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Report on the PhD Thesis of Natalia Szałaj:

‚Synthesis and Biological Evaluation of New Donepezil-Based Benzylamine Derivatives As Multiple Ligands Targeting Cholinesterases and Amyloid Beta'

The doctoral dissertation of Natalia Szałaj (maiden name Guzior) addressed the development of new potential anti-AD active agents originating from donepezil modification, resp. from benzylamine pharmacophore, that have shown promising pharmacological activity, especially two leading compounds **18** and **71** were discovered. The facts that about 36 million people are sick presently with Alzheimer's disease (AD) and the cause of AD is still poorly understood support the importance of the topic covered by this work.

This PhD thesis is based on three experimental papers and two reviews, which were published in impacted International journals (summa IF = **13.604**!) during the period covered by this thesis. All this results have already been evaluated in the prestigious international journals, so my role as a reviewer is therefore very easy.
The thesis that includes 69 pages is well-written and involves Introduction, Aim of the Thesis, Previous Research on Which This Work Was Based, Design Strategies, Synthesis, Biological Evaluation, Concluding Remarks and References. It is accompanied by an extensive Supplements containing 5 reprints of original papers, in four of them Natalia is the first author. All necessary data (^{1}H an ^{13}C NMR, MS; IC_{50}) are included in the corresponding articles. I have found only few inaccuracies, p. 15, etc. „excitotoxicity", fig. 3 rivastygmine.
The Introducing brings the most basic information concerning AD. The aim of the thesis is clearly outlined, the figure no. 9 is a very illustrative from the point of view of medicinal chemistry! Very clear design strategies are demonstrated in figures from papers I-III. Chapter 5 deals with synthesis, with the five series of compounds based on isoindoline or benzisothiazole (saccharin moiety) core. All compounds were evaluated on their activity against cholinesterases using the spectrophotometric Ellman's assay, thioflavin T assay and Cell-Based asssay (both in Ljubljana), and/or the PAMPA-BBB Assay. A conclusion briefly summarizes the main achievements of the thesis. The list of References includes 129 citations.
Finally, the PhD thesis of Natalia Szałaj is a relevant work which offers a number of new facts about new promising compounds against AD and is without any doubt of great interest to the scientific community. In conclusion, let me **recommend this thesis for further procedure** including a public defense.

My questions:

1. According the last results, the Tau protein was found as a driver of AD, is there some connection with your research? What is your meaning about the "diabetes type 3" theory?
2. From the point of drug design I have one question: more compounds with the substitution of benzylamine part on benzene ring in position 4 with an electron donating group (EDG) or electron releasing group (ERG) substitution was not your plan? Only fluorine or chlorine were introduced on benzyl moiety, it was due their halogen binding? What about the connection of halogenation and saccharin moiety?
3. Used neurotoxicity evaluation is OK, some basic information about the cytotoxicity of studied compounds will be useful.
4. Are you thinking about other type of biological screening of prepared compounds?
5. Is there some limit for the access through the BBB of anti-AD drugs from the point of their log P parameters? In the literature it is log $P = 1.8 - 2.2$ for BBB. Is the Lipinsky Ro5 useful in your research?

Opponent: Prof. PharmDr. Martin Doležal, Ph.D.
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