Topics for dissertation theses in doctoral study programme Toxicology (eng)

Téma disertačních prací v doktorském studijním programu Toxikologie (cz)

- for admission procedure on Faculty of Science (University of Hradec Kralove) in academic year 2025/2026
- k přijímacímu řízení na Přírodovědecké fakultě (Univerzita Hradec Králové) v akademickém roce 2025/2026

Design, preparation and evaluation of senomodulators based on JNK inhibition

Design, příprava a testování senomodulátorů založených na inhibici JNK kinasy

Supervisor: prof. Ing. Kamil Kuča, Ph.D.

Consultant: Mgr. Eugenie Nepovimová, Ph.D.

Annotation:

Irreparable DNA damage leads to epigenetic changes in the phenotype of affected cells which is collectively called cellular senescence. The JNK signalling pathway is one of the signalling pathways that leads to cellular senescence. The production and secretion of cytokines and morphogens modulating the tissue microenvironment in an aversive manner is part of the senescent phenotype. Diseases in which senescence plays a significant role include, e.g.: cancer, neurological or neuropsychiatric diseases. For this reason, the development of new molecules modulating the adverse properties of senescent cells - senomodulators - is very demanding. As part of the study, small molecules with senomodulating effect, which could find therapeutic potential in the treatment of some age-related diseases, will be designed, prepared and tested on suitable biological models *in vitro* and *in vivo*.

Aims:

Design of novel molecules.

Synthesis of novel molecules.

Evaluation of novel molecules.

Literature:

Deng, Y., et al. (2023). c-Jun N-terminal kinase signaling in cellular senescence. Arch. Toxicol. 97(8): 2089-2109.

Li, Y., et al. (2024). c-Jun N-terminal kinase signaling in aging. Front. Aging. Neurosci. 16:1453710.

Supercritical fluid chromatography of isomers in metabolomics

Supekritická fluidní chromatografie isomerů v metabolomice

Supervisor: doc. Ing. Miroslav Lísa, Ph.D.

Consultant: RNDr. Oleksandr Kozlov, PhD.

Annotation:

Metabolites are biologically active substances that have a number of important functions in the human body, and their imbalance can affect the origin and development of some serious human diseases such as obesity, cancer or diabetes. The goal of metabolomic analysis is the qualitative and quantitative description of the composition of metabolites (metabolome) in an organism, tissue or cell and the monitoring of their interactions with other molecules, including proteins, other metabolites or toxic compounds. Isomers of metabolites are supposed to have different biological activity in the stereospecific environment of enzymes but are rarely studied in current metabolomic analyses. The goal of this work is the development of new methods for the separation of metabolite isomers using supercritical fluid chromatography and mass spectrometry detection to better understand their biological functions.

Aims:

Development and validation of methods for sample preparation and analysis of metabolite isomers in biological samples.

Study of retention behaviour of metabolites.

Analysis of metabolites isomers in clinical studies.

Literature:

O. Kozlov, E. Horáková, S. Rademacherová, D. Maliňák, R. Andrýs, E. Prchalová, M. Lísa, Direct Chiral Supercritical Fluid Chromatography–Mass Spectrometry Analysis of Monoacylglycerol and Diacylglycerol Isomers for the Study of Lipase-Catalyzed Hydrolysis of Triacylglycerols, Anal. Chem. 95 (2023) 5109 – 5116.

O. Kozlov, N. Štěrbová, M. Lísa, Chiral supercritical fluid chromatography of monoacylglycerol and diacylglycerol enantiomers in biological samples: Adjusting selectivity via column coupling, J. Chrom. A 1740 (2025) 465591.

LC/MS metabolomic analysis of biological samples

LC/MS metabolomická analýza biologických vzorků

Supervisor: doc. Ing. Miroslav Lísa, Ph.D.

Consultant: Ing. Eva Cífková, Ph.D.

Annotation:

Metabolomic analysis deals with the analysis of the metabolome in cells, tissues, organs or organisms. The metabolome represents a very diverse group of substances, metabolites, that enter the metabolic pathways and are important for the growth and normal function of the cell. Knowledge of the composition of metabolites is important for understanding their functions in serious human diseases. Nowadays, metabolites are intensively studied as biomarkers for the early diagnosis of disorders and for monitoring the organism's response to treatment. This work will be focused on the study of metabolism in disorders of the central nervous system, which represent a significant health risk for the entire population and the associated economic impacts. The main goal of the work will be the development of targeted and untargeted methods for the analysis of polar and moderately polar metabolites in biological samples using a combination of liquid chromatography and mass spectrometry.

Aims:

Development of new methods for the preparation of metabolite samples.

Optimization and validation of methods for targeted and untargeted metabolomic analysis.

Study of retention and fragmentation behavior of metabolites.

Analysis of metabolites in biological samples.

Literature:

T. Čajka, O. Fiehn, Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics. Analytical Chemistry 2016 (88) 524−545.

M. Lísa, E. Cífková, M. Khalikova, M. Ovčačíková, M. Holčapek, Lipidomic Analysis of Biological Samples: Comparison of Liquid Chromatography, Supercritical Fluid Chromatography and Direct Infusion Mass Spectrometry Methods, Journal of Chromatography A 1525 (2017) 96-108.

Oxime nucleophiles for reactivation of organophosphate inhibited cholinesterase

Oximové nukleofily pro reaktivaci cholinesteras inhibovaných organofosfáty

Supervisor: prof. PharmDr. Kamil Musílek, Ph.D.

Consultant: RNDr. Dávid Maliňák, PhD.

Annotation:

Organophosphorus compounds (OP) belong to the group of highly toxic irreversible inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Thanks to the inhibition of AChE and BChE, OFs cause disruption of cholinergic functions in the organism which can even cause serious injury or even the death. Causal drugs in OP intoxications are cholinesterase oxime reactivators. Such reactivators contain oxime moieties which under physiologic condition release oxime anion as a functional nucleophile that is able to bind OP moiety and restore cholinesterase function. The main goal of the work will be the preparation and testing of modified oxime nucleophiles that will be optimally able to reactivate both cholinesterases.

Aims:

Design of novel molecules.

Synthesis of novel molecules.

Evaluation of novel molecules *in vitro*.

Literature:

Musilek, K.; Dolezal, M.; Gunn-Moore, F.; Kuca, K. Design, Evaluation and Structure-Activity Relationship Studies of the AChE Reactivators Against Organophosphorus Pesticides. Medicinal Research Reviews. 2011, vol. 31, no. 4, p. 548-575. DOI: 10.1002/med.20192

Zorbaz, T.; Malinak, D.; Marakovic, N.; Macek Hrvat, N.; Zandona, A.; Novotny, M.; Skarka, A.; Andrys, R.; Benkova, M.; Soukup, O.; Katalinic, M.; Kuca, K.; Kovarik, Z.; Musilek, K. Pyridinium oximes with ortho-positioned chlorine moiety exhibit improved physicochemical properties and efficient reactivation of human acetylcholinesterase inhibited by several nerve agents. Journal of Medicinal Chemistry. 2018, vol. 61, no. 23, p. 10753−10766. DOI: 10.1021/acs.jmedchem.8b01398

Synthesis and *in vitro* evaluation of inhibitors of 17β-hydroxysteroid dehydrogenase type 10
Příprava a *in vitro* hodnocení inhibitorů 17β-hydroxysteroid dehydrogenasy typu 10

Supervisor: prof. PharmDr. Kamil Musílek, Ph.D.
Consultant: PharmDr. Ondřej Benek, Ph.D.

Annotation:
17β-HSD10, also termed as amyloid-binding alcohol dehydrogenase (ABAD), is an oxido-reductase enzyme residing in mitochondrial matrix. It can catalyse turnover of numerous substrates, especially steroids and neurosteroids. Besides its catalytic activity, it also acts as a structural component of RNase P. Thus, it is involved in many physiological functions. 17β-HSD10 plays important role in development of several human diseases and its inhibition is considered a potential treatment strategy for Alzheimer's diseases (AD) and hormone-dependant cancer. Only limited number of 17β-HSD10 inhibitors is known to date. Thus, development of novel inhibitors with improved activity and drug-like properties is highly desirable.

Aims:
Design of novel compounds.
Preparation of novel compounds.
In vitro evaluation of novel compounds.

Literature:
Vinklarova, L.; Schmidt, M.; Benek, O.; Kuca, K.; Gunn-Moore, F.; Musilek, K. Friend or Enemy? Review of 17β-HSD10 and Its Role in Human Health or Disease. *Journal of Neurochemistry* **2020**, vol. 155, no. 3, p. 231–249. DOI: 10.1111/jnc.15027
Schmidt, M.; Benek, O.; Vinklarova, L.; Hrabinova, M.; Zemanova, L.; Chribek, M.; Kralova, V.; Hroch, L.; Dolezal, R.; Prchal, L.; Jun, D.; Aitken, L.; Gunn-Moore, F.; Kuca, K.; Musilek, K. Benzothiazolyl ureas are low micromolar and uncompetitive inhibitors of 17β-HSD10 with implications to Alzheimer's disease treatment. *International Journal of Molecular Sciences* **2020**, vol. 21, no. 6, p. 2059. DOI: 10.3390/ijms21062059

Synthesis and *in vitro* evaluation of inhibitors of mitochondrial enzyme cyclophilin D
Příprava a *in vitro* hodnocení inhibitorů mitondriálního enzymu cyklofilin D

Supervisor: prof. PharmDr. Kamil Musílek, Ph.D.
Consultant: PharmDr. Ondřej Benek, Ph.D.

Annotation:
Cyclophilin D (CypD) is a mitochondrial enzyme that regulates opening of the mitochondrial permeability transition pore (mPTP). Excessive mPTP opening is manifested in several diseases associated with mitochondrial dysfunction including ischemia-reperfusion injury or neurodegeneration. Suppression of mPTP opening through CypD inhibition represents a promising approach for treatment of above-mentioned diseases. However, only limited number of CypD inhibitors are currently available - mostly macrocyclic compounds derived from cyclosporin A, which suffer from undesirable physico-chemical properties and low selectivity for CypD over other cyclophilins. Thus, development of novel inhibitors with improved activity, selectivity and physico-chemical properties is a crucial issue to date.

Aims:
Design of novel compounds.
Preparation of novel compounds.
In vitro evaluation of novel compounds.

Literature:
Haleckova, A.; Benek, O.; Zemanová, L.; Dolezal, R.; Musilek, K. Small-Molecule Inhibitors of Cyclophilin D as Potential Therapeutics in Mitochondria-Related Diseases. *Medicinal Research Reviews* **2022**, vol. 42, no. 5, p. 1822–1855. DOI: 10.1002/med.21892
Zemanova, L.; Vaskova, M.; Schmidt, M.; Roubalova, J.; Haleckova, A.; Benek, O.; Musilek, K. RNase T1 Refolding Assay for Determining Mitochondrial Cyclophilin D Activity: A Novel In Vitro Method Applicable in Drug Research and Discovery. *Biochemistry* **2020**, vol. 59, no. 17, p. 1680–1687. DOI: 10.1021/acs.biochem.9b01025

D-Amino Acids in Glioblastoma: Metabolic Insights and Diagnostic Potential

D-aminokyseliny u glioblastomu – porozumění metabolismu a diagnostický potenciál

Supervisor: doc. RNDr. Lucie Zemanová, Ph.D.

Consultant: PharmDr. Rudolf Andrýs, Ph.D.

Annotation:

While L-amino acids are the primary building blocks of proteins, D-amino acids, their mirror images, are increasingly recognized for their biological activity and involvement in various diseases, including cancer. Preliminary data suggests altered D-amino acid profiles in glioblastoma, warranting further investigation into their contribution to tumour progression and metabolic reprogramming. This project will utilize a newly developed, highly sensitive beta-carboline-based chiral derivatization method coupled with LC-MS/MS to quantify D-amino acid enantiomers in biological samples.

Aims:

Establish D-amino acid signatures in glioblastoma.

Identify novel diagnostic biomarkers for this aggressive cancer type.

Literature:

Abdulbagi, M. et al., D-amino acids and d-amino acid-containing peptides: Potential disease biomarkers and therapeutic targets? Biomolecules 11, 1–14 (2021).
Murtas, G. & Pollegioni, L. D-Amino Acids and Cancer: Friends or Foes? Int J Mol Sci 24, (2023).
Armstrong, D. W., Analysis of D-Amino Acids: Relevance in Human Disease. LCGC North America 356–360 (2022) doi:10.56530/lcgc.na.mg4374l5.