



CHARLES UNIVERSITY
Faculty of Pharmacy
in Hradec Králové

Report on the Ph.D. thesis

Student: Mgr. Lukáš Górecki

Title: Development of novel cholinesterase modulators

University of Defence in Brno, Faculty of Military Health Sciences, Department of Toxicology and Military pharmacy

Reviewer: doc. PharmDr. Jaroslav Roh, Ph.D.

In his dissertation thesis, Lukáš Górecki deals mainly with the synthesis of several series of acetylcholine esterase (AChE) modulators and subsequently with *in vitro* evaluation of their properties like inhibition/reactivation of various choline esterases (ChE), their effects on cell viability or potential to cross biological membranes (blood-brain barrier). The dissertation thesis is written in standard way. In the introduction section, the author summarized the current knowledge in the fields important for the objectives of his work. Main part of the thesis consist of the design and synthesis of tacrine inhibitors of ChE, then insect's AChE-targeting inhibitors, and finally three series of the reactivators of organophosphate- and organophosphonate-inhibited AChE. Some of the prepared compounds showed very promising properties and thus will be used as the lead compounds in further *in vivo* studies.

The dissertation thesis has 22 % rate of similarities according to Turnitin system. Although this number looks high, the majority of similarities are in the experimental section (including the sequences of NMR signals etc.) and there are very few similarities in the written text. Therefore, from this point of view, I can say that this work is composed of the original texts and results, which did not appear previously.

The thesis is written in English language. As I am not a native speaker, I cannot objectively evaluate the grammar and style. Nonetheless, I found several mistakes and some formulations are not easily understandable (e.g. abstract *it believed*, in Czech wrong form “nervosvalových spojích”, page 25 - these *leads*, page 33 *have proved effective*, page 32 - *release of a leaving group release*, page 39 – *in addition the parent...*, page 56 - *In the next step was important for the preparation of alkylating intermediates. etc.*).

In my opinion this Ph.D. thesis suffered from 3 major issues.

1. I found that candidate is not the first author (even not second or third) of original experimental-type article in the journal with IF. Lukáš Gorecki is the first author of two official reviews (Arch. Toxicol 2016; Expert Opin. Ther. Pat. 2017) and one review (Arch. Toxicol, 2018) that is presented by the publisher as original article. But I did not find any experimental section there. So my first important question is: Did the candidate perform some experimental work for this article?

2. I am not satisfied with the characterization of many of intermediates and products (no one from any relevant journal would be). It is not enough to present just H NMR. In some cases, it can be tolerated, mainly when the comparison with known data are presented. However, it is not the case in this work.

Moreover, there are several compounds, marked as pure and/or with high yields, but the evidence for structure/purity is missing. This is not possible in the scientific text.

The compound 170 is characterized only with H NMR and the yield is 98%, with no purification. Many authors presented significantly lower yields with another isomer (6-nitro) to be present in the final mixture. Similar problem can be found with compound 174.

Compounds 211 and 226 had the yields >99%. It is nonsense, only possible explanation is that these compounds contain some impurities. Again, any evidence for these results are missing (only H NMR spectra are shown).

3. It was really difficult and tiring to read and understand the discussion part, because author did not use the numbers for the intermediates and some of final compounds, which he commented. So it was time-consuming to look back for the structures and try to find the appropriate ones. E.g. see paragraph on page 90. It is very confusing. Even some mysterious codes like PMS20, PY18 appeared, with no structure or some relevant comment.

Other comments/questions:

1. Abbreviations - chemical formulas are not abbrev. – e.g. PPh₃, MeCN, EtOH
2. Page 19. Author wrote that esteratic site is 17,7Å from Ser200. But figure 2 and scheme 1 show that Ser200 is the part of esteratic site. So what is esteratic site and where is Ser200?
3. Page 21. “Organophosphates bind competitively?” Is it true? Can you comment it?
4. Page 42. What is the meaning of the sentence: “The maleimide moiety was confirmed by the *in vitro* results.”
5. It would be beneficial for the reader to find the ranges of yields in Schemes 9, 10, 11, 15 etc. and yields in table 2, 4 etc.
6. Scheme 15 – conditions h) are not included in the caption
7. Scheme 16 – compound 158 is an ester
8. Table 5 and 7 – some standard, like parent compound tacrine, should be included to allow the comparison of the results of new derivative with it
9. Table 7 – footnote a is not mentioned in the table; same as with c, d in table 8; c in table 9
10. Please, can you comment the pK_a values summarized in Table 18. E.g. for compound **229**.
11. According to exp. section, compounds 229-236 were prepared according to method E. But method E is completely different and cannot proceed to these compounds.
12. Isolation and purification of compounds **234**, **235** and **236** as mentioned in the discussion (page 95) is completely different that those described in experimental section.

13. In the section 4.2 if the conclusion, it would be very welcomed by the reader if some SAR picture/scheme is provided. Again, just the text is not enough for easy understanding.
14. In the figure 28, the compounds are marked with KXXX, so the reader does not know what compounds are they.
15. Page 98, 3rd paragraph – “Unfortunately, all compounds, including the standards, were able to cross MDCK cell line.” This sentence is also quite confusing. Please comment it.
16. Page 97. What is ATCh? Please, can you explain the meaning of the sentence in which ATCh is used?

Additional questions:

1. Page 34. Does the anion in the reactivator play any role in the reactivation potency/ ADME properties?
2. Regarding the designed maleimide insecticides. What about the other cystein residues in the human body. In my opinion, the maleimide would act as non-selective Michael acceptor and will react with various nucleophiles. Did you also check the effects to mammalian cells?
3. The antioxidant activities are shown in table 6. But I did not find any experimental details and I am not sure, what is the meaning of the „% at 10 mM“.
4. Page 89, 4.1.2. I found in exp. Section, that only $AlCl_3$ and $ZnCl_2$ were used. Did you try another Lewis acid, as you indicated in the text?
5. Can you show me the possible interaction of His447 with maleimide part of the inhibitor, which can irreversibly inhibit the enzyme (page 97)? What about the nucleophilic residue of serine in the CAS and its possible reaction with maleimide.

Reviewer can conclude that candidate fulfilled the majority of the objectives and prepared a huge series of compounds with interesting properties regarding the modulation of ChE. I am sure that they will become a crucial part of several research articles. In addition, the overall publication activity of the candidate is very good, despite the fact, that in my opinion he is not the first author of any truly experimental work.

Finally, this Ph.D. thesis can be considered as sufficient for the defense and thus I recommend it for the defense at the University of Defence in Brno.

9. 6. 2019

Assoc. Prof. Jaroslav Roh, PharmD., Ph.D.

Charles University, Faculty Of Pharmacy in Hradec Králové
Department of Organic and Bioorganic Chemistry
Akademika Heyrovského 1203/8, 500 05 Hradec Králové, Czech
Republic



CHARLES UNIVERSITY
Faculty of Pharmacy
in Hradec Králové