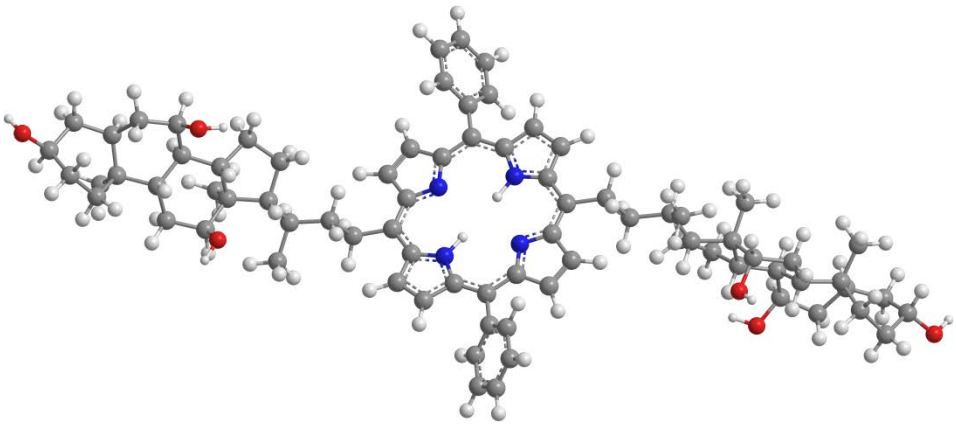

XVIIIth INTERDISCIPLINARY MEETING OF YOUNG RESEARCHERS AND STUDENTS IN THE FIELD OF CHEMISTRY, BIOCHEMISTRY, MOLECULAR BIOLOGY, AND BIOMATERIALS

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Devět Skal Milovy hotel

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APIGENEX medicem pragolab

EXISTENCE OF CIRCADIAN RHYTHMS IN EXPRESSION OF CELL CYCLE GENES AND THEIR CHANGES IN AGING AND COLORECTAL TUMOURIGENESIS

KATEŘINA BALOUNOVÁ[a,b], M. VODIČKA[a,b], P. ERGANG[a], P. KVAPILOVÁ[a], J. PÁCHA[a,b]

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Mammalian endogenous timekeeping mechanism called circadian clock allows the organism to adapt and anticipate natural periodic changes. Circadian clock generates circadian rhythms, which participate in the regulation of a number signalling pathways. Disruption of the circadian regulatory mechanisms seems to be associated with the development and the progression of tumours including colorectal cancer. The progression of tumourigenesis is influenced by age and is linked to disruption of the cell cycle machinery, which is also controlled by circadian clock. Cell cycle is a dynamical process where accurate transitions of cell cycle phases depends on the dynamic expression of the cyclin (CCN)/cyclin-dependent kinase (CDK) complexes. Expression and degradation of these complexes are regulated by CDC25 phosphatases and WEE1/MYT kinases. Therefore, we compared the 24-hr expression profiles of genes encoding the key CCNs (*Ccnd2*, *Ccnd3*, *Ccne2*, *Ccna2*, *Ccnb2*), CDKs (*Cdk6*, *Cdk4*, *Cdk1*, *Cdk2*), CDC25s (*Cdc25a*, *Cdc25b*, *Cdc25c*), WEE1 and MYT1 in healthy colon of young (14 weeks) and old mice (52 weeks) and in chemically induces primary colorectal tumours of 52 week-old mice. Using RT-qPCR we proved circadian rhythmicitys in *Ccne2*, *Ccna2*, *Ccnb2*, *Cdk4*, *Cdk1*, *Cdk2*, *Wee1*, *Myt1*, *Cdc25b* and *Cdc25c* in normal colon of young mice. In contrast, this rhythmicity disappeared during aging, except for *Cdc25b* and was absent also in tumours. In summary, our results indicate circadian regulation of cell cycle machinery and larger effect of aging than neoplastic transformation on these diurnal changes.

Supported by GAUK 1520-243-250318.

SYNTHESIS OF A NEW 2,2'-BIPYRIDINE LIGAND AND ITS APPLICATIONS

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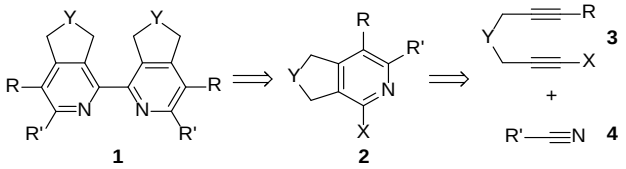
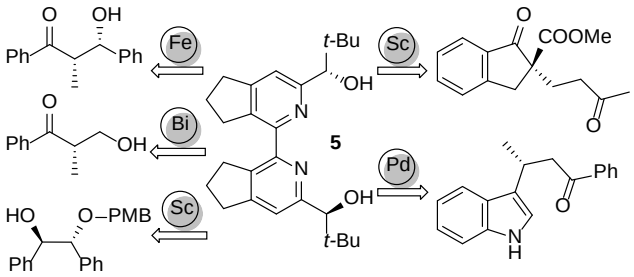
2,2'-Bipyridines and their derivatives form an important class of heteroaromatic compounds with application in various fields of chemistry[1]. Thanks to their unique coordination chemistry and stability, they can be used as ligands in metal-catalyzed racemic and enantioselective reactions.

One of such ligands is bipyridine **1**, synthesis of which is based on two key steps: a) catalytic homocoupling of 2-halopyridine **2** and b) catalytic cyclotrimerization of halodiynes **3** with nitriles **4** developed in our group (Scheme 1)[2].



Scheme 1. **Retrosynthetic analysis of bipyridine 1**

Chiral bipyridine **5** was synthesized by using this approach. Its metal complexes were tested in enantioselective Mukaiyama-aldol reaction, hydroxymethylation, Michael addition, C-H activation of indols, and desymmetrization of epoxides (Scheme 2). In some cases, high enantioselectivity was observed.



Scheme 2. **Application of bipyridine ligand 5**

This work was supported by Czech Science Foundation (reg. No 17-07707S), Grant Agency of Charles University (GAUK 243-250362) and French Embassy in Prague.

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BEE-DERIVED PEPTIDE, DEFENSIN-1, STIMULATES SKIN RE-EPITHELIALIZATION

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Honeybee products, especially honey, have been used in traditional medicine since the ancient times for various skin disorders such as burns and wounds. Honey possesses biological activities (antibacterial[1,2], antibiofilm[3], anti-/pro-inflammatory[4,5], immunomodulatory[5]) creating ideal wound healing environment. Bee defensin-1, antibacterial peptide belonging to the insect defensin group is effective against Gram-positive bacteria. Honeybee defensin-1 was first isolated from royal jelly[6] and named royalisin. Later was discovered that royalisin and defensin-1 are encoded by one gene[7]. Recent findings have shown that defensin-1, as part of royal jelly and honey, plays role in wound healing and exhibits immunomodulatory activity. The aim of this study was to investigate wound closure and re-epithelialization properties of recombinant defensin-1 (rDef-1) *in vitro* and *in vivo*.

Recombinant defensin-1 was prepared in heterologous baculovirus/ insect expression system. Subsequently, rDef-1 was used for *in vitro* and *in vivo* testing. Secretion of matrix metalloproteinase-9 (MMP-9) by HaCaT cell line was assessed by gelatine zymography. *In vitro* scratch and migration assay was performed on HaCaT to measure proliferation and migratory response of keratinocytes to defensin-1. *In vivo* wound closure activity was tested on round excision wounds of Wistar albino rats.

Following results prove the immunomodulatory and wound healing properties of rDef-1. It had potent and dose dependent effect on MMP-9 secretion from HaCaT cells. Furthermore, rDef-1 significantly ($P<0.0001$) stimulated *in vitro* closure in HaCaT monolayer at concentration 0.5 µg/mL and also significantly ($P<0.05$) stimulated cell migration. *In vivo*, rDef-1 significantly ($P<0.05$) promoted wound closure.

In this study, we have shown that defensin-1, besides its antibacterial activity, may accelerate wound healing by affecting keratinocytes proliferation and migration.

This research was supported by a Yamada Research Grant and the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences 2/0007/14.

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PHOTOSWITCHABLE INTRAMOLECULAR HYDROGEN BONDS IN 5-PHENYLAZOPYRIMIDINES

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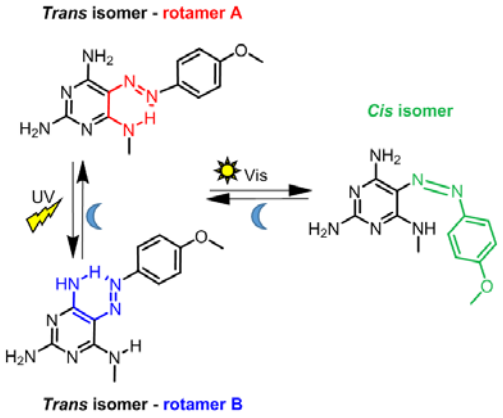
A new series of 5-phenylazopyrimidines with two hydrogen bond donors was prepared by diazotation of various *para* substituted anilines followed by azo coupling with corresponding pyrimidines. NMR spectroscopy with *in situ* irradiation and DFT computations were used to investigate photoswitching behaviour of these compounds.[1]

5-Azopyrimidines form intramolecular hydrogen bonds (IMHBs) with nitrogen atom of the azo group while two stable rotamers of *trans* isomer are formed (Scheme 1). NMR with *in situ* irradiation showed unique reversible photoswitching between both rotamers upon irradiation. Interestingly, upon irradiation, no *cis* isomer was detected probably due to dramatic decrease of *cis*-to-*trans* energy barrier caused by the presence of IMHB. Only *p*-methoxy derivative with the highest energy barrier provided detectable amount of the *cis* isomer. Mechanism of photoswitching between the rotamers was proposed based on DFT computations.



Scheme 1. **Photoswitchable 5-phenylazopyrimidines**

This work has been supported by the Czech Science Foundation (grant no. 15-11223S), Adolf-Messer foundation

and the German Research Council (DFG, TH1115/9-1).

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PHYSIOLOGICALLY RELEVANT VASCULATURE MODELS BASED ON MICROFLUIDIC SYSTEMS

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Vessel inflammation is an initial process preceding the vascular system diseases such as atherosclerosis stroke or myocardial infarction. It can be induced by shear stress disturbances. Thus, vessel lesions and bifurcations are prone to inflammation since the shear stress disturbances can be significant here. This field is worth researching since little is known about the onset of the vessel inflammation.

The aim of our research is to construct a physiologically relevant vessel model with branching and circular cross-section. Since the endothelium is a common element for all the blood vessels the endothelised channel can be taken as a minimal vascular model. By tuning the flow rate and the diameter of the channel we are able to simulate conditions in any blood vessel.

The chips were made from polydimethylsiloxane (PDMS). Channel lumen was modified to promote endothelium cell adhesion. Surface modifications like, oxidation, silanization, and protein coating were tested. Channels were colonized with the mouse endothelial cells (MS-1).

The best surface modification of PDMS to promote endothelial cell adhesion and growth was a combination of oxidation and collagen coating. This allowed a perfect handling the endothelial cells in straight channels. Cells showed high viability and physiological morphology. Currently we are working on bifurcated channels in order to study cell behaviour along the sites of bifurcation.

This work was supported by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR).

SELECTIVE ELIMINATION OF SENESCENT CELLS BY MITOCHONDRIAL TARGETING IS REGULATED VIA ANT2

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Cellular senescence is a form of cell cycle arrest that limits the proliferative potential of cells[1,2]. However, inability of immune cells to subsequently eliminate senescent cells from the organism may lead to inflammation, carcinogenesis or development of age-related diseases[3–5].

Mitocans are agents with anti-cancer activity that induce apoptosis of malignant cells via targeting mitochondria[8]. We have developed several highly specific mitocans with selective mitochondrial uptake driven by high mitochondrial potential of cancer cells[8–10]. Although these agents were intended to eliminate malignant cells, their potential efficacy in targeting cells with increased mitochondrial potential, such as senescent cells, make them intriguing candidates for senolytic agents.

We found that MitoTam, unlike conventional anti-cancer agents, not only kills cancer cells without inducing senescence *in vitro* and *in vivo*, but also selectively eliminates both malignant and non-cancerous senescent cells. In naturally aged mice treated with MitoTam, we observed a significant decrease of senescent markers in all tested organs compared to non-treated animals. Mechanistically, we found an important role of adenine nucleotide translocator 2 (ANT2) in survival of cells treated with MitoTam. Restoration of ANT2 in senescent cells resulted in their resistance to MitoTam, while its downregulation in non-senescent cells sensitized these cells to both MitoTam and oligomycin A as well as CCCP, which underscores the crucial importance of the interplay between ANT2, ATP synthase and the level of mitochondrial potential in maintenance of mitochondrial integrity. The key finding presented here show that simultaneous interference with mitochondrial integrity and ATP homeostasis in senescent cells leads to their effective removal.

The ability to pharmacologically eliminate senescent cells opens the door to study their role in a wide range of relevant (patho)physiological settings and brings a new strategy for the treatment of age-related pathologies or senescence-associated tumorigenesis.

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NEW APPROACH FOR TESTING OF INHIBITORS OF HIV-1 UNCOATING AS A PROMISING TARGET FOR ANTIRETROVIRAL THERAPY

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Proper sequence of events following the entry of HIV-1 to the host cell cytoplasm is crucial for retroviral life cycle. First of the events is disassembly of HIV-1 core, known as uncoating. HIV-1 core is formed by hexameric lattice connected by interactions among N-terminal and C-terminal domains of the capsid protein. The well-balanced stability of the hexameric lattice is one of the major factor influencing not only the process of uncoating, but also the following steps as reverse transcription and cDNA integration to the host cell genome[1,2]. Any interference of this well-balanced stability of the mature HIV-1 core affects the timing of uncoating subsequently resulting in a block of HIV-1 infectivity[3,4]. Despite that uncoating seems to be a suitable target for the antiretroviral therapy, any protocol efficiently monitoring and quantifying the uncoating has not been established yet. To monitor the uncoating of HIV-1 particles in the presence of potential inhibitors we used a combination of three cell-based assays: (i) VSV-G pseudotyped HIV particles as a reporter for HIV-1 infectivity, (ii) total DNA analysis following by qPCR as a reporter of reverse transcription proceeding and (iii) cyclosporine washout assay as a reporter of uncoating timing. In combination with our recently developed *in vitro* stabilization assay for testing of HIV-1 inhibitors, (s-FAITH), this approach provides a useful tool for uncoating inhibitors screening.

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DIFFERENT THERMAL ADAPTATION OF TWO CLOSELY RELATED *BORDETELLA* SPECIES

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Bordetella pertussis and *Bordetella bronchiseptica* are closely related respiratory pathogens. While *B. bronchiseptica* is able to infect mammals generally and also to survive outside the host, *B. pertussis* is exclusively a human-adapted pathogen. Both species produce multiple virulence factors in order to adhere to respiratory epithelia (pertactin, fimbriae, filamentous haemagglutinin), to deregulate immunity system signaling (adenylate cyclase toxin) and therefore to successfully promote the infection. The production of virulence factors is controlled by two-component system, which integrates signals from environment by transmembrane sensor kinase BvgS, phosphorylating a transcriptional regulator BvgA. *In vivo* signals modulating the activity of BvgS are undescribed yet, but in laboratory conditions the system can be switched off by chemical modulators (sulphate, nicitinic acid) or cultivation at temperature below 25 °C, which seems to be the most relevant

factor resembling the environment outside the host. Thermal transitions are one of the major stresses faced by microorganisms as it affects the functionality of cytoplasmic membrane. In order to adapt the stress, composition of fatty acids needs to be adjusted.

To follow the thermal adaptation which *B. pertussis* and *B. bronchispetica* undergoes upon temperature shift, using GC-MS we analysed changes in composition of fatty acids within six hours after the transition from 24 °C to 37 °C cultivation temperature. Interestingly, *B. pertussis* adapted the membrane composition in much lower extent than *B. bronchiseptica*. To further investigate the influence of temperature upshift, we monitored the production of several virulence factors and BvgA transcription regulator by western blot method. In the case of *B. pertussis* the antigens were still produced at 24 °C and temperature upshift did not significantly influence their production in comparison to *B. bronchispetica* which did not produce the antigens at 24 °C and production of virulence factors was induced only upon transfer to 37 °C. These results are in correlation with the membrane adapting capacity of both species. Because the presence of BvgA in phosphorylated form is the determining factor of gene expression and virulence factor production, reflecting the kinase activity of BvgS, we separated phosphorylated and nonphosphorylated form of BvgA using SDS-PAGE with Phos-tag™ and detected both forms by western blot method. Our results verified that BvgS kinase is still active in *B. pertussis* at 24 °C on the contrary with *B. bronchiseptica*. Based on the obtained results we hypothesize that insensitivity of BvgS to thermal changes and retained production of virulence factors in *B. pertussis* may significantly contribute to efficient transition of the disease.

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NEW DISEASE-CAUSING GENE DISCOVERY

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In a large family of Czech origin, we performed linkage analysis and mapped a new locus for an autosomal dominant corneal endothelial dystrophy, Posterior Polymorphous Corneal Dystrophy 4 (PPCD4). No potentially pathogenic variants were identified by whole exome sequence analysis. Using whole genome sequencing, a unique non-coding variant within this locus was identified in a potential regulatory region that segregated with disease.

The whole spectrum of classical and state-of-art methods leading to this discovery will be presented, including linkage analysis, whole exome and whole genome sequencing, *in silico* analysis, reverse transcription-PCR, haplotype analysis, histology, immunostaining and luciferase assay.

Targeted sequencing identified the same variant in three additional previously unsolved PPCD families, including a *de novo* occurrence that suggests this is a recurrent mutation. Two further unique variants were identified in the same regulatory region in unrelated British PPCD families. We demonstrate that these variants identified in PPCD4 individuals induce increased transcriptional activity *in vitro*. Furthermore, although this gene is not expressed in corneal endothelial cells in control tissue, we detected its protein product in the corneal 'endothelium' in PPCD4 patient tissue.

We suggest that mutations inducing mesenchymal to epithelial transition within the corneal endothelium are a convergent pathogenic mechanism leading to dysfunction of the endothelial barrier and disease.

This work was supported by GACR 17-12355S. PS was supported by GAUK 250361/2017 and SVV 260367/2017.

p53 IS INVOLVED IN NEURAL DIFFERENTIATION OF PLURIPOTENT STEM CELL-DERIVED CEREBRAL ORGANOIDS

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Differentiation of cerebral organoids from human embryonic stem cells (hESCs) represents an invaluable method for modelling human neural tissue development *in vitro*. Molecular mechanisms of differentiation have been previously connected to tumour suppressor p53, however its precise role remains unclear. Moreover, how p53 influences human cerebral development has never been described before. Therefore, we aimed to address this question using wild type (wt) and p53 knockout (p53KO) hESCs generated using CRISPR/Cas9 system. Following induction of differentiation into cerebral organoids, phenotype and gene expression variations were assessed. We found that organoids cultured from the p53KO hESCs were significantly larger and had more prominent neuroectodermal morphology than those from the wt hESCs. Additionally, qPCR results showed that p53KO hESC-derived organoids possess higher expression and faster upregulation of neuronal markers such as SOX1, SOX2, β-3-Tubulin, NeuN, and Doublecortin. Finally, Western blotting confirmed that the absence of p53 incited faster downregulation of the pluripotency transcription factors Oct4 and Nanog. Collectively, our results suggest that neuronal differentiation is enhanced in the absence of p53 in brain organoids. We will proceed to evaluate the regulation of neural

differentiation of hESCs without p53 on the molecular level and its implications for human development.

This research was supported by GAMU-Rector's programme to M.B. (MUNI/C/1709/2016), funds from the Faculty of Medicine MU to junior researcher D.B. (ROZV/25/LF/2017), MUNI/A/1369/2016, GACR GJ15-18316Y, and by the National Program of Sustainability II of the Ministry of education, Youth, and Sports of the Czech Republic (LQ1605).

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SMAD4 LOSS IS ASSOCIATED WITH DECREASED OXIDATIVE METABOLISM IN PANCREATIC CANCER AND AFFECTS SENSITIVITY TO MITOCHONDRIAL THERAPY

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Pancreatic cancer is a rapid and aggressive disease with an excessive invasive and metastatic potential. Since there has been no significant progress in the introduction of new therapeutic approaches, pancreatic cancer remains one of the most challenging types of tumours to treat[1]. This is likely caused by a population of resistant tumour cells which are reported to be dependent on functional mitochondria[2]. We have previously shown that mitochondrial targeting may be an efficient way to eliminate cancer cells[3]. At the same time, we believe that loss of Smad4, a mediator of transforming growth factor β (TGFβ) pathway frequently mutated in pancreatic cancer, might be the cause of metabolic reprogramming which alters mitochondrial function in PCCs.

We demonstrated that respiration of Smad4+ PCCs was substantially decreased when TGFβ was applied. On the contrary, Smad4-/- PCCs as well as Smad4 knocked out (KO) cells not only appeared to be insensitive to TGFβ treatment but also showed lower basal respiration level. Furthermore, we observed significant changes in mitochondrial fragmentation of Smad4+ PCCs after TGFβ treatment while Smad4-/- and Smad4 KO PCCs remained unaffected. For this reason, we investigated the responsiveness of PCCs to mitochondrially targeted inhibitor of NADH dehydrogenase, MitoMet, and discovered that it is indeed related to the Smad4 expression profile. Taken together, the Smad4 status seems to correlate with the level of oxidative metabolism in pancreatic cancer.

Our results suggest that loss of Smad4 leads to metabolic reprogramming of PCCs which consequently impacts the sensitivity to mitochondrially targeted therapy. Based on these findings we propose a new treatment approach aimed at efficient elimination of PCCs that rely on distinct energy metabolism.

This work was supported in part by Charles University Grant Agency support (GA UK 1100217) to Z. Ezrova.

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p16/INK4A AND CELL CYCLE REGULATION IN HUMAN EMBRYONIC AND NEURAL STEM CELLS

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Human embryonic stem cells (hESCs) have the ability to unlimitedly self-renew without losing their differentiation capacity. This is underlied by rapid cell division and specific cell cycle regulatory mechanisms. Importantly, length of G1 phase, and activity of specific cell cycle regulators determine the cell fate decision and differentiation[1]. Molecular mechanisms behind this phenomenon are however not well described. In this study, we focused on an important regulator of G1 transition, protein p16/INK4A (p16). Protein p16 acts as a tumor superssor molecule which arrests the cell cycle in G1. During our initial analysis of cell cycle regulators in hESCs and neural stem cells (NSCs), we observed that p16 manifests a different expression pattern than other cell cycle inhibitors. Curiously, its regulation in hESCs and NSCs has not yet been described. Therefore, we focused on several possible regulations of p16 and judging from our functional experiments, it seems that the expression of p16 protein is regulated differently in each cell type. We found that in undifferentiated hESCs, inhibition of miRNA biogenesis pathway significantly upregulates p16 protein levels, suggesting that translation of p16 is inhibited by specific miRNAs. Curiously, we found that the knock-out of p53 protein using CRISPR/Cas9 also upregulates p16 protein expression[2]. Both of these regulatory loops have, to our knowledge, never been described in hESCs. Upon differentiation of hESCs into NSCs, instead of miRNAs, p16 expression becomes directly transcriptionally repressed by

Bmi1 oncogene and level of p16 protein is also actively modulated by proteasomal degradation. Altogether, we have uncovered multiple novel p16 regulations in hESCs and NSCs, which will be closely analysed further.

This study was supported by Masaryk University, Faculty of Medicine (ROZV/24/LF/2016), (ROZV/25/LF/2017), and Czech Science Foundation (GJ15-18316Y and GJ18-25429Y).

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NOVEL APPROACH TO QUINAZOLINE ALKALOIDS TOTAL SYNTHESIS OF ARDEEMIN

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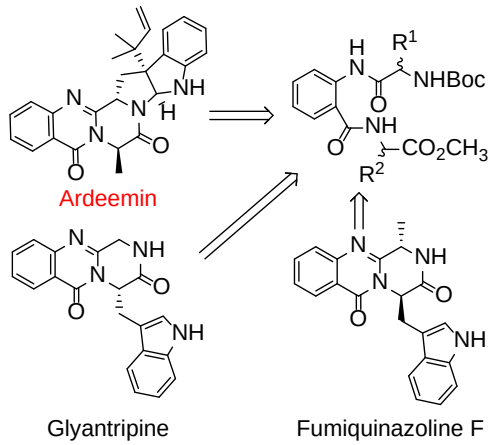
The quinazoline family of alkaloids, having diverse biological activities, is a growing class of secondary metabolites[1]. They are biosynthesized from tryptophan and anthranilic acid by incorporation of an additional amino acid unit. The members of this class of alkaloids exhibit cytotoxic, antiviral and anti-multidrug resistance activities[2]. Therefore, practical methods that allow rapid access to large quantities of these alkaloids and their analogs are needed.

A novel approach to quinazoline derivatives using silica gel mediated double condensation is reported, which is successfully applied to total syntheses of glyantripine, fumiquinazoline F, ardeemin and its analogs.



Scheme 1. **Total synthesis of natural products**

Ardeemin is a complex quinazoline alkaloid challenging organic chemists since 1993, when was isolated from the fungus *Aspergillus fisheri*[3].
We report the shortest synthesis of ardeemin in just 4 steps starting from commercially available materials.

We thank the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences and the Gilead Sciences & IOCB Research Centre for generous funding.

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PROGRESS IN THE SYNTHESIS OF ARYL-*C*-GLYCOSIDES FROM GLYCALS

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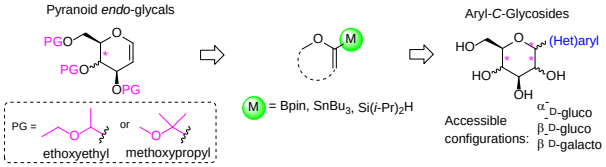
Aryl-*C*-glycosides are an interesting group of carbohydrate derivatives, in which the anomeric *O*- or *N*-glycosidic linkage is replaced by an aromatic substituent connected with a C-C bond. This structural feature makes them an interesting target for medicinal chemistry, because they can serve as *non*-hydrolyzable analogues of glycosides and nucleosides[1,2].

One of the modern synthetic approaches towards the synthesis of aryl-*C*-glycosides involves Pd-catalyzed reactions of metallated glycals with aromatic electrophiles. This method, however, relies upon the lithiation of the C-1 position of glycals with an excess of *t*-BuLi. This step requires very harsh reaction conditions and careful choice of the protection strategy.

In this contribution, significant progress in this problem was made, mainly regarding the use of methoxypropyl (MOP) and ethoxyethyl (EE) protecting groups. This protection excels at very mild conditions of both protection and deprotection procedures and proved to be universally applicable across pyranoid glycals.

The use of C-1 borylated, stannylated and silylated glycals for cross-coupling reactions with (hetero)aryl electrophiles will be discussed, as well as the options for stereoselective oxidation of the glycal double bond. The developed synthetical approach provides aryl-*C*-glycosides of various configurations and was applied to alternative synthesis of Dapagliflozin.



Scheme 1. **Synthesis of aryl-*C*-glycosides from metallated glycals**

This work was supported by the Czech Science Foundation (reg. No. 15-17572S) and in part by financial support from specific university research (MSMT No. 20-SVV/2017).

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CHROMATOGRAPHIC ANALYSIS OF VITAMIN B_1 AND B_6 DERIVATIVES IN WHOLE BLOOD

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B-group vitamins are well studied essential constituents involved in key cellular metabolic functions[1]. Derivatives of thiamine and pyridoxine are incorporated in more than 100 enzymes and enzymatic complexes. Although deficiency of thiamine and pyridoxal-5-phosphate is relatively rare in normal population, it is common in hospitalized patients. Discrepancies in their status may have serious deleterious effects mainly in patients with long-term intensive care[2]. Manifestation is often vague and may be easily overlooked[3]. However, monitoring of thiamine and its biologically active forms thiamine diphosphate and thiamine monophosphate together with pyridoxal-5-phosphate is not widely established in routine diagnostics. Therefore, monitoring of vitamin status has large importance especially in patients with intensive care. Methods for simultaneous determination of thiamine, its mono- and diphosphate derivatives with an active form of vitamin B_6 - pyridoxal-5-phosphate are still not widely established in routine diagnostics[4].

Currently used non-chromatographic methods for the determination of thiamine forms involve time consuming preparation and complicated interpretation of results. Application of chromatographic methods on biological samples, where thiamine derivatives are present at trace levels, still have several drawbacks that needs improvement[5].

An HPLC-FLD method with pre-column derivatization was developed, optimized and validated for the simultaneous analysis of thiamine and its derivatives with pyridoxal-5-phosphate in whole blood. Separation was accomplished by Meteoric Core-BIO C-18 core-shell column. Meteoric core-shell type material is specially optimized for biological applications, with its wider pore size, extended pH stability and higher efficiency. During gradient elution all target compounds were eluted within 15 minutes. Limits of detection are below clinically important values. Recoveries were in the range of 90 to 110% for all analytes. Bioanalytical method will be further implemented into routine practice and used primarily for the determination of thiamine and its derivatives in patients with supplementary nutrition therapy, where fluctuating level of metabolically active vitamins are associated with the occurrence of possible complications.

The study was supported by project SVV 260 412 and by University Hospital in Hradec Králové, 00179906.

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INFLUENCE OF SILVER NANOPARTICLES ON PENETRATION PROPERTIES OF A SKIN BARRIER

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Topical application of drugs represents non-invasive alternative treatment of many diseases. Nanoparticles (NPs) are used frequently to accelerate the penetration of medicaments through skin, for the targeted drug delivery or in surgical implants[1–4]. Moreover, NPs play an important role in cosmetics. Especially oxidic NPs can adversely affect organisms and their functions. Therefore, the development of environmentally friendly NPs that do not exhibit negative effects is necessary. For some medical purposes, silver NPs (AgNPs) are frequently used. AgNPs have antibacterial and anti-inflammatory properties and are used for body and face care and as constituents of various creams, toothpastes and soaps. AgNPs can be also used for the localisation of medicaments[2,3]. The topical usage of AgNPs is very convenient because they are accumulated probably (as Ag^0 or

Ag^+) in skin or *hypodermis* and should not penetrate into the vascular system and/or internal organs[2].

In this experimental study we used solvents ordinarily used in pharmaceutical area namely ethanol, methanol, dimethyl sulfoxide and demineralized water. Gallic acid (GA), which is widely used in dermatology considering the anti-oxidant, anti-bacterial or anti-tumor effects, was selected as a model analyte. AgNPs (with defined mean diameters of 20, 40, 60 and 100 nm) were added both to the pure solvents and corresponding solutions of GA. The samples of pig skin were treated with these systems containing AgNPs and the kinetic series of IR spectra were measured by attenuated total reflection technique to clarify the skin changes. Thousands of spectra in arranged series were evaluated by multivariate statistical methods. The obtained results demonstrate strong influence of AgNPs size on the structural changes of the skin surface. The largest changes of the skin structure were caused by AgNPs with mean diameter of 20 nm which influence strongly the effect of solvents and cause an apparent penetration of the dissolved GA deeper to the skin layers compared to the other systems. This study follows the experiments with vertical Franz diffusion cells where the permeability of the different sizes of AgNPs through the skin samples are tested thoroughly.

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DEVELOPMENT OF ELECTROCHEMICAL *IN VITRO* ASSAY FOR MICRO RNA DETECTION

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It is well-known that early diagnostics of malignant diseases greatly improves chances of successful treatment. Similarly, possibility to effectively monitor treatment response can play crucial role in cancer therapy.

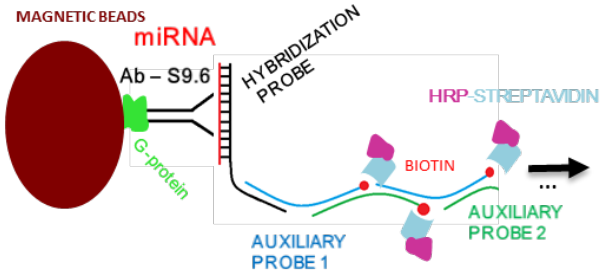
MicroRNA (miRNA) is a class of small non-coding RNA molecules with a wide scale of regulation functions, including cell differentiation, proliferation or apoptosis. Certain miRNAs, termed oncogenic or tumor suppressor miRNAs, were shown to be associated with onset and progression of cancer, which is supported by their different expression profiles in tumour tissues as compared to controls[1].

Present miRNA detection systems are mainly based on fluorescent hybridization reaching high sensitivities, but they often require expensive instrumentation, skilled personnel and complex protocols. Electrochemical (EC) techniques offer fast detection times and relatively inexpensive and simple instrumentation, and thus represent an interesting alternative to standard methods.

Our goal is to develop a cheap, quick and reliable EC assay for detection of up-regulated miRNAs. The assay involves miRNA-specific hybridization probe and two biotinylated auxiliary probes (Scheme 1). Separation of target miRNA from mixture is ensured by protein G-magnetic beads. Connection between magnetic beads and miRNA/probe complex is enabled by S9.6 antibody, which specifically binds RNA/DNA heteroduplexes[2]. EC signal, which comes from an enzymatic reaction catalyzed by horseradish peroxidase (HRP) conjugated to streptavidin[3], is monitored using an amperometry.

We used miR-21 as a miRNA model, which was already described as up-regulated in wide range of tumours and is easily detectable in real samples. Based on this optimized model, we could detect other up-regulated miRNAs on wide panel of cancer cell lines.

We believe that this assay can be potentially useful tool in early cancer diagnostics or when predicting treatment response.



Scheme 1. **Detection system for miRNA – hybridization chain reaction**

The work was supported by the projects GAČR 17-08971S and MEYS - NPS I - LO1413.

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METHOXETAMINE AND ITS METABOLITES SYNTHESIS AND THEIR HPLC AND CHIRAL CAPILLARY ELECTROPHORESIS SEPARATION

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If we modify the structure of drug already regulated by law, we will obtain an unregulated substance that is going to have similar effects as the original drug. Such altered compounds are being called New Psychoactive Substances (NPS). NPS, thanks to the online distribution and temporary legality, have become a phenomenon in recent years and their number is increasing every year. Their regulation poses a great challenge not only to regulatory authorities dealing with drug issues but also to scientific institutions dealing with pharmacology and analysis of this group of substances.

Users are often abusing dissociative anaesthetics (group of NPS) for many of their effects (e.g. dissociation). Due to its mechanism of action (inhibitors of NMDA receptor), dissociative anaesthetics have potential as neuroprotective agents and since recent scientific studies have shown their potential in the treatment of depression, they are currently attracting the attention of many experts. Dissociative anaesthetics are currently associated with a number of deaths and pose a serious health risk to the society[1].

Methoxetamine (MXE) is a dissociative anaesthetic with mild stimulating, anxiogenic and psychomimetic properties that emerged on the drug market during 2010. MXE was sold as a legal alternative for ketamine, which led to its rapid expansion over the market and so far is associated with 22 deaths and 120 intoxications. The fast identification of MXE and its metabolites is a key factor for the successful treatment of intoxication. Therefore, we suggested a convenient preparation method which was used for the synthesis of MXE, seven methoxetamine metabolites and a deuterium labelled derivative as analytical standards. Prepared standards were used for identification and quantification of suggested MXE metabolites in rat urine by LC-MS and for the pharmacokinetics. As metabolic pathways and pharmacological effects may be different for enantiomers, we used capillary electrophoresis as a chiral separation method for the enantioseparation of MXE and its metabolites[1].

This study was funded by a specific university research (project MSMT No. 20-SVV/2018) and by the Ministry of Interior of the Czech Republic (project VG20172020056).

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SYNTHESIS OF SUBSTITUTED INDENO[2,1-*c*]-FLUORENES

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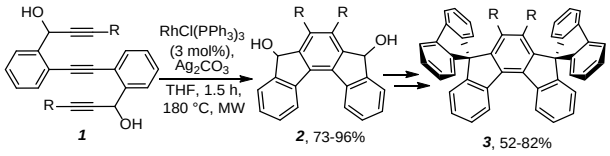
Poly- and oligophenylene derivatives constitute an interesting and important class of molecules that have been intensively studied because of their application in organic electronics[1]. Among them a special attention has been payed to indenofluorenes, dihydroindenofluorenes and their structural analogs. There are existing several indenofluorene isomers possessing five different scaffolds. The synthetic approaches to four of them and further applications have been intensively studied and well developed. However, in the case of the 5,8-dihydroindeno[2,1-*c*]fluorene isomer and its substituted derivatives only a handful of reports have been published[2].

We envisioned that the substituted indeno[2,1-*c*]fluoren-diol ***2*** could be prepared by catalytic [2+2+2] cyclotrimerization of a triyndiol ***1*** and its further conversion to the corresponding spiroindeno[2,1-c]fluorenones ***3*** by using our developed standard procedure in very high yields. (Scheme 1)[3]. The influence of electron donating and electron withdrawing groups as well as extended π-aromatic systems upon their photophysical properties (emission wavelength) were investigated.



Scheme 1. **General synthetic pathway for indeno[2,1-c]fluorenes**

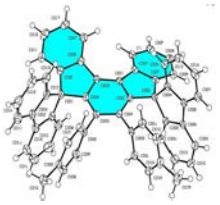


Fig. 1. **Platon plot of *3* showing displacement ellipsoids on 50% probability level**

This work was supported by GAUK (2700-243-250107) and Czech Science Foundation (reg. No. 13-15915S).

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THE JOURNEY TOWARDS MORE POTENT AND MORE WATER-SOLUBLE ANTI-INFLAMMATORY PYRIMIDINES

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As a part of our extensive structure-activity relationship study of polysubstituted pyrimidines with potential anti-inflammatory properties, we were aiming for improved biological properties, namely higher potency to inhibit prostaglandin E_2 (PGE_2) production and increased water-solubility via structural modification of the lead scaffold (**1**).

The first structure-activity relationship (SAR) study was focused on enhancement of the anti-inflammatory efficacy (inhibition of PGE_2 production in C57BL6 mouse peritoneal cells) through modifications of the phenyl moiety in the position C4 of pyrimidine. The key reaction for the synthesis of the target molecules was Suzuki-Miyaura cross-coupling. Firstly, eleven analogues were prepared which showed similar biological activity as the lead, but the most potent compound (bearing benzyloxy moiety attached to the phenyl in the position C4 of pyrimidine) exhibited 62 times higher activity compared to the lead. Based on these results, ten additional derivatives bearing benzyloxy or benzyloxy-like moiety were prepared, resulting in the discovery of **2** with three orders of magnitude higher efficacy to inhibit PGE_2 production compared to lead **1**.

Next, a series of eleven derivatives of the lead bearing a suitable moiety in the C5 position was prepared in order to increase their water-solubility. The synthesis proved to be more challenging, however, all prepared derivatives were more soluble than lead **1**. Unfortunately, the biological data showed that this type of modifications led to slight decrease of anti-inflammatory efficacy. Nevertheless, the most soluble compound, derivative **3**, achieved two orders of magnitude higher solubility than the lead compound while the biological activity remained comparable.

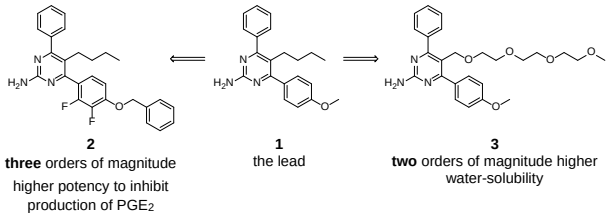

Fig. 1. **Structure of lead 1 and corresponding derivatives developed during this study**

This work was supported by the Institute of Organic Chemistry and Biochemistry (RVO: 61388963) and by Technology Agency of the Czech Republic (TE01020028).

THE FIRST Ru AND Pd CATALYSTS BEARING HELICALLY CHIRAL N-HETEROCYCLIC CARBENES

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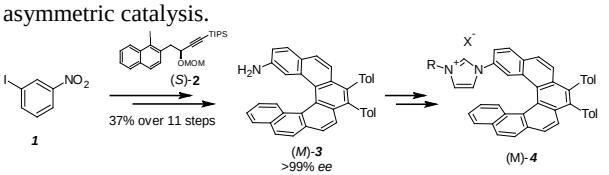
Inherently chiral helicenes can be envisioned as efficient stereoinformation carrying units useful for the construction of chiral catalysts. Aminohelicenes represent versatile building blocks for the synthesis of helical N-heterocyclic carbene (NHC) precursors. Actually, the first examples have already been described[1].

Herein we report on the synthesis of the enantiomerically pure amino[6]helicene (*M*)-***3*** (Scheme 1) on a multigram scale starting from iodonitrobenzene ***1*** and employing enantiopure naphthalene derivative (*S*)-***2*** in an eleven-step sequence. The imidazolium salt (*M*)-***4*** can be prepared from (*M*)-***3*** having either one or two helicene units attached to the central heterocyclic unit. These imidazolium salts were deprotonated and subsequently incorporated into the three different helicene NHC metal complexes (Figure 1), which were tested in asymmetric catalysis.



Scheme 1.

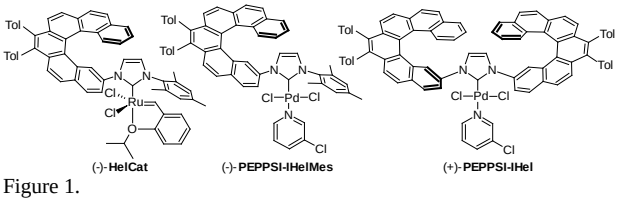


Figure 1.

This work was supported by the Czech Science Foundation (reg. No. 16-08294S), the Institute of Organic Chemistry and Biochemistry CAS (RVO: 61388963), the Faculty of Science, University Potsdam and the Deutscher Akademischer Austauschdienst (DAAD).

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CHARACTERIZATION OF BCA3 PROTEIN

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Human breast cancer-associated gene (*BCA3*) was discovered in mRNA screens of breast and prostate cancer cell lines[1,2]. In human genome, *BCA3* is localized on eleventh chromosome and encodes 210 amino acids. The fact that *BCA3* does not encode any specific structural/functional domain and has no homology to other proteins complicates the prediction of its cellular function. BCA3 has been identified to interact with functionally distinct proteins with different sub-cellular localization comprising: nucleus, cytosol and mitochondria. Over-expressed BCA3 interacts with proteins responsible for apoptosis, angiogenesis and lymphangiogenesis in some cancer cells. Increased BCA3 expression was detected in cardiomyocytes during ischemia, BCA3 helps to protect mitochondria of the cells exposed to hypoxia[2–4].

Among the proteins that has been identified to interact with or to be affected by BCA3 belongs e.g. catalytic subunit of cAMP-dependent protein kinase (PKAc)[1,3]. Based on this interaction, BCA3 protein is also known as A kinase interacting protein (AKIP1).

To understand the cellular role of BCA3 protein we first prepared its recombinant form and studied its physico-chemical properties. Using FoldIndex software©[5,6], I found out that BCA3 is predicted and classified as intrinsically disordered protein (IDP), eventually scaffold protein. I prepared several BCA3 vectors including these with N- and C-terminally located fusion histidine-tags. I expressed a purified BCA3 protein and using biochemical and physicochemical methods such as controlled proteolysis, circular dichroism (CD) or nuclear magnetic resonance (NMR) characterized its properties.

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ANALYSIS OF THE THROMBI MORPHOLOGY

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Cardiovascular diseases (CVDs) are the most common cause of morbidity and mortality in the current society. The presented project deals with strokes, which are among the most common CVDs. The stroke can be divided according to the principle of formation to hemorrhagic and ischemic stroke. The principle of ischemic stroke is the closure of an artery supplying blood and nutrients to the brain[1].

For appropriate therapy and prophylaxis, it is important to know the principle of the stroke formation. Therapy of patients with the stroke caused by thrombus embolization differs from therapy in the case when thrombus formed directly in the brain. If the origin of thrombus is not determined, there is a risk of misapplied therapy, and wrong prophylaxis may lead to a stroke recurrence[2]. To distinguish the principle of stroke formation, it may be helpful to focus on different composition of thrombi. It may be expected because of different mechanism and location of thrombus formation.

Our aim is to analyze the composition and morphology of thrombi to assort them into two groups based on the mode of thrombus formation. To better understand the morphology of thrombi, we focused on the shape and distribution of erythrocytes in the thrombus. We observed that erythrocytes within the thrombi prepared *in vitro* have polyhedral not biconcave shape. This shape is generated due to compression induced by thrombocytes and fibrin fibers. We observed similar trend in thrombi removed from patients. This suggests that contraction of thrombus occurs both *in vitro* and *in vivo*. From this point of view, it is possible to use *in vitro* thrombi for further thrombus analysis. At the same time, it indicates that contraction of thrombus occurs without the mechanical forces generated by blood flow and contraction of the vascular wall.

As a further step, we observed in the *in vitro* formed thrombi the appearance of a thin layer just below the thrombus surface containing intermediate erythrocytes (the intermediate step between biconcave and polyhedral erythrocyte). This layer also has a higher fibrin fiber content compared to the thrombus center. Whether this trend appears in the *ex vivo* thrombi, it is the subject of our further research.

A deeper understanding of the morphology and distribution of individual thrombus components will help us to better understand not only the thrombus composition and possibly also the principle of thrombus formation.

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FLUOROALKYLATED HYPERVALENT IODINE REAGENTS FOR THIOL BIOCONJUGATION

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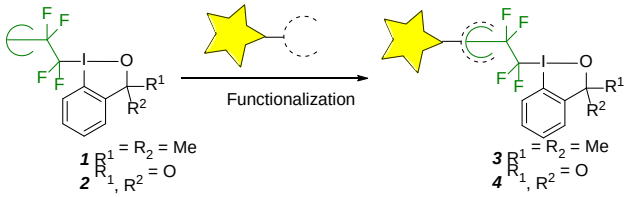
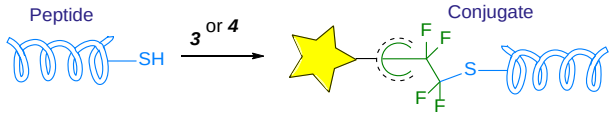
Fluoroalkylated hypervalent iodine reagents based on 1,3-dihydro-3,3-dimethyl-1,2-benziodoxole are used as sources of electrophilic trifluoromethyl[1] or tetrafluoroalkyl groups[2]. In previous work[1,2], exclusive reactivity toward thiols was reported. Other common functional groups (amino, hydroxyl, carboxylate) are unreactive; therefore, we considered studying these compounds as reagents for chemical modification of biological thiol.

The preparation of amino based modular reagents ***1*** and ***2*** allowed the attachment of various biologically relevant functional groups (Scheme 1)[3].



Scheme 1. **Modification of modular reagent *1* and *2***

Finally, the study of modification of natural thiols in water is in progress (Scheme 2). The influence of pH, amount of cosolvent and reaction time is investigated. The aim of the work is to optimize conditions for clean and mild thiol modification and reduction of formed side products.



Scheme 2. **Thiol modification by hypervalent iodine reagents**

We would like to thank Michal Korecký for his help with MS sample preparation. Financial support from the Academy of Sciences of the Czech Republic (RVO: 61388963) and Czech Science Foundation (17-00598S) is acknowledged.

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SYNTHESIS OF BISPYRIDOHELICENES

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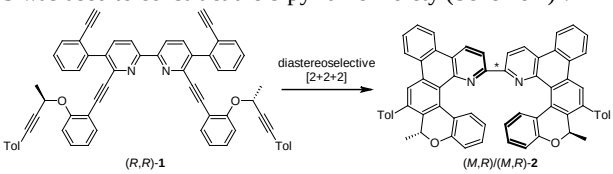
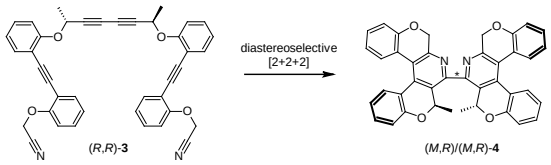
2,2'-Bipyridine ligands belong to the frequently used ligands in catalysis[1]. On the other hand, ligands with helical chirality are far less studied with regard to their application to enantioselective catalysis. We have decided to combine these two priviledged structures and focus on new bipyridines constructed from pyridohelicenes.

Here, we report on two classes of optically pure bispyridohelicenes **2** and **4** connected in positions 2,2 and 7,7, respectively. Their synthesis relies on a diastereoselective approach to nonracemic helicenes employing the 1,3-allylic-type strain control in the formation of the helical backbone from centrally chiral precursors[2]. In the case of bispyridohelicene **2**, a double cyclotrimerization utilizes hexayne **1** with an already incorporated bipyridine unit as the starting material (Scheme 1), whereas for the synthesis of bispyridohelicene **4** co-cyclotrimerization of dicyanotetrayne **3** was used to construct the bipyridine moiety (Scheme 2)[3].



Scheme 1.



Scheme 2.

This work was supported by Czech Science Foundation (Reg. No. 16-08294S) and IOCB CAS (RVO: 61388963).

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SYNTHESIS OF POTENTIAL INHIBITORS OF GOLGI α-MANNOSIDASE II

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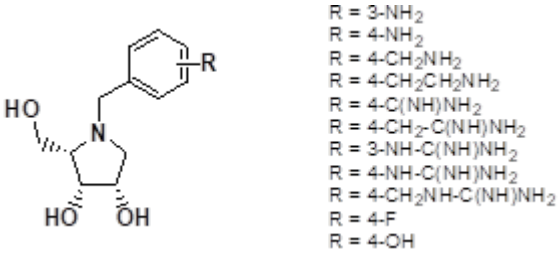
Inhibition of biosynthesis of complex N-glycans in Golgi apparatus influence progress of tumor growth and metastasis. Human Golgi α-mannosidase II (hGMII) has become a target in the development of anti-cancer therapies. All known potent hGMII inhibitors are not sufficiently hGMII-selective due to their undesired inhibition of catabolic lysosomal α-mannosidase (LMan) from the same family GH38.

In our previous research1, seven derivatives, N-substituted polyhydroxypyrrolidines, were synthesized and their inhibitory effect towards *D. melanogaster* homologues of the human GH38 enzymes (dGMIIb and dLMan) has been tested. The most potent structures inhibited GMIIb at the micromolar level [Ki(GMIIb) = 50-76 μM, enzyme assays] with a significant selectivity index of IC50(LMan)/IC50(GMIIb) > 100.

A series of novel 11 derivatives, designed by 3D-QSAR model2, were synthesized (Scheme 1). This contribution deals with their synthesis and inhibitory data towards various GH38 α-mannosidases (dGMIIb, dLMan and JBM).



Scheme 1. **Structure of potential inhibitors of GMII**

This work was supported by the Slovak Research and Development Agency (APVV-0484-12) and Scientific Grant Agency of ME of SR and SAS (VEGA-2/0064/15).

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A STUDY OF ENANTIOSELECTIVE ENYNE METATHESIS

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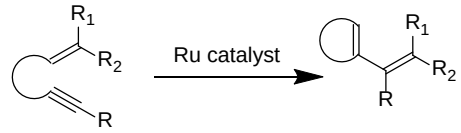
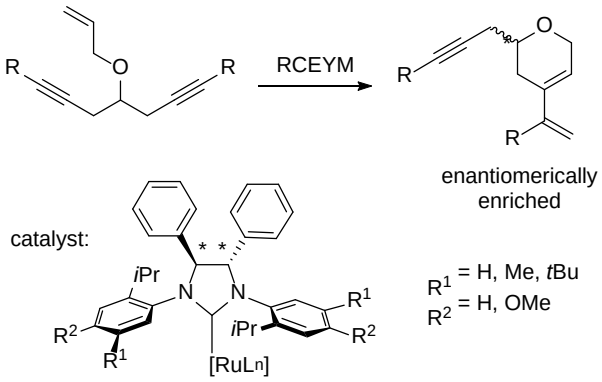
Intramolecular enyne metathesis (RCEYM) is a useful synthetic tool which has been used many times in the total synthesis of natural products or their analogues.[1] The RCEYM forms a 1,3-diene along with a new cycle, while using mild reaction conditions and commercially available ruthenium catalysts (Scheme 1).



Scheme 1. **Intramolecular enyne metathesis[1]**

Enantioselective enyne metathesis with ruthenium complexes has never been described. We have successfully synthesized a small series of prochiral substrates (endiynes and dienynes), which could be utilized in enantioselective RCEYM metathesis. With a group of chiral analogues of the well-known ruthenium-based Grubbs and Hoveyda-Grubbs catalysts, we now explore the enantioselective RCEYM metathesis of endiynes and dienynes, which could afford enantiomerically enriched products (Scheme 2).



Scheme 2. **Enantioselective RCEYM of endiynes**

Financial Support from Specific University Research (MSMT No.20-SVV/2018).

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GTP-DEPENDENT FORMATION OF MULTIMERIC G-QUADRUPLEXES

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Nucleic acid structures known as G-quadruplexes (GQs) are important regulatory elements likely involved in a number of cellular processes. Canonical GQ sequences contain segments of at least three guanosines separated by loops of one to seven nucleotides. Four guanosines assemble in a planar arrangement to form a G-tetrad, and tetrads stack on top of one another to give rise to a GQ. Despite the fact that canonical G-tetrads are formed by guanosines only, GQs have recently been reported to be tolerant to certain substitutions of guanosines in the tetrads. In a previous study, we tested all possible variants of the central tetrad in a monomeric GQ for the ability to form higher-order structures. Dimers were formed from sequences that contained NNGG mutations in the central tetrad, while tetramers contained GGNN mutations in the central tetrad[1].

In the present study we report the discovery of a sequence for which tetramer formation is regulated by GTP. This sequence contains a GGAG mutation in the central tetrad, and in the absence of GTP, three main structures are formed: monomers, dimers and tetramers. Titrating in GTP decreases the concentration of dimers and tetramers while increasing the concentration of monomers.

To investigate the influence of loop sequence on this process, we used site-directed mutagenesis. All possible loop variants with the 1 2 1 architecture were tested for GTP-dependent tetramer formation. Certain mutations in loops inhibited multimer formation, while others resulted in higher-order structures that were not sensitive to GTP. These experiments suggest that loop nucleotides contribute to higher-order structure formation, but further evidence from high-resolution structures is needed.

To investigate possible biological roles of GTP-dependent multimers, a search for sequences in the human genome (build hg19) with the potential to form such structures was performed. For each loop variant identified, the number of examples in GGAG GQs was compared to the number in GGGG GQs. Ten loop variants enriched in GGAG GQs were tested on native gels, and seven formed GTP-dependent multimers. These observations confirm the existence of GQs with the potential to form GTP-dependent multimers in the human genome, and we are currently searching for additional evidence that these sequences are biologically important.

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SELECTED TACRINE-COUMARINE HYBRID MOLECULES: SUBCELLULAR DISTRIBUTION AND BIOLOGICAL ACTIVITY

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Hybrid molecules are multifunctional compounds consisting of two or more pharmacophores/drugs, having specific pharmacological activities. Combination of the same or different types of pharmacophores, could be prepare molecule with combined effect and just these molecules are very requested in treatment of multifactorial diseases[1].

In this study, a series of novel coumarin-tacrine hybrids (**I–IV**) were biologically evaluated for their potential inhibitory effect on topoisomerase I enzyme. The derivatives were analysed against A549 adherent lung adenocarcinoma cells. The presence of the particles was determined through observations of their fluorescence in the green channel. According to our results, the presence of derivatives was detectable predominantly in sample **IV**. In other samples, the fluorescence of the derivatives was not distinguishable from the autofluorescence of the cancer cells. In cells, the derivatives were distributed in cytoplasm and displayed no signs of interference with the cell nuclei. Based on mitochondrial staining and overall distribution of the signal, we were unable to confirm the accumulation of derivatives in mitochondria or in other organelles or membranes. Flow cytometric analysis of derivatives content in A549 cells revealed the cumulative fluorescence of derivatives **I–IV** from the green (FL-1) to the red (FL-3) channel. Derivative **III** was found to display the highest level of fluorescence. Moreover, the total cell number was sharply decreased (more than 50%) in the case of cells treated with compounds **III** and **IV** but viability of the cells was only weaker reduced. The analysis of the cell cycle was determined that compounds **III** and **IV** significantly increased the accumulation of the cell in phase G1, while cells in S and G2 phase was proportional divided. The obtained results could be beneficial in the field of design and development of new cuomarine based agents.

This study was supported by Internal Grant Programme of University of P. J. Šafárik in Košice VVGS-PF-2018-754 and VEGA 1/0016/18.

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EXPLORING THE REACTIVITY OF α,β-UNSATURATED α-BENZOTHIAZOLSULFONYL CARBONYL COMPOUNDS

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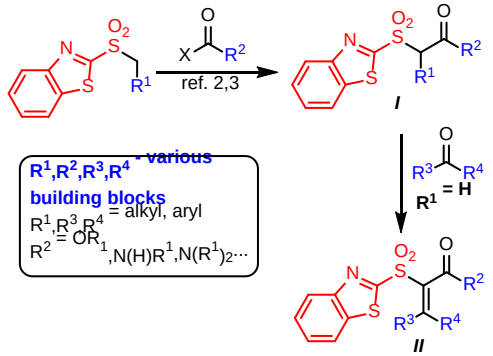
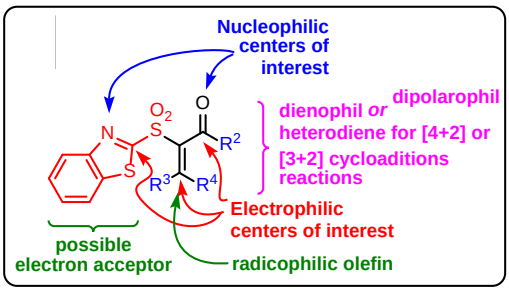
Over the past few years we have been interested in our group in the development and evaluation of various synthetic building blocks and novel method development. One of the goals of our synthetic efforts is to explore and develop the reactivity of polyfunctionalized building blocks in context of Diversity-Oriented Synthesis (DOS)[1]. Within this context the aim of my work is to design compounds having several reactive sites that can be further reacted independently.

Previously we have designed and evaluated the reactivity of β-carbonyl BT-sulfones (*I*)[2,3] (Scheme 1).



Scheme 1. **Our approach to compounds *I* and *II***

Currently my research goal is to explore the reactivity of the next generation of such type of building block – compound *II*. Such molecular framework offers us possibly even more scaffold variations (Scheme 2), and therefore to finely tune its reactivity is rather challenging task.



Scheme 2. **Possible reactive sites of *II***

The financial support by the Ministry of Education, Youth and Sports of the Czech Republic (grant LO1304 and LO1204) as well as by the Internal Grant Agency of Palacky University for O.K. (IGA_PrF_2017_009) is gratefully acknowledged.

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EPIGENETIC DRUG SCREEN OF RITUXIMAB RESISTANT CELL LINE REVEALED POSSIBLE TREATMENT TARGETS

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Standard of care for B-lymphoid malignancies nowadays still relies on the administration of monoclonal antibodies, with CD20 antigen being the prime target. Although effective at first, repeated cycles of anti-CD20 monoclonal antibody therapy often result in the loss of CD20 on the surface of malignant B cells and consequently in therapy resistance and therapy failure[1,2]. We mimicked the situation in patients through chronic exposure of B-lymphoid cell lines to gradually increasing doses of anti-CD20 antibody Rituximab. In this way, we have generated cell lines that are resistant to additional treatment with anti-CD20 antibodies. We could confirm that these resistant cells have downregulated CD20 protein from the cell surface.

Interestingly, Rituximab was suggested to induce epigenetic changes within the CD20 promotor and, consequently, inhibitors of DNA methyltransferases and histone deacetylases were proposed to increase CD20 expression in some lymphoma cell lines[3,4]. Therefore, we have performed a screen with a library consisting of 182 small-molecule compounds targeting various epigenetic modifying enzymes (histone deacetylases, methyltransferases, etc.). We aimed to uncover which epigenetic modifiers were able to enhance the expression levels of CD20 antigen and recover its presence on the cell surface. The most significant increase of CD20 surface density was detected with JAK kinase inhibitor LY2784544. Increase of CD20 surface level was also detected repeatedly using various Aurora kinase inhibitors e.g. GSK1070916, JNJ-7706621, AMG-900 and MK-5108, which could indicate the role of these kinases in CD20 regulation.

Further analysis of mechanisms regulating CD20 expression is needed in order to confirm the effect of detected

inhibitors and thereby to enhance the therapeutic potential of CD20 monoclonal antibodies.

This research has been financially supported by the Ministry of Education, Youth and Sports of CR under the project CEITEC 2020 (LQ1601), by the research grant AZV-MZ-CR 15-33561AA-4/2015 and grant MUNI/A/0968/2017.

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FROM AN OLD DRUG ISONIAZID TO ANTITUBERCULAR AGENTS WITH A NOVEL MECHANISM OF ACTION

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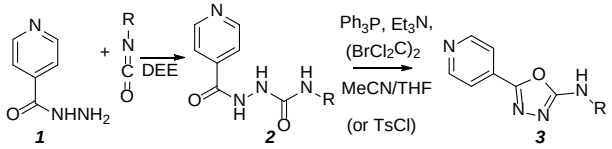
Novel drugs against *Mycobacterium tuberculosis* (*Mtb.*), the causative agent of tuberculosis (TB), are essential. Isoniazid (INH) remains a key anti-TB drug, but an increasing resistance stimulated the development of its derivatives to overcome it[1].

Inspired by anti-TB activity of several oxadiazoles, we proposed efficient two-step synthetic pathway starting from INH ***1*** and leading to 1,3,4-oxadiazoles ***3*** (Scheme 1)[1].



Scheme 1. **Synthesis of isoniazid analogues *2* and *3***

New compounds ***2-3*** are non-toxic for eukaryotic cells (HepG2, MonoMac-6). We evaluated their activity against various myco-bacterial strains (Table 1) and examined mechanism of action. Both hydrazides ***2*** and 1,3,4-oxadiazoles ***3*** showed an improved activity against drug-resistant and nontuberculous mycobacteria (*M. avium*, *M. kansasii*). Moreover, oxadiazoles ***3*** exhibited a uniform activity against INH- and multidrug-resistant *Mtb*. In contrast to ***2***, oxadiazoles ***3*** do not inhibit enoyl-acyl carrier protein reductase (InhA), a target of INH ***1*** responsible for biosynthesis of mycolic acids and cell wall. Thus, they inhibit *Mtb.* selectively *via* an unknown and INH-unrelated mechanism of action that remains to be elucidated[1].

Table 1. Overview of biological activity (MIC in [μM])

Strain/Compounds	**INH *1***	***2***	***3***
Mtb. H37Rv (drug-susceptible)	1	≥1	≥4
Drug-resistant *Mtb.* strains	>250	≥16	≥4
Nontuberculous mycobacteria	>250	≥4	≥8

Surprisingly, this simple chemical modification of an old drug leads to the derivatives with favourable anti-TB activity and by-passing resistance to INH[1]. Structure-activity relationship study of the most active oxadiazole ***3*** (R = dodecyl) is in progress.

This work was supported by GACR project No. 17-27514Y.

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SYNTHESIS AND THEORETICAL STUDY OF FLUOROCYCLOBUTENES AND THEIR RING-OPENING METATHESIS

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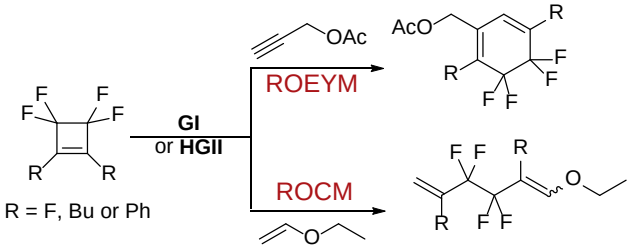
Fluorinated compounds exhibit specific physical, chemical and biological properties. Therefore, they found numerous applications in medicinal, pharmaceutical and polymer industry[1]. One useful tool in synthesis of fluorinated blocks could be ring-opening metathesis (ROM) of cycloalkenes combined with ring-closing, enyne or cross metathesis[2].



Scheme 1. **ROM of fluorocyclobutenes (ROEYM = ring-opening enyne metathesis; ROCM = ring-opening cross metathesis)**

Our study focuses on synthesis of fluorinated cyclobutenes and their utilization in ROM thus introducing CF_2-CF_2 moiety in the product structure (Scheme 1).

The fluorinated cyclobutenes were prepared by reaction of hexafluorocyclobutene with nucleophilic reagents such as RLi or RMgBr. We have also carried out a theoretical study of ^{19}F NMR spectra of fluorocyclobutenes. Surprisingly, the CCSD-DLPNO method gave better correlation with experimental data than DFT methods (Fig. 1).

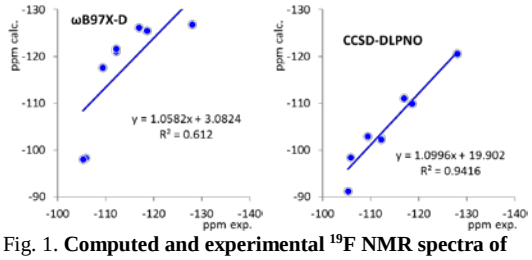


Fig. 1. **Computed and experimental ^{19}F NMR spectra of fluorocyclobutenes (R = H)**

This work was supported by specific university research (MSMT No 20-SVV/2017).

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NMDA RECEPTOR CHANNEL GATING: THE LILI MOTIF OF M3-S2 LINKERS AS A COMPONENT OF THE GATE

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N-Methyl-D-aspartate receptors (NMDARs) are heterotetramers containing two obligatory glycine-binding (GluN1) and two glutamate/glycine-binding (GluN2/3) subunits. These receptors mediate excitatory synaptic transmission in the CNS and it has been shown that dysregulation of NMDARs is involved in the pathophysiology of neurological and psychiatric disorders. Channel opening is the key step in the NMDAR gating that allows the flux of ions across the membrane. Several lines of evidence indicate that the rearrangement of M3 helices in activated receptor makes the central cavity of the channel accessible therefore implying a crucial role of the M3-S2 linkers in channel opening. To answer the fundamental question, what are the initial steps in NMDAR channel opening, we embarked on functional, molecular biology and molecular dynamics studies of GluN1/GluN2B receptors and focused on the M3 and initial segments of the M3-S2 linkers.

The results show that deletion mutations and glycine substitution mutations in the M3-S2 linker of GluN1 and GluN2B subunits profoundly affect the NMDAR channel function: *i.* NMDARs with mutated linkers open spontaneously and as a consequence of receptor activation by a single agonist; *ii.* the effect of deletions is stratified – spontaneous activity and single-ligand induced responses are more pronounced for deletions closer to the M3 helix; *iii.* the degree of spontaneous activity and single-agonist responses, as well as the length of the linker region affected by deletion, differ for GluN1 and GluN2B subunits; *iv.* irrespective of whether deletions have been introduced in GluN1 or GluN2B subunits, application of glutamate or glycine promoted receptor channel activity; *v.* irrespective of whether deletions have been introduced to the M3-S2 linker of GluN1 or GluN2B, responses induced by glycine were (on average) larger than those induced by glutamate. Combining functional data with those of results of computational biology we show that the extracellular channel gate is formed by GluN1(L657) and GluN2B(I655) (LILI motif).

Our data provide new insight into the mechanism of NMDAR ion channel gating and describes LILI motif crucial for the transduction of the energetics of agonist binding to the ligand binding domain to pore opening.

IPLA2 γ ABLATION ALTERS GLUCOSE HOMEOSTASIS AND INSULIN SECRETION IN RESPONSE TO FATTY ACIDS

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Calcium-independent phospholipases (iPLA2s) are a family of enzymes participating in cellular signaling by simultaneously producing free fatty acids and lysophospholipids. Phospholipase iPLA2γ (PNPLA8) is targeted to mitochondria and has been shown to augment GPR40-dependent insulin secretion in β-cell model insulinoma INS1-E cells[1].

Here, we investigated the participation of iPLA2γ on the blood glucose homeostasis and pancreatic β-cell insulin secretion using iPLA2γ-KO mice. Wild type (wt) controls and iPLA2γ-KO mice showed similar cholesterol, triglyceride and basal glucose levels after 6 hours of starving. However, the uric acid levels were increased in iPLA2γ-KO mice. Following the intraperitoneal injection of Intralipid, iPLA2γ-KO mice exhibited a prolonged hyperglycemic state compared to wt mice and an opposite effect given by palmitic acid (Figure 1). Glucose-stimulated insulin release in isolated pancreatic islets

(PI) was moderately decreased in iPLA2γ-KO PI. Physiologically relevant concentrations of palmitic acid stimulated insulin secretion in PI from wt mice, and this stimulation was absent in iPLA2γ-KO PI. In conclusion, the data are consistent with iPLA2γ ablation causing impairment of GPR40-dependent insulin secretion, resulting in prolonged glycemia. Thus, our results support the role of iPLA2γ in regulating insulin secretion *in vivo*.

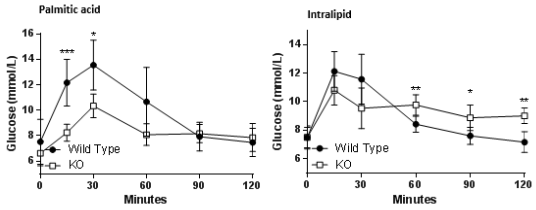

Fig. 1. **Glucose response to palmitic acid or Intralipid at equivalent dose (0.08 mg/g = 1000 µM/animal or Intralipid 1 mg/g). Data expressed as average ± SD, n = 12 per group, * p < 0.05, ** p < 0.01 two-way ANOVA, Sidak's multiple comparison**

This study was supported by the grant GA15-02051S to Martin Jaburek

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INTERVENTION OF STILBENOIDS INTO THE PROCESS OF PERIPHERAL AND NEURONAL INFLAMMATION

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Inflammation is nowadays often associated with so-called civilization diseases, including also some neurogical disorders like dementia, depression or cerebral insults such as stroke that is a major cause of mortality and morbidity worldwide. Monocytes on the periphery and microglia in CNS provide a first line of immunity system defence during the inflammatory process. Seeking after new and safe anti-inflammatory drugs is very actual these days.

The antiphlogistic potential of stilbenoids, natural molecules occurring in food (e.g. peanuts, passion fruit, different types of berries), beverages (white and green tea) and medicinal plants have been since long ago largely investigated[1]. We performed the screening of 38 prenylated and non-prenylated stilbenoids for their anti-inflammatory potential observing different inflammatory markers and cytokines. Lipopolysaccharide stimulated THP1-XBlue™-MD2-CD14 cell line served us as a model of peripheral inflammation. The most of compounds have shown the ability to attenuate the activation of transcriptional factors NF-κB and AP-1, non-prenylated stilbenoids, e.g. piceatannol, pinostilbene, thunalbene *via* inhibition of MAP kinase ERK1/2, p38 and JNK, whereas their prenylated analogues macasiamenene F and AA-CH/F1, newly isolated stilbenoid from *Artocarpus altilis* (Parkinson) Fosberg (Moraceae) *via* inhibition of IκBα degradation. The candidate with promising anti-inflammatory potential, macasiamenene F, isolated from Thailand´s plant *Macaranga siamensis* S.J.Davies (Euphorbiaceae) was selected for more detailed analysis of its anti-inflammatory action in CNS. On mouse microglia BV-2, preliminary assays have shown a beneficial effect of macasiamenene F on cell survival under normal (culture medium) and LPS (1µg/mL)-stimulated conditions. On mouse cortical neurons in culture, evaluation of the influence on production of pro-inflammatory cytokines IL-1ß and TNF-α and neuroprotective effect against oxygen-glucose deprivation (OGD) mediated cell injury are the main goals of our investigation.

This project is supported by Czech science foundation, grant No° 16-07193S.The studies of graduant at University of Nice Sophia Antipolis are financed from French Government Grant provided by Campus France and Embassy of France in Prague, Czech Republic.

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FAST GROWING MARINE BACTERIUM AS A POTENTIAL NEW HOST ORGANISM FOR THE PRODUCTION OF BIOPHARMACEUTICALS

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Current efforts in optimization of production of industrially or pharmaceutically applicable proteins, produced by *Escherichia coli*, rely on standard approaches – media composition, cultivation parameters or different fermentation modes, all with the aim of increasing the yield of target product per unit of biomass with as low as possible input material. With the rise in the field of the synthetic biology, new strain engineering approaches resulted in many advances increasing effectiveness of biopharmaceutical production[1].

Although *E. coli* remains one of the most commonly used production organisms, moving even towards ability to N-glycosylate recombinant proteins[2], a new species of fast growing bacterium – *Vibrio natriegens*, threatens to overtake

the primacy. The obvious advantage is roughly 2.5 times shorter doubling time compared to *E. coli* and the ability of most of the genetic tools available for *E. coli* to be utilized[3]. With much work left to be done in terms of strain development, engineering patterns, vector design and overall growth optimization, the aim of our work is to discover the ability of *V. natriegens* to reach high density cell cultures during various cultivation and fermentation modes.

Our results suggest, that compared to *E. coli*, *V. natriegens* shows superior growth curves during shake-flask as well as batch and fed batch bioreactor controlled cultivations reaching, on average, 1.9 times higher biomass amounts compared to *E. coli*. The ability of this bacterium utilize sucrose and starch even further enhances its potential as a new host for advanced biotech production.

This publication is supported by grants APVV-0061-11 and APVV-15-0466 and is also the result of projects implementation: "Production of biologically active agents based on recombinant proteins" (ITMS 26240220048) and Comenius University Science Park (Bratislava, Slovakia) – 2nd phase (ITMS 26240220086) supported by the Research and Innovation Operational Programme funded by ERDF

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SYNTHESIS OF π-FUNCTIONAL ELECTROACTIVE ARRAYS BASED ON OLIGOPROLINE SCAFFOLDS

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It is known[1] that oligoprolines are able to adopt well-defined three-dimensional (3D) helical structures that resemble the helix of RNA molecules. Owing to a backbone comprised exclusively of tertiary amides, the helical secondary structure of polyprolines is stabilised by only n–π^* [N: ··· C*] interactions between adjacent amide bonds. Consequently, the distance – i.e., the helical pitch – between every third proline residue along the C_3-symmetric backbone can be modulated through a global and solvent-dependent *cis–trans* bond isomerisation (Figure 1a).

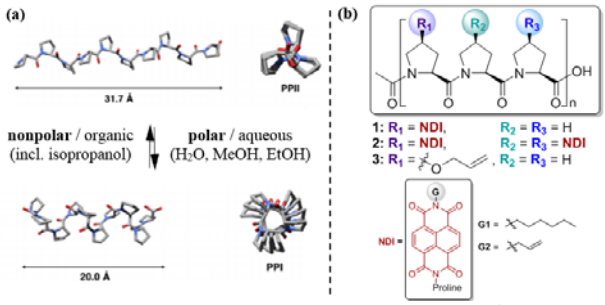

Fig. 1. (a) **Solvent-dependent extension/contraction of oligoprolines**, (b) **NDI-substituted oligoprolines**

Thanks to these properties, oligoprolines can be used conveniently as rigid scaffolds for the 1D organisation of functional groups and self-assembly of supramolecules. The goal of this work is to prepare functional organic π-materials supported by a rigid oligoproline backbone. Tripeptide macromonomers bearing either redox-active naphthalene diimides (NDIs) **1**, **2** or an allyloxy chain **3** (Figure 1b) were prepared and coupled to form oligoprolines between 3 – 15 residues using solid-phase method. Their conformational properties and electrochemical addressability were evaluated as a function of solvent and helical pitch. NDIs organised strictly in 1D dimension provides us an opportunity to observe desirable electronic properties, such as the 1D change-hopping of electron through space, which can be potentially useful within organic electronic device applications. Using ring-closing metathesis, oligoprolines bearing allyl groups (e.g., **3** or **1/2-G2**) can be made more rigid and structurally more robust for device applications.

This work is made possible by an internal UCT Prague "VIGA" research grant (MSMT No 20/2017), Erasmus+ and Royal Society Research Grant (RG160544).

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DIELS-ALDER APPROACH TO SUBSTITUTED DECALINES. SYNTHESIS OF FORSKOLIN ANALOGS

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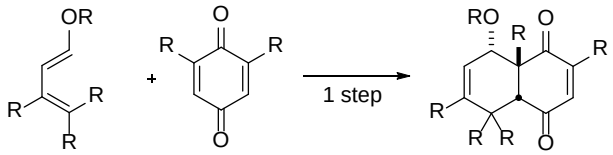
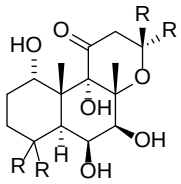
Decalines are basic structural motifs presented in plethora of natural products namely terpenes and steroids[1]. Herein we report Diels-Alder strategy[2] to construct *cis*-decaline scaffolds **1** bearing various substitutions on both ring

A and B (Scheme 1). Preparation of dienes and non-trivially substituted quinones will be mentioned together with scopes and limits of Diels-Alder reaction.



1

Scheme 1. **Synthesis of *cis*-decalines**



2

Heretofore unknown decalines may serve as valuable intermediates for synthesis of natural product analogs for further chemical biology research. Diels-Alder based strategy application in the synthesis of forskolin analogs **2** will be highlighted[3].

This project was funded from EU under Marie Curie International Reintegration Grant 230936 (FP7-PEOPLE-IRG-2008), the European Regional Development Fund under grant FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123, the project Human Bridge for Strengthening Integration of ICRC into European Research Area (ICRC-ERA-HumanBridge, GA 316 345), and the project CZ-OPENSCREEN: National Infrastructure for Chemical Biology (LM2015063).

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CONTROLLED C-C BOND CLEAVAGE OF KETONE ENOLATES AIDED BY NITROSATION

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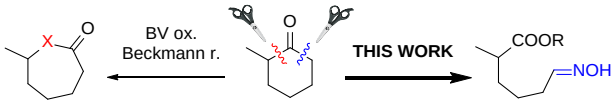
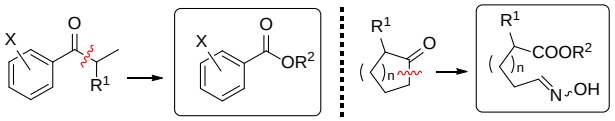
The Beckmann rearrangement and Baeyer-Villiger (BV) oxidation represent traditional ways of cleaving the rather strong C-C bond in ketones. Although reliable and usually quite regioselective, both methods offer little control over the site of cleavage, which is mainly dictated by the nature of the substrate, i.e. configuration of oxime in the Beckmann rearrangement or relative group migratory aptitudes in BV oxidation. Since oxidative cleavage of ketones is a useful synthetic maneuver[1], ways of deliberately controlling the site of cleavage are very desirable. Especially the possibility to direct the cleavage to the side of the less substituted carbon would fill the gap in existing methodology.



Scheme 1. **Cleavage of asymmetrically substituted ketones**

This is addressed by utilizing the known high regioselectivity of kinetic enolate generation by strong amide bases[2]. We found that treatment of ketone enolates with alkyl nitrites at low temperature results in facile C-C bond cleavage producing two new carbon termini with different oxidation states, namely ester and aldoxime. Aldoximes are versatile synthetic intermediates that can be transformed in one step to primary amines, aldehydes, nitriles or nitrile oxides.

Application of this method to a wide range of acyclic and cyclic ketones will be disclosed as well as the possibility to steer the reaction towards formation of alternative *N*-termini like nitriles or oxime ethers. The direct use of the oxime ester functional group couple for follow-up C-C bond forming reactions will be discussed.



Scheme 2. **Cleavage of aromatic and aliphatic ketones**

This work was supported by GAČR (reg. No. 13-40188S).

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NOVEL ANTICANCER (1,10-PHENANTHROLINE)$_2$Cu(II) COMPLEXES ARE INDUCING ER STRESS MEDIATED APOPTOSIS IN OVARIAN CANCER CELLS

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Endoplasmic reticulum (ER) is major cellular organelle primarily responsible for protein synthesis, posttranslational modifications, membrane biosynthesis and calcium ions management. The state of ER stress can be induced by various homeostasis disturbing factors and plays role in a number of civilization diseases including cancer.

Cellular response to ER stress is known as Unfolded Protein Response (UPR). UPR consists of several pathways activated in the case of accumulation of misfolded proteins in the lumen of ER. The final cell response can lead to adaptation or apoptosis and depends on a specific activity of individual pathways.

Novel (1,10-phenanthroline)$_2$Cu(II) complexes were recently synthesized by Tiziana Pivetta et al.[1] These compounds are promising anti-cancer therapeutics, however, the precise molecular mechanism of action is unclear. In this work we studied their effects on ovarian cancer cells.

We show, that these complexes affect the UPR signalling pathways on both protein and mRNA level and influence the cell morphology and cellular ultrastructure. Importantly, we demonstrated that different chemical modulators of UPR could have either synergistic or antagonistic effects on cytotoxic properties with (1,10-phenanthroline)$_2$Cu(II) complexes.

In summary, we characterized cytotoxic effects of new class of anti-cancer candidates and revealed cell structures and molecular pathways involved.

Supported by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by Masaryk University (MUNI/A/1369/2016). We acknowledge the core facility Cellular Imaging (CELLIM), institution CEITEC MU supported by the Czech-BioImaging large RI project (LM2015062 funded by MEYS CR) for their support with obtaining scientific data presented in this paper. We thank prof. J.H. Prehn from Royal College of Surgeons in Ireland for providing fluorescent reporters.

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OCULAR ABNORMALITIES IN THREE FAMILIES WITH *PAX6* MUTATIONS

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Anterior segment dysgenesis (ASD) disorders represent genetically and phenotypically a heterogeneous group of developmental conditions affecting the cornea, iris, and lens. Affected individuals may experience severe visual impairment or even total blindness. These kinds of abnormalities are usually caused by defects in neural crest migration and differentiation during embryologic development. Patients with particular types of ASD have high frequency of mutations in *PAX6* gene, encoding a transcriptional regulator involved in oculogenesis and other developmental processes[1].

We have performed direct sequencing of *PAX6* (NM_000280) in two families with aniridia and whole-exome sequencing in another individual with aniridia. *In silico* analysis was used to predict the effect of identified rare variants; missense mutations were evaluated by PolyPhen2, MutPred, Mutation Taster2, SIFT and SNPs&GO, splicing variants by Human Splicing Finder, NNSPLICE, NetGene2. Pathogenicity of splicing variants was further experimentally confirmed by exon-trapping assay using pET01 vector[2]. Direct sequencing was also used for segregation analysis within the families.

In one proband, we found in a heterozygous state c.622C>T; p.(Arg208Trp) previously reported as disease causing[3]. In two other probands, two intronic variants located in canonical splice sites were identified; c.1183+1G>T and c.1032+1G>A. Both variants were evaluated by prediction tools as pathogenic for loss of splice site and subjected to the exon-trapping assay. Mutations segregated with disease in six other affected family members and were not present in two unaffected first-degree relatives.

Loss of visual functions in ASD disorders is often very severe, therefore establishing molecular diagnosis highly impacts patient management, enabling prenatal and preimplantation diagnostics.

This work was supported by SVV 260367/2017.

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THE ANTIMICROBIAL ACTIVITY OF 1,4-NAPHTHOQUINONES AND THEIR MECHANISM OF ACTION

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The problem of microorganisms resistance to chemical treatment is well known. At this time, more and more infections are not treatable by acquaintanced antimicrobial agents. That is why is more than important to concentrate on research of new compounds which could be crucial solution. Several studies pursue antimicrobial effect of naphthoquinones, which seem to be promising in antibacterial and antifungal activity[1-3]. In our study we focus on 1,4-naphthochinones, its antimicrobial effect and mechanism of action elucidated by analysing proteins of microorganisms which were under its effect.

For the antimicrobial activity testing were chosen 1,4-naphthoquinones juglone, 1,4-naphthoquinone, naphthazarin and also 1,4-naphthoquinones isolated from *Onosma visianii* Clem. (*Boraginaceae*). All compounds were tested against *Escherichia coli, Staphyloccocus aureus*, *Candida albicans* and *C. krusei* using broth microdilution method. For the proteomic studies, microbiall suspension harvested 24/48 hours after treatment were used. Total protein from each sample was purified using the phenol-chloroform isolation, digested by trypsin and was analysed with HPLC-MS/MS.

From the results of MICs is clear, that compounds from the *Onosma* plant are more effective against Gram-positive bacteria, in our case *S. aureus*, than against Gram-negative bacteria (*E. coli*) and fungi (*C. albicans* and *C. krusei*). Two of these compounds were than chosen and tested against MRSA. Against *C. albicans* two isolated compounds showed similar antifungal activity as commercial naphthoquinones naphthazarin, 1,4-naphthoquinone and juglone while juglone and naphthazarin were more effective against *E. coli* and *Candidas* than ampicilin and amphotericin B. First results from the analysing of bacterial proteome shows, that juglone up-regulated proteins of phosphotransferase system, fructose and mannose metabolism, proteins involved in oxidative stress-response and catalyses activity while proteins of pyruvate metabolism and gluconeogenesis were down-regulated.

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CARBOSILANE GLUCOSE GLYCODENDRIMERS: SYNTHESIS AND TOXICOLOGICAL ANALYSES

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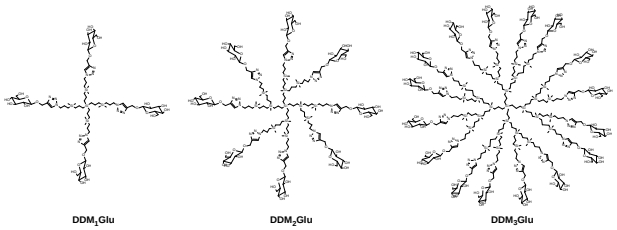
Dendrimers (DDMs) as a class of symmetric nanoparticles are studied for their promising applications in biomedicine[1,2]. Cationic DDMs, mostly investigated in gene delivery due to their ability to form complexes with negatively charged nucleic acids, also exhibit relatively high toxicity[3]. Glycodendrimers (glyco-DDMs) with carbohydrate peripheral moieties proved to be a suitable alternative to positively charged DDMs[2]. Still, the toxicity issues are widely discussed.

Here we present the synthesis, analytical characterization and, to our best knowledge, also the first *in vivo* toxicological data (modified FET, Zebrafish embryos) for novel 1-3rd generation glucose glyco-DDMs (DDM_1Glu, DDM_2Glu, DDM_3Glu) and their comparison with the traditional *in vitro* cytotoxicity assays (MTT, 3 types of rodent cell lines). Overall, the modified FET revealed two to three orders of magnitude difference between the *in vivo* and *in vitro* toxicity of the tested glyco-DDMs.

While, in general, the glyco-DDMs are of great promise as efficient vectors in drug/gene delivery, their developmental toxicity should be further investigated.



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SALTING-OUT ASSISTED LIQUID LIQUID EXTRACTION AS A SUITABLE APPROACH FOR DETERMINATION OF NEW SYNTHETIC DRUGS IN NEONATAL MECONIUM

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In recent years there has been a dramatic increase in the availability and abuse of new synthetic drugs (NSD). These compounds, also called "designer drugs" or "legal highs" are obtained by modifying structures of traditional drugs while keeping or even enhancing their properties. Their use is particularly popular among young adults, including women in childbearing age.

The abuse of drugs during pregnancy can have a negative effect on the child development, but no method of NSD detection in neonates has been published and these substances are still excluded from standard toxicological screenings. Meconium (first neonatal stool) is a promising material because it indicates intrauterine drug exposure from a longer period of time than blood or urine. As it is complex matrix, however, sample preparation is a critical step before the instrumental analysis itself.

The aim of this study was to determine a proper sample extraction technique for meconium samples using principles of salting-out assisted liquid-liquid extraction (SALLE). The optimization included selection of acetonitrile as a suitable solvent and combination NaCl and Na_2SO_4 as salting-out agents. We also developed an LC-MS/MS method for determining selected NSD, such as mephedrone and MDPV, in the extracts. Reversed phase LC analysis was performed on an Agilent EclipsePlus C18 column and MS method was carried out in a positive ESI mode using MRM (multiple reaction monitoring) with two transitions for each analyte. Negative samples were futher tested for the presence of traditional drugs, such as amphetamines.

The presented method allowed us to significantly reduce volumes of toxic solvents in comparison to other extraction techniques, such as conventional liquid-liquid extraction (LLE), and is less time-consuming than solid-phase extraction (SPE). Furthermore, analysis time less than 15 minutes per sample makes this method suitable for determination of both NSDs and other drugs of abuse in neonatal meconium.

FUNCTIONAL ELECTRON TRANSPORT CHAIN IS NECESSARY FOR STRESS RESISTANCE IN QUIESCENT CELLS

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Mitochondria are organelles central to energy metabolism as well as to cell death. Surprisingly little is known about mitochondrial function in quiescent cells, despite the fact that cellular metabolism is substantially remodeled on entry into quiescence. In this work we studied the role of functional electron transfer chain (ETC) in the cell's adaptation to the quiescent state. As the experimental model, we used endothelial-like cell line EA.hy926 depleted in mtDNA (ρ0 cells) or knocked down for the essential mtDNA-specific transcription factor TFAM. Both these models are deficient in ETC and cannot support mitochondrial respiration, but undergo normal contact inhibition. Preliminary results demonstrated a paradoxical increase in glucose consumption and lactate production in ETC-deficient quiescent cells compared to their proliferative counterparts. Moreover, unlike control cells, quiescent cells lacking the functional ETC were highly susceptible to reactive oxygen species (ROS) inducers such as isothicyanate (PEITC). This is surprising, as the ETC-deficient quiescent cells, similar to quiescent cells with functional ETC, showed elevated activity of the antioxidant defense (SOD2 and GPx1). Interestingly, we observed significantly reduced autophagic flux in quiescent ρ^0 cells. We therefore used autophagy inhibitors and knocked down ATG5, an essential component of the autophagic machinery, reducing autophagic flux in ETC-functional quiescent cells to the level observed in ETC-deficient cells. This manipulation resulted in increased ROS-induced cell death in quiescent cells, recapitulating the ETC-deficient phenotype. Our results suggest that quiescent ETC-deficient cells are metabolically stressed, leading to compromised autophagic flux and reduced protection from ROS. Insufficiency of stress-response pathways like autophagy is therefore a major consequence of ETC dysfunction in quiescent cells. Studies are ongoing to further elucidate this phenomenon.

PROTEOMIC PROFILE OF HEK-293 CELLS OVEREXPRESSING MAGNESIUM TRANSPORTER SLC41 A1.

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Magnesium (Mg^{2+}) plays an essential role in diverse spectrum of biochemical reactions and its deficiency has detrimental effects on cellular physiology[1]. Intracellular Mg^{2+} homeostasis is sustained by Mg^{2+} transport systems including transmembrane Na^+/Mg^{2+} exchanger A1 from solute carrier family SLC41 that is currently the only known ubiquitously expressed system responsible for Mg^{2+} efflux in cells[2]. Overexpression of SLC41A1 leads to increased cellular Mg^{2+} efflux capacity as was previously demonstrated in HEK-293 cells[2]. Recently we have reported that A1 overexpression alters pro-survival cell signalling and leads to complex but yet still unclear structural changes in cells[3]. Currently nothing more is known about the impact of Al overexpression on the overall physiology of the cells.

The main focus of our study was on proteomic profiling of A1 overexpressing HEK-293 cells in time dependent manner to identify proteins with significantly deregulated levels and subsequent mapping of their interactions to sort out the most affected metabolic pathways. Using 2D-gel electrophoresis and further Maldi-TOF/TOF mass spetrometry approach we identified in total 45 significantly deregulated proteins. The most abundant were proteins of cellular and metabolic processes with binding and catalytic activity. We found that most afftected were processes involved in cellular stress response and detoxification of reactive oxygen species. Several proteins were found to have a reference to neurodegenerative disorders.

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DECIPHERING THE MYSTERY OF TERMINATING RIBOSOME

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Recent research has shown that eukaryotic translational initiation factor eIF3 (eukaryotic initiation factor 3), besides its well characterised role in translational initiation, is also involved in translational termination and programmed stop codon readthrough[1]. High percentage of geneticaly inhereted diseases is caused by non-sense mutations causing the development of shortened, non functional proteins[2], hence it is important to gain insight at the proteins involved in the process. However, which of the small ribosomal proteins play a physiologically important role in anchoring the eIF3 to the ribosomal surface and perhaps even modulate its function remains to be explored. We have subjected several ribosomal proteins that are known to interact with eIF3 to genetic and biochemical screening and some mutants have shown interesting phenotype. Our most promising candidate is protein RPS3 located near the mRNA entry channel and interacting with the TIF35[3]and TIF32[4] eIF3 subunits. RPS3 and it´s 2 mutants K108E and R116D have shown opposite phenotypes in a dual lucipherase assay testing stop codon read-through. The *in vivo* and *in vitro* protein-protein binding experiments were carried out with this mutants and possible models of this delicate protein interplay occuring at terminating ribosome will be discussed

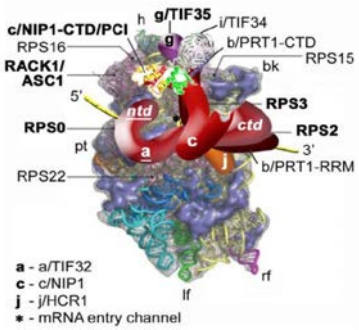

Fig. 1. **40S ribosomal subunit with bound eIF3**

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ROLE OF CRIPTO-1 SIGNALING IN CENTROSOMAL CYCLE REGULATION IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESC) are pluripotent cells derived from five- or six-day-old blastocyst-stage embryos. We have reported previously that hESC cultured *in vitro* develop centrosomal amplifications that lead to formation of aberrant mitoses. We have also unraveled that this phenomenon of multicentrosomal mitoses vanishes with prolonged time in culture and with initiation of differentiation, and it is strongly affected by the culture substratum. The molecular mechanism driving the centrosomal instability, however, remains unknown. Curiously, the supernumerary centrosomes can be induced by media collected from cultures of early passage hESC. Recent studies have shown that Cripto-1 (CR-1) has multiple binding partners and can modulate a variety of intracellular signaling pathways including those converging at components of mitotic apparatus. Here we have accomplished a series of experiments aimed at unravelling of molecular mechanisms underlying these phenomena. To investigate the link between Cripto-1 and occurrence of supernumerary centrosomes, we have increased and decreased Cripto-1 signaling by recombinant Cripto-1 protein and by anti-Cripto-1 blocking antibody. In concordance with our hypothesis, increased Cripto-1 signaling resulted in dramatically increased percentage of multicentrosomal mitoses

Better understanding of precise molecular mechanisms of centrosomal metabolism may help to develop better strategies for propagation of stable and safe bioindustrial and clinical grade cultures of hESC.

This study was supported by funds from the Czech Science Foundation (15-11707S), from the Faculty of Medicine of Masaryk University (MUNI/A/1369/2016), and from the National Program of Sustainability II (project no. LQ1605, MEYS CR).

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LIQUID CRYSTALS AND MAGNETIC NANOPARTICLES WITH MODIFIED SURFACE

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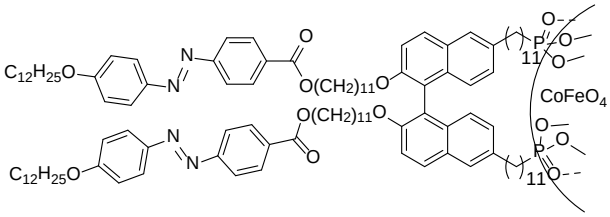
Liquid crystalline (LC) materials form mesophases between solid and liquid states. In mesophase their supramolecular structure can be tuned using mechanic or electric stimuli. In the case of magnetic field – even strong fields show no significant influence on LCs properties. Therefore, mixtures of magnetic nanoparticles (MNPs) and LCs are at high interest nowadays[1,2].

In this study we present mixtures prepared from LCs and MNPs modified with multifunctional ligands (Scheme 1), which serve not only as fixators of NPs in LCs structure, but also as substitute of chiral additives and enable target mixtures modulation with UV-light.



Scheme 1. **Ligands structure**

Ligands design, synthesis, optical purity determination and photoisomerisation process parameters will be presented. NPs surface modification and characterisation will be presented as well. Target mixtures preparation, host-guest structure relationship and modified NPs additives effect on hosts physical properties will be also discussed.

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TESTING OF NANOFIBROUS POLYMERS AS ALTERNATIVE TO RESTRICTED ACCESS MATERIALS FOR ON-LINE EXTRACTION OF BIOLOGICAL SAMPLES IN COLUMN SWITCHING CHROMATOGRAPHY SYSTEMS

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Restricted access materials (RAM) are a type of extraction sorbents. They are used primarily in biological sample preparation because they allow to remove proteins from low molecular weight compounds in one extraction step. The RAM are characterized by two types of surfaces – outer for interaction with macromolecular proteins and inner for extraction of low-molecular analytes. Hydrophilic groups on outer surface avoid an access of macromolecules into the solid phase. The inner surface is created with porous structure where analytes are retained. The use of RAM leads to automation of analytical process due to direct injection of proteinaceous sample and on-line connection of extraction step with flow separation method.

Many research groups are looking for new restricted access materials, but nobody has tried nanofibers although they seem to have good assumptions for this use. Therefore, we have tested two different nanofibrous polymers as a "restricted access material". We have chosen the nanofibers with optimal mechanical and chemical properties for this project - polycaprolactone (PCL) and polycaprolactone combined with polyvinylidene difluoride (PVDF). An extraction efficiency and proteins removal were tested on the three groups of analytes (parabens, anti-inflammatory drugs and insecticides) in milk and human serum matrices. A column cartridge 25×4.6 mm was filled with nanofibers and placed to a column switching chromatography system where the extraction and separation were carried out. Recoveries, matrix and standard calibrations, long term repeatability and life-time of extraction columns were evaluated.

OVERPRODUCTION AND CHARACTERIZATION OF THE FIRST ENZYME OF A NEW ALDOXIME DEHYDRATASE FAMILY IN *BRADYRHIZOBIUM SP.*

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Almost 100 genes within the genus *Bradyrhizobium* are known to potentially encode aldoxime dehydratases (Oxds), but none of the corresponding proteins have been characterized yet. Aldoximes are natural substances involved in plant defence and auxine synthesis, and Oxds are components of enzymatic cascades enabling bacteria to transform, utilize and detoxify them. The aim of this work was to characterize a representative of the highly conserved Oxds in *Bradyrhizobium* spp. which include both plant symbionts and members of the soil communities. The selected oxd gene from *Bradyrhizobium* sp. LTSPM299 was expressed in *Escherichia coli*, and the corresponding gene product OxdBr1 was obtained as an N-His6-tagged protein (monomer, 40.7 kDa) with 30–47% identity to Oxds characterized previously. OxdBr1 was most stable at pH ca. 7.0-8.0 and at up to 30 °C. Its substrates were some (aryl)aliphatic aldoximes. Some of the reaction products of OxdBr1 are substrates of nitrilases occurring in the strains of the same genus. Regions upstream of the oxd gene contain genes encoding a putative aliphatic nitrilase and its transcriptional activator, indicating the participation of OxdBr1 in the metabolic route from aldoximes to carboxylic acids. The determination of OxdBr1 substrate specificity allowed us to hypothesize on its possible roles in the aldoxime-nitrile metabolism and to propose its potential uses for organic syntheses.

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project COST LD15107) and Charles University (project GAUK 352217).

EMBRYONIC STEM CELL HEPATIC DIFFERENTIATION: A NOVEL MODEL FOR ADVANCED *IN VITRO* TOXICOLOGICAL TESTING

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Chemical compounds of natural or synthetic origin in the environment present potential threat to ecosystem as well as human health. Based on historical incidents and precautionary principle, regulations dictate the necessity and means of toxicity testing of new or previously untested chemical entities. Time and cost requirement *in vivo* testing is gradually replaced by alternative *in vitro* human-relevant models promising more efficient toxicity screening[1]. Due to advances in cell biology in the recent decade, monolayer *in vitro* models of cancer-derived cell lines with limited physiological relevance are being replaced with novel models based on stem

cells, 3-dimensional cultures and co-cultures, which can more reliably recapitulate *in vivo*-like cellular and tissue characteristics[2]. Driven by climate change and high loads of nutrients into aquatic ecosystems, the environmental occurrence of toxic cyanobacteria and cyanobacterial toxin cylindrospermopsin (CYN) is rapidly increasing[3]. Inhibition of protein synthesis[4], induction of oxidative stress[5] and DNA double strand breaks[6] were reported as the major mechanisms of CYN-induced cellular damage, however, the tissue-specific effects of CYN responsible for acute and chronic liver damage have not been fully elucidated yet.

In our research we successfully established and characterized human embryonic stem cell-based hepatic differentiation. The effects of CYN on hepatic progenitors, immature and mature hepatocyte-like cells were studied by assessing cytotoxicity and related mechanisms known to be relevant for hepatic differentiation and liver damage. CYN cytotoxicity and cell death mechanisms depended on the cell maturity, with apoptosis being more frequent during the earlier stages of differentiation, while necrosis became more prevalent in the mature hepatocyte-like cells. However, HNF4α-positive immature cells were specifically sensitive to CYN cytotoxicity. Surviving HNF4α-negative immature hepatocytes were characterized by impaired cell functions (decreased albumin secretion, accumulation of lipid droplets), and limited ability to further differentiate, these effects might contribute to the disruption of liver tissue development, renewal and regeneration. Our study demonstrates that stem cell-based *in vitro* model provide a unique opportunity to study tissue-specific effects of environmental toxicants such as CYN, and improve our understanding about the role of stem cells and hepatic differentiation in CYN-induced liver damage and diseases.

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EFFECT OF H247A MUTATION ON NAMPT ENZYME ACTIVITY AND CELLULAR METABOLISM

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Nampt is described as an enzyme involved in the synthesis of NAD from nicotinamide. It catalyzes the transfer of phosphoribosyl group from 5-phosphoribosyl-1-pyro-phosphate to nicotinamide forming nicotinamide mononucleotide and pyrophosphate. The precise mechanism of this reaction has not been clarified yet. It is supposed that autophosphorylation of histidine at the 247th position (H247) is necessary for the enzyme catalysis of Nampt. For this reason, the H247A mutation (H247→A247) is referred to as "H247A-mutant enzymatic-dead Nampt"[1]. Our objective has been to verify this assumption.

HepG2 cells were prepared with the production of Nampt wild type (WT) and Nampt with point mutation H247A. The measurement of the enzyme activity of Nampt in cell lysate was followed by fluorescence detection of NAD, which is generated as the final product of the entire biosynthetic pathway from nicotinamide. Compared with the control, Nampt-WT exhibited an increased enzyme activity. Surprisingly, its enzyme activity value also coincided with the activity of Nampt-H247A.

We also determined the intracellular NAD content by LC-MS. It was increased in both Nampt-WT and Nampt-H247A, compared with the control.

Viability of the prepared cells, determined via the enzyme activity specific inhibitor of Nampt FK866 was also increased compared to the control cells. However there were no significant differences between viability of the Nampt-WT and Nampt-H247A cells.

The results suggest that the designation 'H247A-mutant enzymatic-dead Nampt' is not accurate, because Nampt-H247A still possesses enzyme activity in the cell lysate and the amount of intracellular NAD is increased in both Nampt-WT and Nampt-H247A. Moreover, the viability of the cells with specific inhibition of Nampt corresponds to Nampt-WT cells. This indicates that autophosphorylation of the H247 is not essential for enzyme activity of Nampt.

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NON-TOXIC RECOMBINANT PLANT DEFENSIN AND ITS ANTIFUNGAL ACTIVITY

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Plant defensins are small and cysteine-rich peptides that belong to the group of antimicrobial peptides (AMPs). Their main function in the plant defence system is protecting cell integrity during biotic stress. Here, we present a plant defensin – limen, which has been described in the literature so far as an isolated and superficially characterized peptide from lima beans with antifungal activity[1] and as one of the many defensins originally from *Fabaceae*. We expressed this peptide in a prokaryotic system, verified and scaled up its effect on pathogenic fungi, including a human pathogen (such as *Candida* sp.). To confirm limen's activity *in vivo*, transformation of the barley was chosen. Transgenic plants were obtained using *Agrobacterium*-mediated transformation and the recombinant production of limen peptide in tissues was verified by molecular techniques. Young barley plants were repeatedly exposed to Fusarium oxysporum spores. Increased resistance to the most important pathogen was followed using chlorophyll measurements as indicators of fusariosis starting. Focusing on the food safety of genetically modified plants, cytotoxicity was followed in extracts from transgenic stressed plants. We found no or a lower cytotoxic effect on human keratinocytes and human embryonic kidney cells than extracts from the non-transformed plants. This result may contribute to the better acceptance of GM plants by customers.

This work was supported by GACR project No. 15-22276S.

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DRUG EFFICACY IN 3D TUMOR MODELS

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The efficacy of drugs is affected by local gradients of nutrients, oxygen, growth factors or pH in tissues. To address these parameters in human solid tumors, three dimensional (3D) models rather than monolayers of cancer cells are necessary[1].

We aimed to assess the cytotoxicity of disulfiram complexed with copper ions (DSF/Cu^{2+}), a drug that is currently being tested in preclinical studies, to colon carcinoma HT-29 cells and compare it with a clinically used drug, 5-fluorouracil (5-FU). We confirmed the increase of DSF/Cu^{2+} cytotoxicity in acidic pH compared to physiological pH on monolayers[2]. However, this effect is decreased in hypoxic environments. To determine the cytotoxicity of these drugs to 3D models, we used MTT and ATP assays and calcein-AM/propidium iodide (PI) staining (Fig. 1).

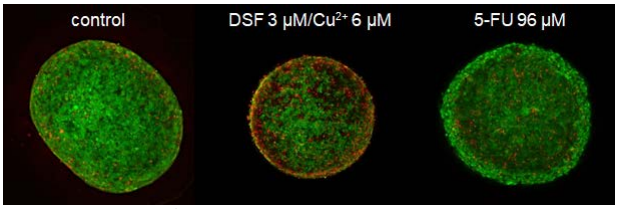

Fig. 1. **Viability of HT-29 cells in 3D models exposed to tested drugs. Green (calcein): viable cells. Red (PI): non-viable cells**

All these methods showed that DSF/Cu^{2+} is more cytotoxic than 5-FU, even at lower concentrations. As DSF was complexed with Cu^{2+}, we are also focusing on the distribution of this element in 3D models.

We established a 3D tumor model for the analysis of cytotoxicity of anti-cancer drugs and used it successfully for DSF/Cu^{2+} and 5-FU.

This work was funded by the projects no. LQ1605 and LQ1601 from the National Program of Sustainability II (MEYS CR) and no. MUNI/A/0824/2017 and MUNI/G/0974/2016 of Grant Agency of Masaryk University.

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GLUCAN PARTICLES AS DRUG DELIVERY SYSTEM FOR CURCUMIN AND THEIR POTENTIAL ANTI-INFLAMMATORY EFFECT *EX VIVO* AND *IN VIVO*

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β-Glucans, polysaccharides consisting of glucose polymers, have already shown their immunomodulatory and anti-inflammatory potential in many *in vitro* and *in vivo* studies[1]. Glucan particles (GP) - hollow porous *Saccharomyces cerevisiae* cell walls consisting of β-1,3-D-glucans, can moreover be used as drug delivery system[2]. The aim of our study was to assess usability of GP as drug delivery system for curcumin and if there is any synergic anti-inflammatory effect.

In our experiment, we used glucan particles prepared by the partial extraction of yeast cell components as described in literature[3] and then loaded with curcumin. Primary porcine immune cells were used for *ex vivo* experiment. We evaluated production of reactive oxygen species (ROS) of neutrophils and peripheral blood mononuclear cells, in which we also measured production of IL-1β. Dextran sodium sulphate induced colitis in rats was used as a model of *in vivo* intestinal inflammation. The activity of disease was monitored, the colonic tissue was evaluated macroscopically, histologically, and by performing immunodetection to determine levels of proteins associated with inflammation and antioxidant enzymes.

Encapsulated curcumin in GP exerted better therapeutic effectivity than curcumin or GP alone or curcumin in physical mixture with GP. It lowered significantly ROS and IL-1β production in cells, delayed onset of disease in rats and reduced the disease activity index. Encapsulated curcumin also reduced the expression of TNF-α, IL-1β, IL-6 and CAT.

Obtained results indicated the strong potential of GPs as carrier for anti-inflammatory natural drugs and combined with curcumin they seem as promising therapeutic strategy for treatment of inflammatory diseases.

This work was supported by Ministry of Health of the Czech Republic, grant nr. 16-27522A.

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PYRONIN FLUOROPHORES WITH NON-AROMATIC SUBSTITUENTS IN POSITION 9: STRUCUTURE-OPTICAL PROPERTIES RELATIONSHIP

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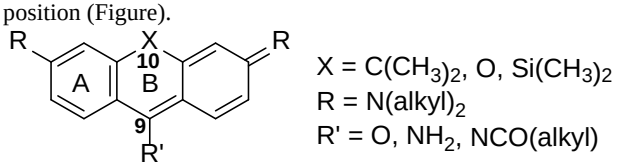
Pyronin and xanthene analogs are popular fluorescent dyes with broad applications in bio-medical sciences. In the past decade, we have introduced a series of their analogs with non-aromatic substituents in the C-9 position as visible light-activatable photoremovable protecting groups[1], CO releasing pharmaceutically interesting compound[2], fluorophores for bioimaging of cell-structures[3] or a biocompatible clickable pH sensor[4].

To further map the scope and properties limitations in such systems, we developed a small library of pyronins with selected p-block element atoms or groups in the C-10 position and O- or N- based[5] non-aromatic substituents in the C-9 position (Figure).



Electronegativity of the B-ring member X was found to play a role in the emission properties of the dye. On the other hand, electronic properties of a substituent on the nitrogen atom on the C-9 position influence especially the absorption band position.

As a result, we suggest that both the C-9 and C-10 positions are the key points for fine-tuning of optical properties of xanthene/pyronin analogs and future design of such dyes.

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1,3,5-TRIAZINE AS ELECTRON WITHDRAWING GROUP IN PUSH-PULL SYSTEMS

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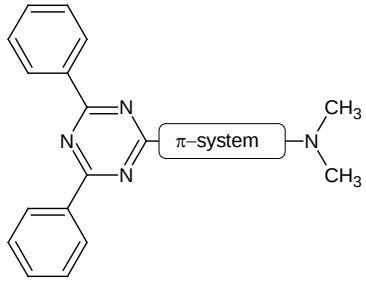
This work deals with the synthesis and study of properties of new derivatives of 1,3,5-triazine. The 1,3,5-triazine was used as an acceptor of electrons and dimethyl amino

group as a donor of electrons in target molecules with π-conjugeted system as a bridge. The π-linkage has been formed by systematic extension of the π-conjugated path by ethenylene, ethynylene and 1,4-phenylene subunits. Knoevenagel condensation and cross-coupling reactions were used for preparation of eight new push-pull chromophores (7 compounds have not been described in the literature yet). In the prepared chromophores were studied effect of 1,3,5-triazine as electron withdrawing group in push-pull systems and physico-chemical properties depending on the conjugated system between donor and acceptor. New compounds were identified by ^{1}H and ^{13}C NMR, IR and MALDI (and 5 chromophores were identified by RTG analysis). The properties of target molecules were investigated by cyclic voltammetry, UV/Vis spectroscopy, differential scanning calorimetry, and by the semi empiric calculations.



Scheme 1. **General structure of chromophores**

The prepared chromophores have good fluorescence properties, thermal stability and they are soluble in commercial organic solvents. These facts it is giving good probability to use chromophore as an organic photovoltaic solar cells (OPVC) or light-emitting diodes (OLED). The picture is shown solvent effect of chromophore with ethenylbiphenyle backbone between acceptor and donor moiety.

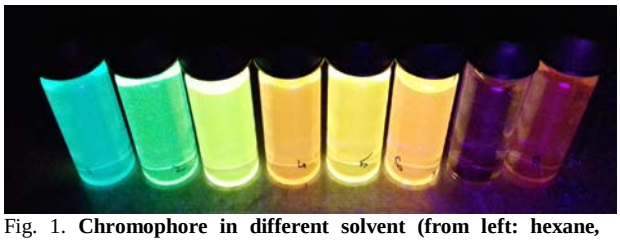


Fig. 1. **Chromophore in different solvent (from left: hexane, toluene, dioxane, THF, chloroform, DCM, acetonitrile and acetone)**

N-GLYCOSYLATION REGULATES TRAFFICKING AND FUNCTION OF THE NMDA RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that play a critical role in excitatory transmission in the central nervous system of mammals. These receptors are important for synaptic plasticity and memory formation. However, the abnormal regulation of NMDARs is associated with a wide variety of neurological and psychiatric disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease or schizophrenia[1,2]. It is generally believed that both the number and type of NMDARs present at the neuron's cell surface are regulated at multiple levels[3,4]. Previous studies have shown that a posttranslational modifications such as phosphorylation and palmitoylation regulates trafficking of NMDARs, however, little is known regarding the *N*-glycosylation of the NMDARs. Using a combination of electrophysiology, microscopy and biochemistry in two experimental model systems – lines of human fibroblasts derived from patients with various forms of congenital disorders of glycosylation (CDG) and rat hippocampal neurons, we studied the effects of impaired *N*-glycosylation machinery on the trafficking of NMDARs. Our research findings show that the *N*-glycosylation pathway regulates multiple steps in the trafficking of the NMDARs into the excitatory synapses, both on the endoplasmic reticulum level and on the neuronal cell surface. Finally, we observed that surface mobility of the NMDARs was profoundly change in the presence of lectins- conA, WGA and AAL. Together, our data show that the *N*-glycosylation is essential for proper functioning of glutamatergic excitatory transmission in the mammalian brain.

This work was supported by the Grant Agency of the Czech Republic (14-02219S) and the Grant Agency of Charles University (1520-243-227060).

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POLYHYDOXYALKANOATES – MICROBIAL POLYESTERS WITH MULTIPLE PROTECTIVE FUNCTIONS FOR BACTERIAL CELLS

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In this work, we investigated whether and how polyhydroxyalkanoates (PHA) protect bacterial cells against harmful effects of various environmental stress factors such as pH, temperature, UV exposure, osmotic pressure etc. Poly(3--hydroxybutyrate) (PHA) are polyesters produced in form intracellular granules by numerous bacteria, the polymer content of bacteria can reach up to 90% wt of cellular dry matter. We proved that PHA granules are not used only as carbon and energy storage but granules can serve also as protectant[1]. At first, PHA synthesis and degradation in microbial cells are simultaneous processes. It means that PHA accumulating cells contain substantial amount of monomers such as 3-hydroxybutyrate (3HB), which serves as chemical chaperone and efficient cryoprotectant[2]. Furthermore, another mechanism of protectivity is associated with unique biophysical properties of native PHA granules. For instance, their light scattering properties provide protection against UV irradiation or their unique liquid-like properties provide protection to cells exposed to sudden changes in external osmolarity[3]. Therefore, PHA accumulation is very valued strategy for bacteria cells to survive under various conditions[4].

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PROVIRAL SILENCING IN GENE BODIES

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Integration into host genome is an important step in a retroviral life cycle. For that reason, retroviral vectors are considered as a tool for a gene therapy. Thus, there is a huge effort for development of safe and efficient vectors.

Not every integrated retrovirus, so-called provirus, is transcriptionally active. It seems that majority of proviruses are silenced. We distinguish two types of silencing, so-called early silencing occurs up to three days after infection and after that we talk about late silencing. Epigenetic mechanisms regulating host genome are involved in this process. However, the exact mechanisms remain poorly understood.

It was shown that avian sarcoma leucosis viruses (ASLV) integrated in close proximity to promoters of active genes, which are marked by trimethylation of lysine 4 of histone 3 (H3K4me3), are stable and protected against methylation by DNA methyltransferases (DNMTs). Proviruses integrated in intergenic regions are effectively silenced by mechanisms independent of DNA methylation[1]. Generally, intergenic regions are highly methylated, but they have lower CpG density because of higher rate of C to T mutations at methylated CpG dinucleotides[2]. Whereas proviruses integrated in gene bodies, transcribed regions of genes, are methylated by activity of *de novo* DNMTs, especially DNMT3b, and transcriptionally silenced. The further from promoter proviruses are, the more prone to silencing[1].

In my study, I focus on proviruses integrated in gene bodies of active genes. It was published that histone modification H3K4me3 prevents DNMT3b from binding to DNA[3]. On the contrary, trimethylation of lysine 36 of histone 3 (H3K36me3), a mark of gene bodies of active genes, which increases with a proximity to promoters, is a site preferentially bound by DNMT3b[4]. One of the aims of my study is to find out whether long terminal repeats (LTRs), which flank provirus and contain regulatory sequences, are marked by H3K36me3, especially 5' LTR, and if so, whether knock-down of histone methyltransferase Setd2, which is responsible for H3K36me3 modification, has influence on stability of proviral expression and the level of DNA methylation.

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INNOVATIVE GALVANIC CELL SETUP UTILIZING PRINCIPLES OF BIPOLAR ELECTROCHEMISTRY

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Output voltage of a single galvanic cell is thermodynamically limited; the voltage generated from electrochemical reactions on each of the half-cells cannot exceed certain value. Thus, multiple galvanic cells connected in batteries are employed for the applications where higher voltage is necessary. However, the trend of modern technology is miniaturization and the dimensions of serially connected galvanic cells in batteries can thus be a limitation.

Herein, enhancement of galvanic cell output voltage using principles of bipolar electrochemistry is introduced[1]. This phenomenon applies an addition of a conducting object into an electrolyte resulting in changes of behaviour of a cell. It is practically capitalized on an insertion of a short circuit between anode and cathode where the short circuit gains corresponding charge on its poles. In our case, the short circuit can be considered as a conductor with an anode and a cathode on its extremities. So, the final layout consists of series of electrodes forming anode-cathode pairs consecutively and it is all immersed in a single volume of an electrolyte (Fig. 1 left). The effect is demonstrated on a Daniell cell, which is as a typical example of galvanic system. Output potential of the Daniell cell is 1.10 V under standard conditions. The actual modified version of Daniell cell enhances the potential up to 190% of its standard value (up to 2 V, Fig. 1 right)[2].

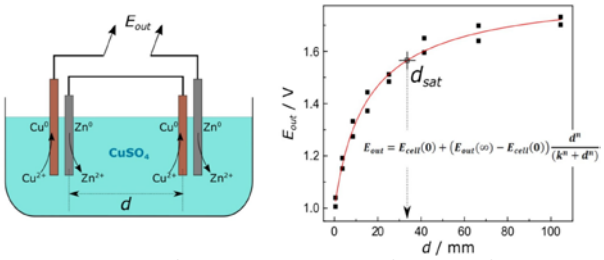

Fig. 1. **Illustration of the galvanic cell modification (left) and the output voltage dependence on its magnitude (right)**

This approach is applicable in cases where higher voltage supply is needed, but only one volume of electrolyte is available. It might serve as a power supply for wet-sensitive detectors (flood detector or activation of life jackets). It can also be utilized in the area of implantable biofuel cells where only single compartment of the electrolyte – human body – is utilizable.

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BIOLOGICAL SAFETY AND TISSUE DISTRIBUTION OF (16-MERCAPTOHEXADECYL) TRIMETHYL-AMMONIUM BROMIDE-MODIFIED CATIONIC GOLD NANORODS

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Gold nanorods have a great potential to be used in biomedicine and its associated disciplines. Especially an ability to convert absorbed near infrared light into localized heat makes GNRs a promising tool for tumor cells elimination in so-called photothermal cancer therapy. However the material, surface charge and coating of the nanoparticle can induce toxicity. In this study we examined the effects of cationic GNRs coated with (16-mercaptohexadecyl) trimethylammonium bromide (MTAB) on cellular homeostasis *in vitro* and their toxicity and tissue distribution *in vivo* in mouse[1].

As a result, no genotoxicity, induction of autophagy, destabilization of lysosomes, alteration of actin cytoskeleton and cell migration were observed even after cellular accumulation of high amounts of MTAB-GNRs sufficient to induce photothermal effect. Inductively coupled plasma mass spectrometry revealed major accumulation of MTAB-GNRs in spleen followed by lungs and liver. In blood, injected MTAB-GNRs interact with thrombocytes and are transported via the bloodstream to the red pulp of spleen in the form of GNRs-thrombocyte complexes. Despite clear accumulation in spleen, no acute toxic effects of MTAB-GNRs administered as 10 or 50 μg of gold per mice, as well as no pathological changes of spleen structure and splenic immune cells populations were observed.

In conclusion, no toxic effects or acute effects of MTAB-GNRs were observed both *in vitro* and *in vivo*. Their excellent features make them optimal for photothermal cancer therapy and imaging.

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THE ROLE OF WIP1 PHOSPHATASE ON CHROMATIN

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Cells are constantly exposed to diverse factors that cause DNA damage, which may lead to development of genome instability and tumor generation[1]. To avoid these deleterious consequences after DNA damage, cells activate the signalling pathway DDR (DNA damage response), in which p53 is an integral component. Wip1 phosphatase (also called PPM1D) directly dephosphorylates p53 and terminates DDR. It is still not known by which mechanism Wip1 controls p53-dependent transcription directly at promoters and what are Wip1 p53-independent functions[2–4].

In this study we focus on the role of Wip1 on chromatin since we have found that Wip1 is mostly chromatin bound and that it binds histones, especially histone H3.1 and H3.3. We have also discovered which region of Wip1 is responsible for this interaction. We have detected that Wip1 interacts with H3.3 histone chaperone DAXX which can be also dephosphorylated by Wip1[5]. Using proximity biotinylation assay and mass spectrometry we identified potential interactors of Wip1 and found evidence that Wip1 may be associated with telomeres. Potential roles of Wip1 interaction with histones, histone chaperone DAXX and telomeres will be discussed.

This work was supported by Ministry of Education Youth and Sports (CZ-OPENSCREEN, LO1220)

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OPTIMIZATION AND COMPARISON OF 1,5 DAN MATRIX APPLICATION METHODS FOR MASS SPECTROMETRY IMAGING OF LIPIDS

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MALDI MSI is a modern analytical technique capable to monitor a spatial distribution of compounds within the target tissues[1]. Selection of a suitable matrix and a deposition technique is a critical step in MALDI MSI. In present work, we compared three techniques of matrix deposition. Sublimation and spraying with two automatic sprayers based on different principles (ImagePrep – Bruker; iMatrixSpray – Tardo GmbH) were used for 1,5 DAN matrix applications. The methods were optimized and evaluated for the analysis of lipids in the negative mode in the mouse brain.

Best method was then used for study of lipid changes in THY-Tau22 mouse model of neurodegeneration and age-matched controls. Datasets were studied using statistical software SCiLS Lab 2016b (SCiLS GmbH, Germany). THY-Tau22 mouse model is a model of Alzheimer's disease (AD). It is a neurodegenerative disorder and it is pathologically characterized by the accumulation of hyperphosphorylated tau neurofibrillary tangles and β-amyloid plaques in the brain[2,3.] THY-Tau22 mice are a model for the tau aggregation only.

The sublimation method for 1,5 DAN (5 min, T=140 °C) was found highly irreproducible because of the matrix sublimation in the vacuum of the ion source in the mass spectrometer. Even after the number of laser shots per pixel during measurement was lowered for faster data acquisition, the stability of the signal was unacceptable. The spraying techniques provided more promising results. The best solution for spraying was 10 mg/mL 1,5 DAN in 70% acetonitrile. Both sprayer methods yielded reproducible datasets with about the same number of detected lipids in mass range 500–2 000 m/z and at similar intensity. However, iMatrixSpray has several technical advantages, specifically, a faster matrix deposition and a provision of smaller matrix crystals, which leads to high spatial resolution.

We did not find any lipid increased or decreased in the THY-Tau22 mice model and PCA analysis showed no separation between the two datasets in both ion modes. The results indicate that lipid changes in AD are not associated with the accumulation of hyperphosphorylated tau tangles.

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ROLES OF FIBROBLAST GROWTH FACTOR (FGF) SIGNALLING IN BRANCHING MORPHOGENESIS OF MAMMARY EPITHELIUM: ANALYSIS THROUGH FGF2 HYPERSIGNALLING

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Mammary gland consists of epithelial parenchyma and surrounding stroma whose interactions are crucial in mammary branching morphogenesis – a key process of mammary gland development. Branching and elongation of epithelial tubes are regulated by stromal cells through signalling with soluble molecules. A crucial one is fibroblast growth factor (FGF). When FGF signalling is deregulated, it can lead to breast cancer, therefore it is necessary to fully understand how mammary development is regulated by FGF on molecular level.

We induced FGF hypersignalling using hyperstable FGF2 molecule (FGF2-STAB), whose receptor specificity and enhanced thermostability we had characterized in 6 BaF3-cell lines, each expressing a single isotype of FGF receptors 1-3 (FGFR), a widely used tool in FGF research.

In 3D mammary epithelial organoid culture, FGF2-STAB showed an unprecedented potency to induce organoid branching at ten times lower concentration than wild type FGF2 (FGF2-wt) and after a single or short-time exposure to FGF2-STAB. In standard culture settings (1 nM FGF2, medium changed regularly) FGF2-STAB promoted epithelial growth to large hyperplastic organoids with massive branches formed by multiple layers of luminal cells (KRT8+) and a single layer of basal cells (SMA+, KRT5+, KRT14+). Basal cells, unlike in normal branches, covered the entire massive branches.

Further analysis of the hyperplastic phenotype revealed global overactivation of FGFR downstream signalling pathways (MEK-ERK, AKT, STAT3 and PLCγ) and their different roles in epithelial morphogenesis and pattern formation. Moreover, we uncovered a cooperation of FGFR signalling with insulin signalling pathway in the induction of hyperplastic phenotype.

Our findings will contribute to better understanding of the roles of FGF signalling in normal mammary gland development and cancer initiation.

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ANTHELMINTICS IN PLANTS – THE EFFECT ON TRANSCRIPTOME

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Anthelmintics, the drugs against parasitic worms, are widely used in human and veterinary medicine, nowadays. The usefulness of anthelmintic drugs is indisputable, but at the same time they pose a risk to ecosystems. With excrements of treated animals, anthelmintics can get into the environment and there affect non-target organisms – free-living invertebrates and wild plants. In our project, the most frequently used anthelmintics (albendazole, fenbendazole, flubendazole, ivermectin, monepantel) were used, and different plant species were tested, also the model plant *Arabidopsis thaliana* (wild type, *Brassicaceae*).

The presented work is the part of this project. The aim of the study is to get informations about the effects of anthelmintics on hydroponics cultures of *Arabidopsis thaliana* and changes in plant transcriptome. The broad-spectrum benzimidazole anthelmintic fenbendazole was first used. Hydroponics cultures were stressed by 5 μM fenbendazole. The effects were studied after 24 and 72 hours of stress. Microarray analysis were performed. For general expression at the transcription level were used Agilent-based microarrays. genes was increased

Exposure to fenbendazole in 5 μM concentration resulted in up-regulation of 104 and down-regulation of 64 transcripts in roots after 24 hours, up-regulation of 10 and down-regulation of 20 transcripts in roots after 72 hours. Significantly stronger response was recorded in rosettes, where transcription of 193 genes were increased and 272 genes were decreased after 24 hours, 393 genes were increased and 403 genes were decreased after 72 hours.

This project was supported by the Czech Science Foundation (GA ČR, grant No. 18-08452S).

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SYNTHESIS OF CHIRAL C_2-SYMMETRIC BIS(TRIALKOXYSILANES) AS PRECURSORS FOR HYBRID ORGANOSILICA MATERIALS

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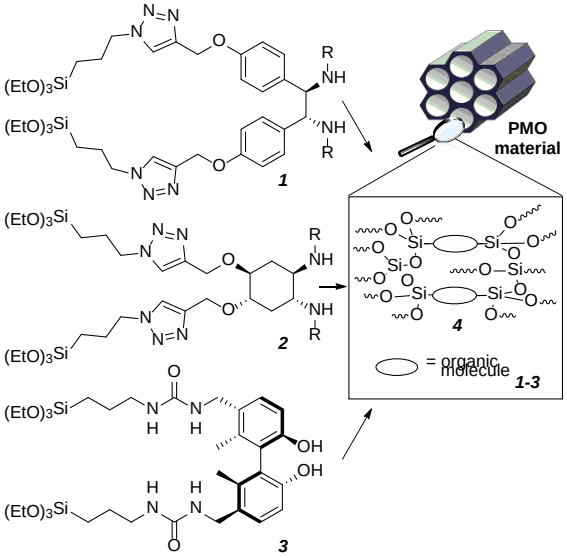
Many biologically active compounds (fragrances, flavours, hormones, enzymes or drugs) are optically active and usually only one of the stereoisomers has the required effect. One of the option, how to prepare the desired enantiomer, is the use of optically pure catalysts. Hybrid organic-inorganic organosilica materials[1,2] bearing optically pure organic moieties have been already successfully tested as mechanically and chemically robust heterogenous catalysts of enantioselective reactions. Here, we present the synthesis of three new chiral C_2-symmetric bis(trialkoxysilyl) derivatives ***1-3*** as suitable precursors for bridged periodic mesoporous organosilica (PMO) materials ***4*** (scheme 1). Design of the precursors ***1-3*** is inspired by successful homogeneous catalysts with structure modifications allowing their connection to the solid silica framework. Those precursors will be used in preparations of PMOs, which will be tested as solid, stable, filterable and reusable heterogeneous catalysts of enantioselective reactions.



Scheme 1. **Bis(trialkoxysilanes) *1-3* - precursors of PMO materials**

Financial support from Czech Science Foundation (reg. No 18-09824S) and specific university research (MŠMT No. 20-SVV/2017) is gratefully acknowledged.

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SYNTHESIS OF NEW CYCLODEXTRIN DERIVATIVES FOR CATALYSIS AND THEIR MOLECULAR MODELING

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Cyclodextrins (CDs), cyclic cone-shaped oligo-saccharides, have become worldwide attracting compounds in research and industry recently[1]. CDs perform ideal skeleton for catalysis, with generally non-toxic, chiral background, and the application in stereoselective and enantioselective organic reactions has been lately investigated[2]. New α-CD derivatives ***1***, ***2*** with Cinchona alkaloid moieties were prepared and tested in enantioselective reactions for chiral alcohols preparation (Fig. 1).

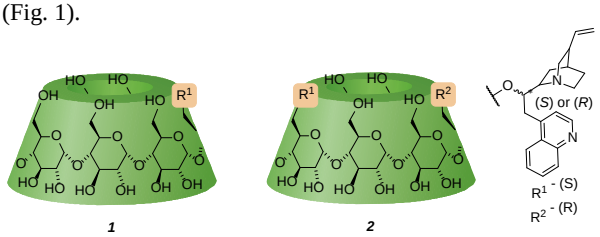

Fig. 1. **Prepared monosubstituted and disubstituted α-CDs *1*, *2* on primary rim with Cinchona alkaloids**

Also, the catalytic activity of prepared α-CD derivatives ***1***, ***2*** was investigated *in silico*[3] (Fig. 2) and compared with experimental results.

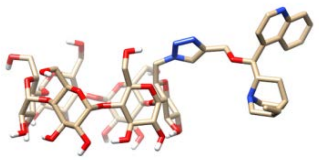

Fig. 2 **Computational model of prepared α-CD catalyst *1***

The support of the Grant Agency of Charles University (277015) is gratefully acknowledged.

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TOWARDS UNDERSTANDING EVOLUTION: A NOVEL FUNCTION BY A SINGLE-POINT MUTATION

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The process of evolution is usually understood as the change of an organism's characteristic. However, this phenomenon can be studied in advanced details at molecular level by focusing on single molecules which are present in an organism's body. The field of molecular evolution is following the rise of molecular biology, sequencing, and molecular phylogeny[1]. The aim of this study is to observe the process of divergent evolution and how different functions evolved from a common ancestor.

Two chosen enzyme groups – haloalkane dehalogenases and *Renilla* luciferases – share a high degree of sequence similarity and thus, are evolutionary related[2]. Despite the fact the luciferase contains all five amino acid residues necessary for dehalogenase reaction, it lacks the hydrolytic dehalogenase activity and is not able to transform halogenated compounds to alcohol products. On the other hand, it catalyzes a chemically distinct oxidoreductive reaction resulting in the production of visible light, known as bioluminescence[3].

Using phylogeny-guided protein engineering, the sequence of the luciferase enzyme (oxidoreductase) was modified and the full catalytic cycle for the dehalogenase activity (hydrolase) was introduced into this protein. At the same time, the original luciferase activity was significantly impaired, indicating that during evolution, one of the activities had to be partially sacrificed in order to enhance the second one.

Strikingly, all these dramatic functional changes were achieved by only a single-point mutation located just next to the catalytic machinery. These results show how evolution can take advantage of simple modifications of existing protein structures and switch between even very diverse functions easily within individual mutations.

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ENDOPLASMIC RETICULUM IN OVARIAN CANCER, STRESS, AND SENESCENCE

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Endoplasmic reticulum (ER) is a principal cellular organelle which is essential for cellular function and survival. Perturbation of ER homeostasis results in a condition known as ER stress and triggers a set of adaptive signalling pathways called unfolded protein response (UPR). UPR primarily activates pathways alleviating ER stress, however in case of long-lasting or too strong ER stress, the UPR switches its signaling upon apoptosis. Thus, it is not surprising that ER stress and UPR are both involved in various physiological of pathological events including differentiation, cancer, or ageing.

In our study[1], the ER-associated tumor suppressor TUSC3 has been shown to mediate ER stress and UPR in ovarian cancer cells and its loss promoted extensive tumor growth in mice model while *in vitro*, it caused increased proliferation and adhesion of ovarian cancer cells. This prompted us to investigate the role of UPR signaling in normal ovarian surface epithelium (OSE), a major source of ovarian malignancies. OSE forms a simple layer of cells covering the ovary, actively participates in the ovulatory cycle, and is regularly undergoing physiological cycles of wounding and repair. OSE is also constantly exposed to the variety of stressful signals coming either from the inside of the ovary or extrinsically from the peritoneal cavity. Therefore, understanding molecular mechanisms of OSE regeneration and aging is fundamental for deciphering various pathologies, including ovarian dysfunction, infertility, and cancer.

Our results indicate that murine OSE undergoes rapid onset of replicative senescence and during this process accumulates expression of UPR markers (such as BiP, CHOP, or PERK) as well as TUSC3 expression. Similarly, ER stress induction by tunicamycin alleviates cell proliferation and causes increased expression of senescent markers while alleviation of ER stress has the opposite effect.

In conclusion, our results indicate a biologically relevant link between UPR, senescence, and cancer that may contribute to better understanding of ovarian and age-related pathologies extending the portfolio of druggable molecular targets.

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COMPARISON OF TRANSGENIC AND NONTRANSGENIC CROPS EXPOSED TO VARIOUS TYPES OF STRESS

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Plants are constantly exposed to a wide range of environmental stresses such as drought, high salt, soil contaminants, heat and extremes of temperature. Environmental stress presents a major challenge in our quest for sustainable food production as it reduces the potential yields as high as 70 % in crop plants[1]. As has been previously many times demonstrated, transgenic plants can serve as a satisfactory solution for many global problems. However, their applications are still very limited because of unfounded fear, lack of knowledge, misunderstanding or commercial and political lobbyism. Here, we demonstrate that transgenic plants have more advantages then have been previously thought. Even if they should increase the yield of crops by their resistance or tolerance to biotic and abiotic stress factors, they could be less toxic then non transgenic plants exposed to the same stress. We demonstrate that both food and feed prepared from transgenic plants could be even healthier without any negative interference for consumers. The influence of healthy nutrition is still growing up; therefore, our hypothesis that some transgenic crops are better prepared for climatic changes with additional benefits for consumers represented by less changes of their metabolome could have a great impact on GMO acceptance.

For this purpose, we investigated several toxicity studies assays: resazurin assay, micronucleus assay, haemolytic assay and endocrine disruption assay.

Our results demonstrate that a lower toxicity was detected in the case of stressed transgenic plants[2] in comparison to stressed non transgenic plants. Therefore, we can summarize that the GM-plants are better prepared to cope with the environmental stressor and thus the plant response to the stress is less intensively.

We demonstrate, that the effect of the transgene should help to overcome the stress and therefore, the defense, immune response of the plant does not have be so strong.

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MODULATION OF 17β-HYDROXYSTEROID DEHYDROGENASE TYPE 10 ACTIVITY AS A POTENTIAL TARGET IN NEURODEGENERATIVE DISORDERS

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Mitochondria have essential role in cells and are important player in many neurodegenerative disorders[1]. Some mitochondrial proteins, including 17β-hydroxysteroid dehydrogenase type 10 (HSD10), have been shown to be able to interact with β-amyloid in Alzheimer´s disease. This binding result in increased oxidative stress and neuronal impairment[2].

Level of HSD10 was found to be increased not only in Alzheimer´s disease but in some types of cancer. Thus, inhibition of HSD10 may be a novel target for treatment of neurodegenerative diseases or prevention of cancer growth[3].

HSD10 enzyme was recombinantly produced in *E. coli* and purified using standard chromatographic methods. The enzymatic assay was performed spectrofotometrically in 37 °C using microplate reader and kinetic parameters of enzyme were determined. Number of novel compounds based on 1-(benzo[d]thiazol-2-yl)-3-phenylurea, targeted to HSD10, were tested whether they can modulate its enzymatic activity[4]. Based on activity assay, inhibitors with increased inhibitory ability were selected and their IC_{50} constants and type of inhibition were determined. These compounds will be further studied with implications to neurodegenerative disorders and cancer.

This work was supported by the Ministry of Health of the Czech Republic (No. NV15-28967A) and Specific Research Project of Faculty of Science, University of Hradec Kralove (No. 2103-2018).

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SELECTIVITY OF ONE-POT INTERACTION OF α-CARBONYL CH-ACIDS, DMFDMA AND ACTIVE METHYLENE NITRILES

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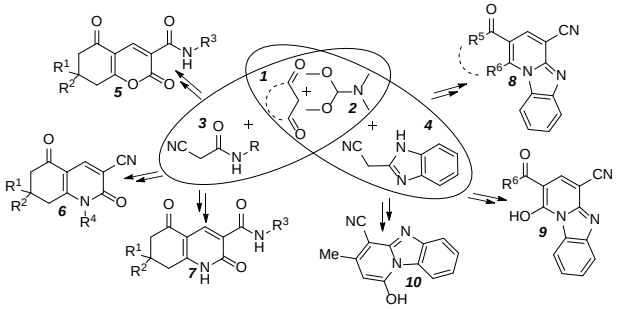
Multi-component and one-pot reactions are of high interest in modern organic chemistry. The use of highly-functionalized reactants in such reactions may lead to several chemotypes of individual reaction products obtained from one set of reactants. In our work we studied alternative pathways of one-pot stepwise interaction between carbonyl CH-acids ***1***, DMFDMA ***2*** and methylene active nitriles ***3*** and ***4***. In the case of N-substituted cyanoacetamides ***3*** selective synthesis of 2-pyrons[1] ***7*** and 3-cyano-2-pyridones ***9*** were developed avoiding formation of the side product ***8***. When 2-cyanomethyl-benzimidazole[2] ***10*** is used, different reaction directions are observed (formation of products ***11***-***13***) depending on the structure of the initial carbonyl CH-acids **1** and applied reaction conditions. Quantum chemical calculations using DFT B3LYP/aug-cc-pvdz method[3] allowed us to support our proposed reaction mechanism and rationalize tautomeric properties of intermediate 2-iminopyrans and related 2-imino-coumarins.



Scheme 1. **One-pot interaction of α-carbonyl CH-acids, DMFDMA and active methylene nitriles**

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WHEN SUGAR MATTERS: COMPARATIVE TRANSCRIPTOME ANALYSIS OF ACIDOPHILIC BACTERIA *Streptococcus mutans* GROWN ON DIFFERENT SUBSTRATES

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Acidophilic bacteria *Streptococcus mutans* is the primary etiologic agent of human tooth decay[1]. This cariogenic microorganism breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth.

In this study we have established the effects of various sugars on the regulation of gene expression in bacterium *S. mutans* growth on tryptic soy broth by employing whole transcriptome shotgun sequencing (RNA-Seq). Bacterium *S. mutans* was grown in a 5% CO_2-aerobic atmosphere at 37 °C on solid and liquid tryptic soy broth with cariogenic sugars represented by sucrose, fructose, glucose, galactose, lactose and non-cariogenic sugar xylitol. Gene expression was monitored by Illumina sequencing technology after a 12-hour growth of *S. mutans* on cariogenic or non-cariogenic sugars and compared to control culture grown without carbohydrates when results were considered as significant at padj <0.01.

Bacteria grown on lactose showed only minor changes in gene expression compared with control bacteria grown on tryptic soy broth. Bacteria grown on galactose showed upregulation of genes involved in galactose metabolism. Another group of sugars was represented by fructose and glucose where genes involved in glycolysis were upregulated. The largest changes in gene expression were observed in bacteria grown on medium with xylitol and sucrose. Bacteria grown on xylitol showed downregulation of genes involved in glycolysis, carbon metabolism and oxidative phosphorylation. In agreement with previous studies[2], bacteria grown on medium with sucrose showed strong upregulation of genes involved in glycolysis and synthesis of secondary metabolites. The obtained data helps to understand the impact of individual sugars on *S. mutans* metabolism involved in one of the most common chronic diseases affecting the human population.

The study was supported by funds provided by the Faculty of Medicine MU to junior researcher Petra Borilova Linhartova and grant AZV 17-30439A.

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ACUTE TOXICITY OF (*R*)-PULEGONE AND (*R*)-MENTHOFURAN IN HUMAN LIVER SLICES AND THEIR INFLUENCE ON miRNA EXPRESSION CHANGES *EX VIVO*

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Monoterpenes (*R*)-pulegone (PUL) and (*R*)-menthofuran (MF) are major constituents of several mint (*Mentha*) species and their derived volatile oils, including peppermint (*M. piperita*), spearmint (*M. spicata*), European pennyroyal (*M. pulegium*) and American pennyroyal (*H. pulegioides*). They are used for flavouring foods and drinks, in herbal medicinal products and cosmetics. MF is the major metabolite of PUL in the body and they both display similar hepatotoxicity in rodents. A literature review of cases of human intoxication with pennyroyal oil (PUL content 62–97 %) indicate that ingestion of 10 mL (corresponding to ca. 5.4–9 g PUL, ca. 90–150 mg/kg bw for a 60-kg person) resulted in moderate to severe toxicity. This is very vague range and despite a large number of PUL and MF toxicity and metabolism studies, vast majority of them are limited to rodents, making it difficult for regulatory authorities to apply the information to humans.[1] In our experiments, 5 human liver samples received from surgery were used to gain precision-cut liver tissue slices, which were cultivated for 24 hours in the presence of PUL and MF (100-750 µM). Their toxicity was determined by the ATP content in liver slices. While PUL showed similar toxicity in all of the samples, the MF treatment decreased viability of slices only in 2 out of 5 samples in tested concentrations. The half maximal effective concentration for PUL was determined to be 293 µM, which corresponds to 80.5 ± 11.7 mg/kg (calculated for a 65-kg person with 1.3 kg of liver weight); and ≥ 418 µM for MF. We are planning to also determine influence of these hepatotoxins on the expression of liver enriched miRNAs (e.g. 122-5p, 885-5p, 192-5p, 125b-5p), since these short non-coding RNAs play a significant role both in physiology and pathology and there is to our knowledge no such a study validating this model on miRNA level.

The study was supported by by the Czech Science Foundation (grant No. 18-09946S)

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REMARKS AND NOTES

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