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 2024/2025
- k přijímacímu řízení na Přírodovědecké fakultě (Univerzita Hradec Králové) v akademickém roce 2024/2025

Design, preparation and evaluation of senomodulators based on mTOR inhibition

Design, příprava a testování senomodulátorů založených na inhibici mTOR

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 mTOR 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:

Mongelli, A., et al. (2020). Treating Senescence like Cancer: Novel Perspectives in Senotherapy of Chronic Diseases. Int J Mol Sci 21(21).

Weichhart, T. (2018). mTOR as Regulator of Lifespan, Aging, and Cellular Senescence: A Mini-Review. Gerontology 64(2): 127-134.

Design, synthesis and evaluation of small molecules affecting aging and age-related diseases

Design, syntéza a testování malých molekul ovlivňujících stárnutí a stářím-podmíněných onemocnění

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

Consultant: Mgr. Eugenie Nepovimová, Ph.D.

Annotation:

Aging is a complex process. By delaying the onset of individual specific symptoms of aging, it is not possible slow down this process. Currently, senolytics are available, but they show a number of unwanted and toxic effects, such as diabetes-like side effects, short-term effects, gastrointestinal problems or limited penetration through the blood-brain barrier. Considering that the population is aging and at the same time the number of elderly people suffering from some of the age-related diseases is increasing, the design and preparation of small molecules affecting the aging process is medically and socially very necessary. As part of the topic, small molecules with the potential to comprehensively affect the aging process will be designed, prepared and evaluated.

Aims:

Design of novel molecules.

Synthesis of novel molecules.

Evaluation of novel molecules.

Literature:

Bjedov I, Rallis C. The Target of Rapamycin Signalling Pathway in Ageing and Lifespan Regulation. Genes. 2020;11(9):1043. doi: 10.3390/genes11091043

Weichhart, T. (2018). mTOR as Regulator of Lifespan, Aging, and Cellular Senescence: A Mini-Review. Gerontology 64(2): 127-134.

Supercritical fluid chromatography of isomers in metabolomics

Supekritická fluidní chromatografie isomerů v metobolomice

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 human body, and their imbalance can affect the origin and development of a number of 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 for a better understanding of 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:

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, M. Holčapek, High-Throughput and Comprehensive Lipidomic Analysis Using Ultrahigh-Performance Supercritical Fluid Chromatography−Mass Spectrometry. Analytical Chemistry 2015 (87) 7187−7195.

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.

LC/MS metabolomic analysis of biological samples

LC/MS metabolomická analýza biologických vzorků

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

Consultant: Mgr. Maria Khalikova, CSc.

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, M. Holčapek, High-Throughput and Comprehensive Lipidomic Analysis Using Ultrahigh-Performance Supercritical Fluid Chromatography−Mass Spectrometry. Analytical Chemistry 2015 (87) 7187−7195.

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

Reactivators of cholinesterases with enhanced CNS bioavalability

Reaktivátory cholinesteras se zvýšenou CNS biodostupností

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

Consultant: RNDr. Dávid Maliňák, PhD.

Annotation:

The oxime nucleophiles that are used for the causal treatment of organophosphorus intoxication are charged molecules are usually administered by intramuscular injection. They are well water soluble, but they are also well known for limited blood-brain barrier permeation and CNS bioavailability. For this reason, their effect on reactivation of brain cholinesterases is negligible. To overcome this limitation, various systems were used including uncharged reactivators or nanovehicle delivery systems. The main purpose of the work will be modified cholinesterase reactivators that could spontaneously permeate to CNS or could be encapsulated into variable delivery nanosystems and released in CNS. For this purpose, modified monocharged oxime reactivators will be designed, prepared and evaluated *in vitro*, *ex vivo* or *in vivo*.

Aims:

Design of novel molecules.

Synthesis of novel molecules.

Evaluation of novel molecules.

Literature:

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

Tesarova, B.; Musilek, K.; Rex, S.; Heger, Z. Taking advantage of cellular uptake of ferritin nanocages for targeted drug delivery. Journal of Controlled Release. 2020, vol. 325, no. 1, p. 176-190. DOI: 10.1016/j.jconrel.2020.06.026

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.; doc. RNDr. Lucie Zemanová, 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.; doc. RNDr. Lucie Zemanová, 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

Immobilised enzymes - useful tool in biocatalysis and bioremediation

Imobilizované enzymy - účelný nástroj v biokatalýze a bioremediaci

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

Consultant: PharmDr. Rudolf Andrýs, Ph.D.

Annotation:

Enzymes are natural catalysts that have evolved to work under physiological conditions for millenniums. They are biocompatible, biodegradable and usually originate from renewable resources. Enzymatic reactions can be conducted under mild conditions while maintaining high rates and selectivity. Unlike classical chemical reaction, they usually do not require any additional functional group protection or activation, which makes them more economic and energy efficient.

Although modern technologies enable the preparation of enzymes with improved properties (e. g. increased stability at different pH) it is still a rather demanding process. Especially a lack of long-term operational stability and difficult recovery of the enzymes are the main drawback preventing their full industrial use. However, these drawbacks can generally be overcome by immobilisation of the enzymes. More convenient handling, facile separation from the product, efficient recovery and re-use or enhanced stability, under both storage and operational are the main advantages of enzyme immobilisation.

Aims:

Development of various biocatalyst immobilised on the magnetic and non-magnetic particles.

Application of immobilised enzymes as biocatalysts in the process of drug synthesis.

Application of immobilised enzymes in biomediation

Literature:

P. Grunwald, Industrial biocatalysis, Pan Stanford Series in Biocatalysis, 2015, ISSN: 978-981-4463-88-1.

R. A. Sheldon, S. van Pelt, Enzyme immobilisation in biocatalysis: why, what and how, Chemical Society Reviews 2013, 42, 6223-6235.

Duan S. et al. The structural and molecular mechanism of type II PETases: a mini review. Biotechnology Letters, 45, 1249-1263.

Selected PDZ domains in synthetic fusion proteins

Vybrané PDZ domény v syntetických fúzních proteinech

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

Annotation:

The PDZ protein domains are found in more than 500 human proteins, often in multiple repeats. Its main function is an interaction with short peptide sequences (5-10 amino acids) that are usually localized at the C-terminus of proteins. This results in the formation of stable or, more commonly, transient protein complexes, which are then involved in various biological processes such as cell signalling, adhesion, formation of cell junctions, etc.

Recently, it has been described that the specificity of PDZ domains can be regulated by a mechanism of dynamic allostery, which probably significantly influences its function. Since PDZ domains are usually part of multidomain proteins, there is a question how the surrounding domains may influence PDZ function. Domain fusion techniques using various peptide linkers can be used to design and prepare synthetic two-domain fusion proteins that can be used to study and understand changes in PDZ domain specificity by another domain. Such approach may help to design synthetic PDZ domain proteins with completely new or improved function targeted to specific applications in the future.

Synthetic two-domain fusion proteins containing selected PDZ domain and a second artificial or functional domain will be designed and prepared in the recombinant form. These proteins will be characterized and studied by different methods to describe and understand the changes in function of the contained PDZ domain *in vitro* or at the cellular level.

Aims:

Design and preparation of selected synthetic fusion proteins containing the PDZ domain.

Description of changes in PDZ domain function *in vitro* or *in cellulo*.

Further characterization of the found changes.

Literature:

Yu, K.; Liu, C.; Kim, B-G.; Lee, K-Y. Synthetic fusion proteins and their application, Biotechnology advances. 2015, vol. 33, p. 155-164. DOI: 10.1016/j.biotechadv.2014.11.005

Liu, X. and Fuentes, E.J. Emerging themes in PDZ protein signaling: Structure, function and inhibition. Interantional review of cell and molecular biology. 2019, vol. 343, p. 129-218. DOI: 10.1016/bs.ircmb.2018.05.013

Stevens A. O. and He Y. (2022) Allosterism in the PDZ family, Int J Mol Sci, 23, 1454. DOI: 10.3390/ijms23031454

Study of a role of selected anthelmintic drugs in STAT3 signaling in gliblastoma

Studium role vybraných anthelmintik v STAT3 signalizaci v glioblastomech

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

Consultant: PharmDr. Hana Navrátilová, Ph.D.

Annotation:

Glioblastoma (GBM) is the most aggressive type of diffuse gliomas, which are most common primary cerebral neoplasias in adults. Unfortunately, treatment efficiency of GBM is low so novel molecular targets and mechanism are widely searched and studied. One of them is Signal transducer and activator of transcription 3 (STAT3). It is a transcription factor belonging to STAT family proteins composed of seven members. STAT3 play a role in several cellular pathways – STAT3 induces transcription of target genes in nucleus but also influence microtubule dynamics in cytosol. Its regulation of microtubule dynamics is mediated by microtubule depolymerizing protein stathmin.

Drugs from the group of anthelmintics (flubendazole, mebendazole) are tested as potential drugs for treatment of GBM, it was proved that they potentiate cytotoxicity of clinically used drug temozolomide. Exact mechanism of action is unknown but it appears that they induce microtubule network collapse leading to inhibition of proliferation through STAT3 signalling. It is necessary to study their mechanism on molecular level using

Aims:

Preparation of recombinant protein as STAT3, stathmin and their activation protein (e.g. JAK) and their suitable mutants as well as isolation of native tubulin

Study interactions of tested compound with particular proteins using in vitro methods

Functional assays for determination of phosphorylation of STAT3, tubulin polymerization assay

Literature:

Ng, D.C., et al.,Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin. J Cell Biol, 2006. 172(2): p. 245-57.

Skarkova, V., et al., Evaluation of glioblastima cell dissoaciation and its influence on its behaviour. Int J Mol Sci, 2019. 20(18).

Patil, V.M., et al., Mebendazole plus lomustine or temozolomide in patients with recurent glioblastoma: A randomised open-label phase II trial. EClinicalMedicine, 2022. 49: p. 101449.