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Assessment of the doctoral dissertation of Katarzyna Szczepańska entitled "The Search for Novel Histamine H_3 Receptor Ligand in the Group of Piperazine Derivatives"

Dear Prof. Wesołowska,

Thank you for your letter of 27 February 2020, in which you asked me to review the above dissertation. Hereby, I confirm that I have sufficient knowledge, experience and qualification to prepare the requested review. I have been Full Professor of Pharmaceutical Chemistry for more than 20 years working at the University of Bonn in this position since 1998. I have published more than 400 original papers, review articles and patents in the broader field of Pharmaceutical and Medicinal Chemistry including Molecular Pharmacology, and I would like to point out that there are neither impediments of technical or legal nature nor a conflict of interest or bias that would prevent me from performing the evaluation of the thesis submitted by Ms Szczepańska.

The thesis by Katarzyna Szczepańska consists of five articles along with a comprehensive and timely introduction, a description of the background of the thesis and the aims of the studies, and a chapter on methodology divided into ligand design and pharmacological studies. The thesis is a cumulative dissertation, but it additionally contains some so far unpublished work. Ms Szczepańska is first author of three original publications that have already appeared and of one review article on the subject while she is a co-author on another publication, in which one of the compounds was pharmacologically evaluated in *in vivo* studies. Moreover, Ms Szczepańska is a co-author on four additional publications on a different subject, which are not part of the submitted thesis.

The scientific work by Katarzyna Szczepańska focuses on a specific class of G protein-coupled receptors, which belong to the rhodopsin-like subfamily of GPCRs, namely histamine receptors. The main aim of the thesis was to develop specific ligands for the histamine H_3 receptor subtype. While for histamine H_1 and H_2 receptors important drugs are already on the market, the histamine H_3 receptor has been discovered very late and only in recent years potent ligands for this receptor subtype have been developed. The group of Prof. Dr. Katarzyna Kieć-Kononowicz had previously developed several series of histamine H_3 recep-

tor ligands, and in the present thesis, Ms Szczepańska focused on the optimization of previously developed lead structures termed DL76 and DL77. In a systematic way she designed new target structures modifying the different parts of the molecules. This systematic methodology led to the successful development of highly potent histamine H_3 receptor antagonists with very high affinity and potency in the low nanomolar range. Moreover, the best compounds were evaluated for their physicochemical and pharmacokinetic properties and further optimized with respect to these properties, which are important for *in vivo* efficacy. In fact, one of best compounds, compound **9** or KSK19, showed potent *in vivo* efficacy in animal studies, e.g. in the passive avoidance test indicating a penetration into the central nervous system and CNS activity. Moreover, this compound was evaluated in animals treated with a high-fat diet and was found to lead to significant lower weight gain in comparison to a control group. This opens new therapeutic applications of histamine H_3 receptor ligands.

Ms Szczepańska's thesis starts with a comprehensive and up-to-date introduction into the field of histamine and its receptors including molecular aspects of structure biology, signaling and expression and an excellent review on currently known ligands for the histamine H_3 receptor subtype including potential application in disease for such compounds (chapter 1). Chapter 2 describes the background of the thesis presenting previous work that has been done in the group of Prof. Kieć-Kononowicz and on which the thesis has been based. Chapter 3 describes the aims of the study in a well-structured and concise way. Chapter 4 contains a detailed description of the methodology used, including ligand design of the four different series of compounds that were synthesized in the present study. This chapter in particular and the whole thesis in general clearly showe the very structured approach pursued by the candidate. The synthetic methods and strategies are explained in several informative schemes followed by the description of the *in vitro* studies such as affinity determination, selectivity measurements, functional characterization using highly innovative technology, metabolic studies as well as permeability, hepatotoxicity and enzyme interaction. In chapter 5 the results are presented and discussed on the basis of the current literature. The presentation is again excellent, well-structured, e.g. important results are highlighted by color, and it has been a pleasure to read and very easy to understand the procedure and the results and their significance. Chapter 6 contains concluding remarks which put the most exciting results in a larger context and highlight the most interesting findings. Chapter 7 contains a list of more than 100 references on the subject including the latest development in this field. Very helpful is chapter 8, which contains a table with structures of all final compounds along with their histamine H_3 receptor affinities. This list of 80 final products is impressive, especially when considering also the high potency of many of the compounds. These parts of the thesis are followed by the 5 articles authored and co-authored by Ms Szczepańska.

The thesis by Katarzyna Szczepańska deals with a very important and timely subject in medicinal and pharmaceutical chemistry. She has used modern methodologies to achieve her aims and has shown that she is able to perform academic research highly successfully. She obtained very potent histamine H_3 receptor ligands, which were shown to be active in animal models of disease. I am very impressed not only by the amount of research the candidate has performed but

also by the very high quality of her work. Finally, I want to point out once more that the introduction into the topic and the presentation of the results are excellent and well-structured and that it has been a great pleasure for me to read the thesis.

I highly recommend the Collegium Medicum of the Jagiellonian University of Krakow to accept the thesis by Katarzyna Szczepańska as a fully valid and excellent doctoral dissertation, and to promote Ms Szczepańska and award her the doctoral degree for her important work.

Yours sincerely,

Prof. Dr. Christa E. Müller