG– construct.

Based on this finding, pH of SC and an activity of secretory phospholipase ($sPLA_2$) were evaluated. SC pH was optimal and increased $sPLA_2$ activity was confirmed. This means, that conversion of phospholipids into fatty acids by $sPLA_2$ is another mechanism how to acidify SC for ceramide formation. Nevertheless, the increased level of free fatty acid causes decreased skin lipid order which was proved by ATR-FTIR spectroscopy. Disorder and higher mobility of hydrophobic chains has a negative effect on skin barrier since it becomes more permeable for lipophilic substances.

In conclusion, we found a feedback mechanism between filaggrin and fatty acid-based acidification pathways in the skin. The lower ceramide levels previously described in atopic dermatitis are probably caused by mechanisms independent of filaggrin.

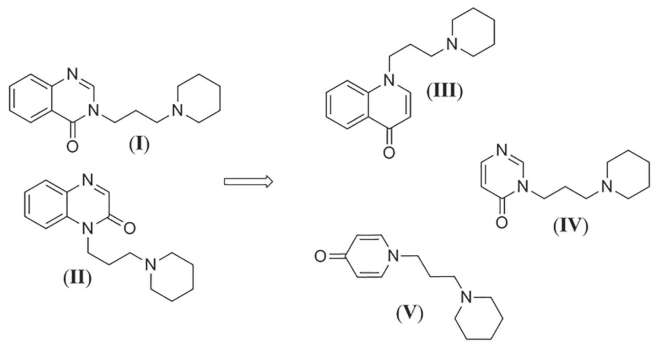
The study was supported by the Czech Science Foundation (project No. 207/11/0365) and Charles University (SVV 267 001).

PREPARATION OF HETEROCYCLIC COMPOUNDS WITH BRONCHODILATORY ACTIVITY

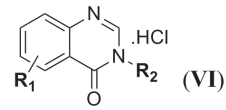
SOCHOVÁ, P.,[1] MACHOVÁ, K.,[1] VOPRŠÁLOVÁ, M.,[2] ŠPULÁK, M.[1]

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

Two most active compounds from previous screening were selected as model structures, *N*-alkylquinazolinone (**I**) and *N*-alkylquinoxaline (**II**).[1,2] The "active" (piperidine-1-yl) propyl fragment was attached to structurally related heterocycles to examine relationship between the bronchodilatory effect and a) the number of nitrogen atoms (quinolinone **III**) and b) the presence of benzene ring (pyrimidinone **IV** and pyridinone **V**).

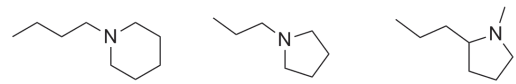


The replacement of N3 in quinazoline by a carbon atom caused a significant decrease of activity; the omission of benzene ring resulted in complete loss of the bronchodilatory effect. Therefore, we decided to modify ring A in quinazoline bearing an "active" fragments with halogen and methoxy groups which would result in the following series (**VI**):



(**VI**)

R$_1$ = 7 - Cl, 7 - Br, 7 - MeO, 6 - MeO, 6,7 - diMeO

R$_2$ = 

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.

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This work was supported by the Czech Science Foundation (project No. P207/10/2048) by Charles University in Prague (SVV-267-001).

STUDY OF LIPIDS MEMBRANES CONTAINING CERAMIDE PRECURSORS AS MODELS OF SKINS DISEASES

Staňková, K., Pospíšilová, M., Školová, B., Pullmanová, P., Vávrová, K.

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

Ceramides are important determinants for both water-retention function and permeability-barrier function in the stratum corneum. They are synthesized from their precursors, i.e. sphingomyelin and glycosylceramide by the enzymes sphingomyelinase and glucocerebrosidase. Their reduced levels have been found in the skin diseases e.g. in atopic dermatitis, and are also a causative factor for the dry and barrier-disrupted skin.

The aim of this study is to prepare model skin lipid membranes simulating the defect of sphingomyelinase and glucocerebrosidase and study their permeability. We expected that the models with precursors will be more permeable than normal skin model. Models of stratum corneum lipid membranes were prepared as equimolar mixture of ceramides or precursors in different ratios, cholesterol, fatty acid mixture and 5% of cholesterol sulfate. This lipid mixture was dissolved in hexane/ethanol 2:1 (*v/v*) at 4.5 mg/ml concentration and then slowly applied on Nuclepore polycarbonate filters with 15 nm pores under the nitrogen stream using Linomat V. the formed lipid layer was 11 μm thick. These lipid films were heated above of phase transition (90 °C) for 10–15 minutes and then slowly cooled down to room temperature for about 3 hours which ensured formation of lamellae similar to skin. Then they were incubated for 24 hours to 32 °C. The permeation of these model membranes was evaluated using permeation of theophylline and indometacin and electrical impedance in Franz diffusion cell with 0.5 cm^2 available diffusion area. The amount of model drug permeated through the membranes was evaluated using HPLC.

The results of our experiment were surprising. The permeability of the membranes with an increasing level of the precursors (sphingomyelin and glukosylceramide) was lower than the permeability of membranes simulating healthy skin. The reason for this finding will need further study, probably using a model containing the full skin ceramide fraction.

The study was supported by the operational programme ECOP, registration number CZ.1.07/2.3.00/30.0061, Increasing of the R&D capacity at Charles University through new positions for graduates of doctoral studies, Czech Republic, Czech Science Foundation (207/11/0365 and 13-23891S), and Charles University in Prague (SVV 267 001).

AN HPLC-MS/MS METHOD FOR DETERMINATION OF ANTI-CANCER AGENT BP4ET AND ITS MAIN PHASE I METABOLITES IN PLASMA AND ITS APPLICATION TO *IN VIVO* STUDY

SUPRUNOVÁ, V.,[1] STARIAT, J.,[1] RICHARDSON, DES R.,[2] KOVAŘIKOVÁ, P.[1]

[1]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Department of Pathology and Bosch Institute, Iron Metabolism and Chelation Program, Bosch Institute, University of Sydney, Sydney, New South Wales, 2006 Australia
e-mail: suprunov@faf.cuni.cz

Despite the substantial advance in cancer treatment, the rising incidence of malignant diseases urgently calls for novel treatment regimes. Thiosemicarbazone derivatives, including 2-benzoylpyridine-4-ethyl-3-thiosemicarbazone (Bp4eT, Fig. 1), are currently under intensive research for their promising antiproliferative effect on tumor cells observed *in vitro* and *in vivo*. The unique mechanism of anticancer activity is based on inhibition of ribonucleotide reductase mediated by chelating the iron from its active site. Moreover, the redox activity of iron and copper within the complex of thiosemicarbazone as well as the effect on various transcription factors further contribute to cytostatic activity. In previous studies it was identified the oxidation of thiosemicarbazone sulphur as the principal biotransformation pathway of Bp4eT, leading to metabolites of semicarbazone (M1) and amidrazone (M2) structure.

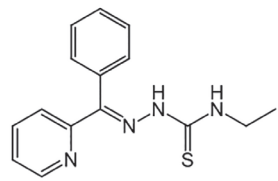

Fig. 1. A chemical structure of Bp4eT

The aim of this work was to develop and validate a bioanalytical LC-MS/MS method for determination of Bp4eT and its major metabolites in rat plasma (M1, M2) and to utilize the method for analysis of plasma samples from the pilot pharmacokinetic study.

The samples were treated by solid-phase extraction, the eluate was evaporated to dryness by nitrogen stream. The residuum was reconstituted in 50% ACN and injected. The separation of analytes was achieved on Discovery HS C18 column (75 × 4.6 mm, 3 μm, Supelco) using 2 mM ammonium formate and acetonitrile (40:60, *v/v*) as a mobile phase. Prior to each analytical run, 20 μL of an aqueous solution of EDTA (20 mM) was injected and eluted to the waste in order to maintain sensitivity and repeatability. Ion-trap mass spectrometer with electrospray ionization source was utilized as a detection technique. The method was successfully validated over the range of 50–800 ng/mL for Bp4eT, 5–100 ng/mL for both *E* and *Z* isomers of M1, and 25–400 ng/mL for M2. Following validation parameters were determined: selectivity, calibration curve, precision, accuracy, extraction recovery, long-term and post-preparative stability, dilution integrity and matrix effect.

Finally, the method was successfully utilized for the analysis of plasma samples from *in vivo* experiment in rats (dose 3 mg/kg, n = 4). The concentration-time profile for Bp4eT and metabolite M2 was acquired and the basic pharmacokinetic parameters for the parent compound were calculated.

The study was supported by the grant IGA NT 12403-3/ 551 2011 and by the grant SVV 267 001.

SYNTHESIS OF CARDIOPROTECTIVE IRON CHELATORS DERIVED FROM DIETHYLENETRIAMINEPENTAACETIC ACID

ŠŮS, J., EISNER, T., ROH, J., VÁVROVÁ, K., HRABÁLEK, A.

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

Anthracycline antibiotics such as doxorubicin or daunorubicin are widely used anticancer drugs. However, the administration of anthracyclines is connected with high risk of cardiotoxicity. Chronic anthracycline cardiotoxicity is characterized by dilated cardiomyopathy, with subsequent development of left ventricular contractile dysfunction and congestive heart failure. It is supposed that the complexation of anthracyclines with intracellular iron leads to the formation of reactive oxygen species, which causes serious tissue damage especially in myocardium. The only clinically used drug preventing anthracycline cardiotoxicity is dexrazoxane (DXZ). It was argued that its mechanism of action involves iron-chelating properties of ADR-925, the main metabolite of DXZ (Fig. 1). However, recent studies showed that its mechanism of action is more complex.

In this study, we designed several potential cardioprotective iron chelators derived from diethylenetriaminepentaacetic acid (DTPA, Fig. 1) and investigated several synthetic approaches for their preparation.

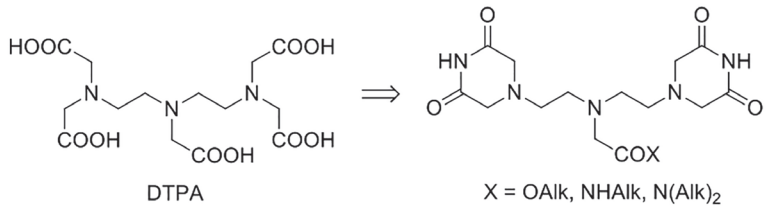

Fig. 1. Analogues of DXZ derived from DTPA

Furthermore, sufficient amount of ADR-925 was synthesized to clarify the role of this metabolite in cardioprotective effect of DXZ *in vivo*.

This project was supported by the Charles University in Prague (Project UNCE 33/2012 and SVV 267 001).

SYNTHESIS OF 1-(3-METHOXYPHENYL)-*N*-METHYLIMIDAZO[1,2-*A*] QUINOXALIN-4-AMINE AND STUDY OF ITS CHEMICAL PROPERTIES

VALÁŠKOVÁ, L.,[1] DELEUZE-MASQUEFA, C.[2]

[1]Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Laboratoire de Chimie des Biomolécules et de l'Environnement, Pharmacochimie et Biomolécules, Faculté de Pharmacie, Université Montpellier I, 15 av. Charles Flahault, BP 14491, 34 093 Montpellier Cedex 5, France
E-mail: valaskl1@faf.cuni.cz

Melanoma is malign tumor usually located in the skin, mucous membranes or rarely in other parts of the organism. Every year the prevalence of this tumor is growing. Tumors which are detected in early stages can be successfully removed, but when metastasis appear treatment of this type of cancer is difficult. Some tumors (e.g. on problematic places on face) cannot be removed by surgery, even if they are soon detected. In those cases, topically administered anticancer drugs can be used. One of those substances is imiquimod (ALDARA®; Fig. 1), possesses antiviral, immunostimulating and cytotoxic activity. Limiting factor of this substance is its toxicity – it can be used only topically. The research group of prof. Pierre-Antoin Bonnet deals with the synthesis of imiquimod analogues. Synthesized molecules belong to three chemical groups, which differ in the orientation of imidazole moiety. Their lead structures, providing higher *in vitro* cytotoxic activity against human melanoma cells than imiquimod, are EAPB0203 and EAPB0503 (Fig. 1).[1]

Main problem of EAPB0503 is its poor solubility in water. In this work I focused on the synthesis of EAPB0503 and its water-soluble salts.

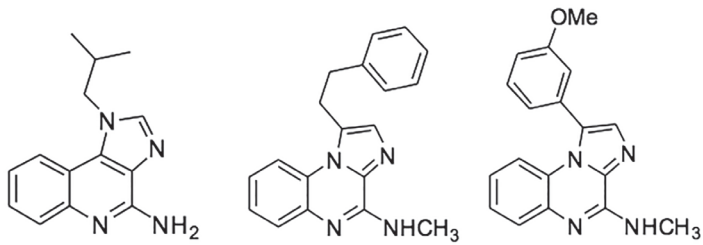

Fig. 1. Structures of imiquimod, EAPB0203 and EAPB0503

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LIQUID PAEDIATRIC PREPARATIONS 1. SOTALOL HYDROCHLORIDE

KŘÍŽ, T.,[1] KLOVRZOVÁ, S.,[2] ŠKLUBALOVÁ, Z.[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
[2]Hospital Pharmacy, University Hospital Motol, Prague, Czech Republic
e-mail: tomas.kriz@gmx.com

Sotalol hydrochloride (SOTCL) is used in a treatment of arrhytmia from the age of neonates. Extemporaneous preparation is essential in paediatric therapy due to a lack of commercially available pharmaceuticals. The capsules are the main used extemporaneous paediatric dosage form in the Czech Republic until now.

Formulation of a paediatric oral solution with SOTCL 5 mg/ml for an extemporaneous compounding in a hospital pharmacy was the aim of this work. The target was to use as low as possible amount of excipients to provide a three-month physico-chemical and microbial stability and a suitable taste of the preparation.

Sotalol hydrochloride is well soluble in an aqueous vehicle; within pH of 4 to 5 has a good chemical stability[1]. After the preformulation study, eight aqueous formulations of sotalol hydrochloride 5 mg/ml were compounded using citric acid dibasic sodium phosphate dodecahydrate to stabilize pH value, potassium sorbate 0.14% w/v as a preservative, and simple sucrose syrup and/or sodium saccharin, respectively, as sweeteners. The properties of the preparations: density, osmolality, and index of refraction were estimated. The solutions were stored at two temperatures of 20–25 °C and/or 2–8 °C, respectively. At time intervals of 1 – 3 – 7 – 14 – 30 – 60 – 90 days, the pH value was measured. The content of SOTCL was determined by a measurement of content of chloride using argentometric potentiometric titration.

All studied formulations showed stable pH values and no visible change in appearance for 90 days at both temperatures of storage except for an aqueous sotalol hydrochloride solution without any additives. The use of dibasic sodium phosphate dodecahydrate buffer solution is not necessary to ensure stable pH value.

Some of the formulations are proposed as suitable candidates for a validated stability study using high performance liquid chromatography (HPLC) with a determination of the concentration of sotalol hydrochloride and potassium sorbate.

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STUDY OF INFLUENCE OF CROSSLINKING ON THE ABSORPTION PROPERTIES OF HYALURONATE NANOFIBRES

LEBEDOVÁ, V.,[1] RŮŽIČKOVÁ, J.,[2] ŠKLUBALOVÁ, Z.[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic
[2]Contipro Group, a.s., Dolní Dobrouč, Czech Republic
e-mail: lebedovv@faf.cuni.cz

Hyaluronic acid and its derivates belongs to biopolymers which are currently intensively studied in many medical applications. Micro and nanofibers have suitable properties to be used as dressings in healing of injuries. Liquor handling (absorption and donation) properties (capacity and rate) are important properties of wound dressing materials.

In this experimental work, we studied the properties of the new synthesized hyaluronate derivates (HAD). To modify properties of the aqueous solutions of HAD, polyethylene oxide (PEO) 400 000 was used. The solutions had an appropriate surface tension, conductivity, and viscosity required to prepare nanofibres. The nanofibres were manufactured by electrospinning method using 4spin (Contipro, Czech Republic).

The samples of nanofibres were crosslinked by UV radiation for 60 minutes (HAD60) and/or 10 minutes (HAD10). To study the influence of crosslinking on the absorption capacity of nanofibres, the modified free swell absorbency method (WO 93/12275/A1)[1] was used in which samples were immersed in a bath containing water and/or a saline solution for 5 minutes (liquor/fibre ratio of 75:1), the excess liquor was removed by draining, and amount of liquid absorbed was then estimated by weighing. The nanofibers crosslinked for 60 minutes had significantly larger absorbency of both liquid media than nanofibers crosslinked for 10 minutes. The moisture affinity of nanofibers HAD60 for water (35 g/g) and saline solution (17 g/g) were comparable with those of commercial dressing made of cellulosic fibres (32 g/g and/or 19 g/g, respectively).

The results showed that the newly synthetized derivates of hyaluronate crosslinked by UV rays for 60 minutes are an appropriate material for further investigation.

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The study was supported by Student grant SVV 267 001.

ANALYSIS OF DRUG RELATED PROBLEMS IN HEALTHCARE FACILITY

ORNSTOVÁ, E., DOSEDĚL. M., MALÝ, J.

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

Background: A Drug-Related Problem (DRP) is an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes.[1] Their occurrence among inpatients is not rare and can interfere in patient safety.

Aims: Aim of the research was to analyse and evaluate the incidence of DRPs in healthcare facility.

Methods: Research was conducted in specialized medical institution in Pardubice region, facility for long terms rehabilitation after neural illnesses, musculoskeletal disorders and severe injuries. Data for further analysis and evaluation were from medical records. Following data were collected: patient's characteristics, diagnosis, using drugs, selected laboratory markers. DRP found were classified according to modified Pharmaceutical Care Network Europe classification for DRP, version 5.01. Research results were evaluated by descriptive statistics.

Results: Medical records of 182 patients (43% of men) were revised and contained in the results. An average age was 57.6. Number of medications per patient was 6.1 on average. Overall number of found DRPs was 383 resulting in average 2.1 DRP per patient. Most frequent DRPs were classified as "Drug choice problem" (46%) or "Dosing problem" (36%). Followed by "Unspecific problems" (14%), "Drug use problem" (2%), "Interaction" (1%) and "Adverse reaction" (1%). Top rated DRPs were missing drugs in prescription even when the indication was clear (e.g.: absence of ACE inhibitors or statins at patients with high cardiovascular risk). Detected DRPs were described, statistically evaluated and discussed with physicians.

Conclusion: Incidence of DRPs is very significant. Pharmacist's intervention can lower the number of DRPs and increase the efficiency of a treatment by optimizing drug therapy.

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This study was supported by the Charles University in Prague (Project SVV 267 005).

PATIENTS WITH OBSTRUCTIVE PULMONARY DISEASE OR LOCAL ADVERSE EFFECTS ASSOCIATED WITH INHALED FORMULATIONS

ŠIMANDL, O.[1,2]

[1]Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic
[2]Česká lékárna, a.s., Lékarna Dr. Max – Ostrov nad Ohří, Czech Republic
e-mail: ondrej.simandl@seznam.cz

Asthma (AB) and chronic obstructive pulmonary disease (COPD) can be ranked among obstructive pulmonary diseases. Reversible airway obstruction on the basis of bronchospasm due to chronic eosinophilic inflammatory response only occurs in AB, irreversible obstruction on the basis of chronic bronchitis and emphysema occurs in COPD. The prevalence of both diseases in the Czech Republic (AB ≈ 8%, COPD ≈ 6%) and in the world is increasing, so we often meet these patients in pharmacies.[1,2]

Pharmacist's duty is not only to dispense the drug, but also to provide qualified pharmaceutical care, especially during the dispensation of the drug in the form of inhaler. Pharmacist should provide information to the patient about the application, maintaining the cleanliness and operability of the inhaler. Pharmacist should also warn the patient of possible adverse reactions (ADRs) associated with the use of the drug, that often resulting from the wrong application method, and the possibilities of their reduction.[3]

This work presents the results of a five-week survey (questionnaire method) focused on ADRs associated with the treatment of obstructive pulmonary disease with inhaled drug forms (IDFs). The survey took place in the most frequented public pharmacy (catchment area ≈ 20,000 inhabitants / 6 pharmacies) in late November and December 2012.

Population consisted of 35 patients (21 men, 14 women) diagnosed with AB (23) and COPD (12), and treated with at least one inhalar. The most common inhalors were Aerosol MDI form (26), Turbohaler (9), Diskus (9) and least frequent were Respimat SMI (3), the Aerolizer (1) and Easyhaler (1). ADRs associated with IDFs applications were detected in 15 patients (10 AB, 5 COPD), some of them were combined. The most often occured ADRS were irritation of the throat (7 AB, 2 COPD), hoarseness (5 AB, 2 COPD), candidosis (4 AB, 0 COPD) and xerostomia (2 AB, 2 COPD). Those problems occured lways in connection with the use of inhaled corticosteroids (ICS), mostly from beclomethasone (9 AB, 5 COPD).

Another important group of drugs in the IDFs were bronchodilators – SABA / SAMA ev. their combination (16 AB, 10 COPD) or LABA / LAMA (2 AB, 1 COPD), from which is expected significantly lower risk of local adverse reactions than ICS. In 16 (10 AB, 6 COPD) patients were used together with the above medications alone or a in combination of ICS + LABA. The local adverse reactions were most frequently associated with MDI aerosol systems (13) and the Turbohaler (5), from active substances naturally ICS – budesonide (5) and beclomethasone (5). This can be seen as a subject for reflection while dispensing IDFs and proprietary medicinal product containing inhaled corticosteroids.

33 patients were educated about proper application by the doctor, only 2 by the pharmacist (also educated by the doctor). Despite their treatment, patients assessed on

a scale 1 to 10 average mark 7 (AB 7.3, 6.5 COPD), indicating gaps in providing not only medical, but also pharmaceutical care, especially in the area of training how to apply the medicine IDFs.

To complete the survey, investigation was also attended pharmacists too (3). None of them had practical training of applications IDFs during their studies. Only 1 of them graduated from this course in lifelong training, and one other has an interest in this course. They all said that they educate the patient, but does not verify whether the patient handle can actually handle the application. They all unanimously indicate as the cause the lack of time and privacy during the expediture. Here is also a space for better quality of pharmaceutical care while dispensing the drug, especially when one considers that the average interval of patient's appointmen at the doctor is one in 4 months. During this time the patient's condition is not professionally monitored and the dose is not adjusted according to the current condition (e.g. no patient with the diagnosis AB is using Peak Flow Meter to correct the dose of bronchodilator). A few patients (around 3, this question was not included in the questionnaire) interupt their treatment because of ADRs associated with IDFs and led to subsequent severe exacerbation.

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The survey was materially supported firms AstraZeneca Czech Republic s.r.o., Boehringer Ingelheim Česká republika, Novartis Europharm Ltd. a Teva Pharmaceuticals ČR.

SELF-REPORTED COMPLIANCE WITH ORAL BISPHOSPHONATES IN POSTMENOPAUSAL WOMEN

TOUŠKOVÁ, T.,VYTŘÍSALOVÁ, M.

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

Objectives: Oral bisphosphonates (oBIS) are frequently used in the treatment of osteoporosis. However, low adherence to the treatment significantly affects effectiveness. The study aim was to assess adherence with oBIS among Czech women in common clinical practice.

Methods: A cross-sectional multicentre questionnaire survey was performed in consecutive secondary care female patients aged ≥ 55 years. Two dimensions of adherence were studied: adherence based on Osteoporosis Specific Morisky Medication Adherence Scale (OS-MMAS) and compliance with dosing instructions for safe and effective use of oBIS.

Results: A total of 515 self-reported or nurse-assisted questionnaires were obtained (response rate 95%). As much as 457 questionnaires were used for analysis of adherence.

Respondents (mean age 69 years) were treated with alendronate (N = 73, 16%), risedronate (N = 63, 14%) – once a week dosing interval – and ibandronate (N = 321, 70%) – once a month dosing interval. Based on OS-MMAS, 68% of respondents showed high adherence. Only 44 % of respondents were compliant to all five dosing recommendations. The two dimensions of adherence were not associated with each other. Similar mean compliance rates were found for once a week and once a month formulations. Compliance with dosing instructions (score) correlated positively with education (P = 0.001).

Conclusion: Low adherence to oBIS is striking. Adherence to ibandronate is not better than to once a week formulations. It is needed to implement intensive counselling on compliance with dosing instructions within pharmaceutical care.

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