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ASSESSMENT OF THE THESIS OF Mr Adam HOGENDORF

Mr Adam Hogendorf has delivered a doctoral dissertation entitled "Aromatic basic groups in the design and synthesis of serotonin receptor ligands" pertaining to his PhD thesis at the Jagiellonian University, Krakow, Poland.

The general scientific context of the work is medicinal chemistry, that is the design, synthesis and biological evaluation of novel molecules to probe life mechanisms and develop drug candidates.

In this particular study, the ultimate objective is the development of 5-HT6 serotonin receptor antagonists to treat Alzheimer's disease and 5-HT7 receptor agonists to treat cognition impairment and neuropathic pain.

The main originality of the work resides in its strategy that consists in replacing the classical basic amine groups of 5-HT ligands by aromatic, more or less basic motifs. Interestingly, such an approach has never been exploited in the serotonin field. Adam Hogendorf was successful in discovering potent and specific 5-HT receptor ligands carrying these structural fragments.

Overview of the results and discussion:

In a first chapter, the PhD student presents a review of literature on different points related to his study: survey of the amine groups generally observed in 5-HT receptor ligands and more specifically on 5-HT6 and 5-HT7 receptors; the 5-HT6 receptor pharmacology and its ligands;

the 5-HT7 receptor pharmacology and its ligands; 2-aminoimidazole and 1-alkylimidazole in medicinal chemistry; and the non-basic or low basicity ligands of G Protein Coupled Receptors (GPCRs) and other target proteins.

In a second short chapter, the specific aims of the PhD study are presented as two workpackages (WPs):

- WP1 – the employment of 2-aminoimidazole in the design of serotonin receptor ligands, in particular a series of 5-HT6R antagonists;
- WP2 - the application of a multi-component reaction protocol in the synthesis of 1-alkylimidazole-based 5-HT7 receptor agonists.

Results and discussion are presented in Chapter 3. It is subdivided as follows:

- WP1: Chemistry of 2-aminoimidazoles and 2-aminothiazoles and structure-activity relationship study of the prepared compounds on the main target receptor (5-HT6R) in parallel with closely related off-targets (5-HT1A, 5-HT2A, 5-HT7 and D2 receptors). The absorption, distribution, metabolism, excretion and toxicity (ADMET) of the best compounds were studied leading to the selection of a lead compound for *in vivo* evaluation in Alzheimer's disease animal models. The best compound, 131i, is a selective 5-HT6 receptor antagonist, active on a scopolamine-induced cognitive impairment assay in rats, alone or in a synergistic combination with an existing drug. A model of its putative binding mode has also been proposed and discussed.
- WP2: Chemistry and pharmacology of 1-alkyl-5-aryl-1H-imidazoles and structure-activity relationships study of the prepared compounds on the 5-HT7 target receptor. New synthetic routes have been developed with success. The binding profiles of all new compounds on 5-HT1A, 5-HT2A, 5-HT6, 5-HT7 and D2 receptors have been produced. Potent, specific non-basic 5-HT7 agonists have been discovered and further studied: binding mode, ADMET, extended pharmacological profile. The best lead compound, 149l, showed an interesting activity *in vivo* in animal models of cognition and neuropathic pain.

The overall conclusions were presented and discussed in the last Chapter 4.

- WP1: The structure-activity and structure-ADME study of approximately 70 compounds (47 included in the thesis) showed that 2-aminoimidazoles could be modified in various manners while retaining 5-HT6R activity and providing different ADME properties.
- WP2: The conjugation of 1-alkylimidazoles with the indole-3-yl cores yielded a series of highly active 5-HT7R low-basicity ligands (~150 compounds, 61 included in the thesis). Non-basic compounds with high selectivity for 5-HT7R and agonist profiles were discovered whereas all known serotonin receptor agonists at that time were highly basic compounds. These pharmacodynamic properties were associated to high water solubility, good metabolic stability and high blood-brain barrier permeability of lead compounds. Lead compounds 149m and 149l are the first selective, functionally potent, orally bioavailable and brain penetrant 5-HT7R agonists. They

underwent a number of ADMET and behavioural studies which clearly showed their superiority over all the previously used molecular probes for studies of 5-HT7R functions.

Evaluation of the work presented:

General comments:

This work is an important contribution to the medicinal chemistry of 5-HT receptor ligands and beyond, to the design of novel aminergic GPCR ligands.

The scientific project was ambitious and very well defined: the discovery of novel and drug-like 5-HT6-R antagonists and 5-HT7-R agonists. There was a clear need for such pharmacological probes to explore the physiological functions of these two receptors and pave the way towards clinical candidates for associated diseases.

The strategy looked simple: replace the primary, secondary or tertiary amines classically present in 5-HT receptor ligands by conjugated cyclic moieties covering a wide range of basicity. As any great idea, such an approach looks obvious afterwards but had never been explored before....

This project required the integrated contribution of a multidisciplinary team including expertise in molecular modelling, organic synthesis, molecular pharmacology, ADMET and *in vivo* pharmacology. It is very rare in academia to cover such a large range of disciplines and more particularly to take into account the bioavailability of the compounds prior or in parallel to *in vivo* animal studies. The team and the PhD candidate must be congratulated for this integrated approach.

A huge amount of work has been accomplished and is reported in the PhD dissertation. Elegant chemical strategies were designed and implemented, leading to the production of 208 intermediate or final compounds. The final compounds have been tested for their binding affinity on several GPCRs allowing an interesting structure-activity relationship profiling. As said above, I do appreciate that the physicochemical and pharmacokinetic characteristics of the most interesting compounds have been studied. This is neither common nor easily achievable in an academic environment. It reinforces the conclusions drawn from the *in vivo* animal studies and our understanding of underlying mechanisms of action.

On the other extreme, I also appreciate the efforts to understand the binding mode of the ligands and to explain their affinity and selectivity.

Overall, this project was very successful since the objectives have been met: original, potent, selective and bioavailable 5-HT6 antagonists and 5-HT7 agonists have been designed, produced and fully characterized (pharmacodynamics, pharmacokinetics and activity in animal models of targeted diseases). The results are of great significance in the field and led to a first important publication.

I wish to congratulate Mr Adam Hogendorf and his project team members for the work delivered.

Concerning the PhD document, it is very well written and presented. The introduction of the project is well documented and the results are clearly presented and discussed. The experimental part is informative with relevant analytical data.

I have some minor comments on possible improvements of the organisation and content of the manuscript (see below) but the manuscript is excellent as such.

Minor comments and questions:

As said above, the work is excellent and very comprehensive. As reader, one is always tempted to ask for more ... The goal of the following comments is not to criticise but instead, to open the discussion for the defence.

- In the acknowledgements, Mr Hogendorf fairly quotes the names of several colleagues who contributed to the project. It would have been interesting to precise what was exactly his contribution beyond chemical synthesis (which represents already a huge amount of work). Did he contribute experimentally to the modelling, physicochemistry, pharmacology and pharmacokinetics studies?
- It would have been interesting in introduction to have a short but detailed presentation of aminergic GPCR binding sites: sequence alignment of the receptors studied or discussed in the thesis (5-HT receptor subtypes, D2 receptor); available reference GPCR crystal structures; identification of all residues present in the orthosteric binding sites; description of the residues known to interact with ligands beyond Asp3:32. This information would have been useful to support the discussions on affinity and selectivity.
- Some elements are sometimes not explicitly given to justify the options that have been taken. For instance, what were the criteria to select the 5-HT1A, 5-HT2A and D2 off-target receptors and not other receptors for compound screening (binding)? How are chosen the benchmark compounds (for example LuAE58054 and DB 742457 instead of AVN-211)? How was developed the pilot compound AH-42, at the origin of the 5-HT6 study? What is the rationale for the indole-imidazole design (an agonist pharmacophore? Former hits?).
- Apart from few quotes and data in the text, I could not find experiments and data showing the antagonist activity of the 5-HT6-R ligands and the agonist activity of the 5-HT7-R ligands.
- Many "satellite" studies are described in details in the Experimental Part: *in silico* studies, *in vitro* pharmacology (binding and functional assays), ADMET studies and animal models. It would have been useful to describe in few sentences the principle of these studies in the Results and Discussion part to help the reader making his opinion on their relevance and on the relevance of results.
- It would have been interesting to measure experimentally the pKa of the most representative and diverse structures.

As said above, these comments are minor and aim at stimulating the discussion during the defence. The dissertation document in its current form is very good and does not need supplementations or corrections.

Overall rating and recommendations.

The thesis document submitted by Mr Adam Hogendorf is well written and presented. It deals with a very important and interesting project that has been addressed with relevant and original strategies and methods. The results are excellent. This is an outstanding contribution to the field of serotonin receptors and drug design. Results have been discussed in depth with a clear presentation of potential and limits.

There is no doubt that Mr Hogendorf has acquired experience and maturity in medicinal chemistry and that his scientific potential is quite high. He clearly meets the criteria stipulated in the act of academic degrees to obtain the PhD diploma.

I do recommend accepting the thesis with great credit and allowing the defence.

Marcel HIBERT
Professor