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Reviewer's Report on the Ph.D. Dissertation Thesis of Katarzyna Szczepańska entitled „The search for novel histaminę H_3 receptor ligands in the group of piperazine"

In the central nervous system, histamine participates in the regulation of more complex cerebral functions (sleep-awake, cognitive processes, feeding behavior) as well as triggers compensatory mechanisms under life-threatening conditions (hypovolemic shock, hypoglycemia, dehydration). All that needs the interaction of histamine with other neuronal systems. Histamine H_3 receptors presynaptically located on histaminergic neurons (autoreceptors) or other neurotransmitters neurons (adrenergic, serotonergic, dopaminergic, cholinergic) (heteroreceptors) control the release and synthesis of the stored neurotransmitter. Thus, H_3 receptor ligands may be an important pharmacological tool allowing modulation of various signaling systems in the CNS. Progress has lately been made in the understanding of the H_3 receptor role. Clear indications for the potential therapeutic use of H_3 receptor agonists and antagonists are now available based on experimental studies. Particularly, the H_3 receptor antagonists/inverse agonists can become useful drugs for the treatment of narcolepsy, epilepsy, obesity, neuropathic pain, schizophrenia or memory and learning deficits.

The aging population and the related increase in the number of patients with neurodegenerative diseases and encompassing the countries of a highly-developed obesity epidemic, by all means, justify the desirability of searching for highly potent and selective non-imidazole H_3 histamine receptor antagonists/inverse agonists.

The initial development of potent H_3 receptor antagonists focused on extensive modification of the natural ligand histamine and resulted in a series of very potent imidazole-containing H_3 antagonists. Unfortunately, it has appeared that many of them have found application as useful pharmacological tools only. The problem with the imidazole ligands is the poor CNS penetration because the imidazole ring is a strong H-bond acceptor and donor. One potential liability of imidazole-based drug candidates is the possibility for mechanism-based inhibition of hepatic CYPs (cytochrome P450), caused by imidazole nitrogen complexation to heme iron in the active site of the enzyme. Since these enzymes are a major route of clearance for most medicines, drugs that are cytochrome P450 inhibitors perpetrate drug-drug interactions by reducing or preventing the clearance of co-administered medicines. Additionally, the inhibition of CYPs by imidazole-based H_3 antagonists can interfere with adrenal steroid synthesis via inhibition of heme-containing enzymes.

For these reasons, many laboratories all over the world, including the Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, took a challenge to design and synthesize highly potent and selective non-imidazole H_3 histamine receptor ligands that easily enter the brain and do not interfere with the metabolism of endogenous compounds and xenobiotics.

The main goal of this thesis was to obtain a series of novel, active and selective, histamine H_3 receptor ligands, whilst maintaining favorable physicochemical properties and ADMET parameters. As lead compounds two piperidine derivatives DL76 and DL77 - previously synthesized in the Department of Technology and Biotechnology of Drugs - were chosen for further structural modifications. Aforementioned, lead compounds possessed high H_3 receptor

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affinity and selectivity, as well as a promising anticonvulsant and pro-cognitive pharmacological profile in several rodents model.
The dissertation presented by Katarzyna Szczepańska describes research in the field of medicinal chemistry performed using the widely recognized methods for new drug design.
The Ph.D. Thesis of Katarzyna Szczepańska is well structured and correctly presented. It consists of 8 main chapters written in English, the first and second of which (Introduction and Background of the thesis, respectively) contains a review on histamine itself, H_3 receptor function and its ligands. Such a selection of the discussed group of topics is deeply justified because it allows, among others locate the Ph.D. student's experimental work against the background of achievements in current research on the search for non-imidazole H_3 receptor antagonists and is based on data from about 85 references.
In chapters 3 (Aim of studies) and 4 (Methodology), the Ph.D. student describes the objectives of her doctoral dissertation and the ways of achieving them, i.e.
a) Ligand design supported by molecular modeling based on the known structure of active derivatives,
b) Synthesis of newly design chemical compounds involving three major modifications of the general structure presented in Table 2 i.e. - replacement of the piperidine ring with different 4*N*-heterocyclic substituted piperazine groups; elongation of the alkyl chain length from 2 to 8 methylene groups; introduction of various substituents as methyl, ethyl, phenyl, benzoyl in the aromatic ring in "eastern part" of the compound.

Recent years have brought several review articles regarding selective non-imidazole H_3 histamine receptor ligands and their chemical, pharmacological and pre-clinical properties Although a number of the active compounds reached the advanced clinical trials, some were rejected due to adverse effects and/or physicochemical properties. In these tests non-imidazole ligands caused among others;

- phospholipidosis
- drug-hERG channel interactions
- weak BBB penetration
- high lipophilicity •
- weak pharmacokinetic properties •
- cytochrome P450 interactions

Therefore, considering the above adverse effects, for the most active compounds in each series, the following studies were performed:
c) *In vitro* pharmacological evaluation of obtained ligands
- all newly synthesized compounds were tested in H_3 receptor in vitro binding studies and to confirm their selectivity affinity at histamine H_1, dopamine D_2, muscarinic M_1 and, α_1 adrenergic activation was carried out to identify whether the most active compounds of receptors was determined,
- functional char each series are antagonists, inverse agonist and/or partial agonists,
- determination of selected ADMET parameters like

the metabolic stability of selected compounds was initially examined using computational tool MetaSite,
permeability profile - for selected compounds their ability to penetrate across lipid membranes was estimated by a parallel artificial membrane permeability assay
hepatotoxicity – the potential antiproliferative activity was examined in vitro using hepatoma HepG2 cells,

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the risk for phospholipidosis - for prediction of potential PLD of selected ligands from the second series the LYSOD-ID Red Detection kit was used,
influence on cytochrome P4503A4 activity – the luminescence-based CYP3A4P450-GloTM test was used to assess the potential risk of drug-drug interactions of the most active ligands of synthesized series,
and finally
d) SAR's analysis of newly obtained piperazine derivatives and identification of the most active and selective compounds for in vivo test
Chapter 5 (Results and discussion) represents the most extensive part of the dissertation, where the author clearly and comprehensively presents the results of research based on peer-reviewed publications in the European Journal of Medicinal Chemistry, Bioorganic & Medicinal Chemistry and, Bioorganic Chemistry, which Katarzyna Szczepańska briefly summarized in Chapter 6 (Concluding remarks)
This Ph.D. thesis is based on four experimental paper and one unpublished results, and one review paper, which was published in International Journals (sum IF=19.409!) during the period covered by this thesis. To the extensively and thoroughly discussed results, copies of original articles with supplementary materials were attached.

In four of five papers, Katarzyna Szczepańska is the first author leaving no space for questioning on her leading input to the concept, experiment design, and execution, interpretation or writing in each manuscript.
It proves, that Katarzyna Szczepańska is a researcher with an excellent qualification for the Ph.D. title.
An unquestionable achievement of the doctoral dissertation presented for review is the synthesis of ten very active compounds from the obtained group of eighty derivatives. The compound that has the highest affinity for the H_3 receptor is derivative 77. SAR analysis and computer modeling have shown which modified fragments of lead compounds have increased activity

Summing up, the main objectives of the paper have been fulfilled. The results are well presented and their interpretation is at a high scientific level. All experiments are well arranged and measurement techniques and methods are correctly applied.

However, I have some remarks and comments related to the text in the paper "KSK-19 - Novel histamine H_3 receptor ligands reduce body weight induced obese mice".
If I good understand the authors expected that KSK19, with significant antagonistic properties at the H_3 receptor, would cross the blood-brain-barrier and blocking the H_3 receptors would trigger histamine release, which, by acting on H_1 receptors would induce loss of appetite resulting in a decrease of food intake and reduced body-weight. Based on the obtained data, it was shown that compound KSK19 after multiple administration reduced body-weight. Many laboratories examine increases in histamine levels via in vivo microdialysis. Is there a plan to carry out this type of experiment for compound KSK19? It would be strong evidence of this compound in vivo activity.

In my opinion, the reviewed Ph.D. Thesis fulfills all requirements posed on theses aimed for obtaining a Ph.D. degree and demonstrates the Author's ability of critical thinking and scientific teamwork in organic chemistry, biochemistry, and pharmacology. Therefore, I strongly recommended the Pharmaceutical Sciences Discipline Council of the Jagiellonian University in Krakow to accept the dissertation "The search for novel histaminę H_3 receptor ligands in the group of piperazine" and to permit Ms. Katarzyna Szczepańska for the public Ph.D. defense.

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