

**POSUDEK ŠKOLITELE STUDENTA  
K OBHAJOBĚ DISERTAČNÍ PRÁCE**

Student: Mgr. Lukáš Górecki  
Školitel: PharmDr. Jan Korábečný, Ph.D.  
Školitel-specialista: -  
Studijní program: toxikologie  
Forma studia: prezenční  
Ročník: 4.

Název disertační práce: Development of novel cholinesterase modulators

Lukas Gorecki started his scientific career as postgraduate student at the Department of Toxicology and Military Pharmacy in 2015. The title of his thesis is „Development of novel cholinesterase modulators”. The topic is very up-to-date given both the long-term scientific plan of the Department and escalated global terrorism threat of nerve agents (NAs) misuse.

The major goal of student was to develop novel small molecules in different fields of research that are generally associated with acetylcholinesterase (AChE) enzyme. Student work can be divided into several distinct topics. Initially, Lukas developed novel tacrine-phenothiazine hybrids rationally designed on the basis of dual-binding site approach that could be potentially applicable against organophosphorous (OP) intoxication as prophylactic agents or as novel, so-called multi-target directed ligands, useful for the treatment of Alzheimer’s disease or related neurodegenerative disorders. The project was carried out in collaboration with prof. Maria Laura Bolognesi (University of Bologna, Italy) where Lukas spent four months. Within his contribution, he experimentally faced new challenges trying to combine two various scaffolds, i.e. tacrine and phenothiazine, or their differently substituted analogues, into one single entity. Indeed, he developed very elegant protocol to get final tacrine-phenothiazine heterodimers. Note that, the approach differed in each subset based on the linker length used. In another project of his thesis, student concentrated on the synthesis of small tacrine-based molecules to inspect their affinity to cholinesterase enzymes as well as *N*-methyl-D-aspartate (NMDA) receptors. This work can be conceived of high importance given the fact that OP intoxication is accompanied by inflammatory and excitotoxicity processes mediated via this type of receptors. Completely different goal was established to find selective insecticides. It is well-known that the most of the insecticides nowadays act though the inhibition of hydroxyl serine residue. This residue forms indispensable part of catalytic triad machinery of AChE enzyme. However, the selectivity between human and insect species impose a serious obstacle. To this end, the major goal was to develop human-safe and insect-selective insecticides. In the student’s work, Lukas foresaw new insect selective inhibitors by targeting cysteine residue near the entrance of the AChE cavity gorge. In this case, the cysteine residue is very specific target present only in insect. Accordingly, student applied multi-step

synthesis to develop novel insecticides, some of them displaying truly insect-selective profile. The majority of the work within the thesis, obviously, is devoted to the development of therapeutics in OP intoxication. From this point of view, student worked on the synthesis of mono-charged and uncharged reactivators, out of which some analogues from mono-charged family yielded as the most potent ones, i.e. having broad biological profile against different OPs, known to date. These excellent results encouraged us to further continue with this project. Lukas carried out large scale synthesis of the selected, most promising compounds with broad profile against different OPs. This laborious work should be cheered when taking into account multistep synthesis approach comprising of more than 10 steps with optimized reaction conditions. Last but not least, Lukas invented novel tacrine-oxime reactivators, simply known as tacroximes. These novel agents are built on tacrine scaffold with attached oxime moiety at position 7- thus allowing the tacrine conversion from inhibitor into reactivator. The idea behind is somewhat unique, not previously described in the literature. Furthermore, as a part of Lukas's 5-months scientific mission in University of San Diego, California, US, Lukas established a strong collaboration with experts in the AChE biochemistry field, prof. Zoran Radić and prof. Palmer Taylor working there also on the topic of the uncharged reactivators related to RS194B.

To summarize all the aforementioned, Lukas's thesis truly reflects all the hard work behind. He was capable to run more 1,000 reactions, which can be nicely documented by high number of final products (more than 100). This in my opinion totally outnumber the scope of classic work considered as dissertation thesis and normally could be even separated into two independent theses. The uniqueness of Lukas approach can be also well-documented by his scientific enthusiasm. He was not only dedicated organic chemist, but he was also able to learn and run *in vitro* biological experiments. Accordingly, he established biological activity of uncharged reactivators during his scientific mission in US.

As a scientific writer, he has published three scientific papers being the first author there. I would like to also point out there that two of these papers were published in Archives of Toxicology, which has impact factor over 6,0 and belongs to one of the top-ranked journals in Toxicology. Besides all that, Lukas extensively contributed into several other articles. His total number of published articles nowadays reaches 11 (according to WOS). We expect that this is not the final one since we are mostly in the process of data collection for all of these compounds developed during his postgraduate career and definitely will publish them in journals with impact factor.

To conclude, Lukas is hard-working, motivated, self-consciousness and self-disciplined student. The presented dissertation thesis is in accordance with intern University regulations (rector's precaution UO, No. 3/2018) and I approve it for defense.

Datum: 9.4.2019

Podpis školitele: .....

PharmDr. Jan Korábečný, Ph.D.