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Review Report Ph.D. Thesis of Marcin Józef Drop

entitled

"Flow chemistry methods in the search for new 2-arylpyrrole derivatives as 5-HT$_6$ receptor ligands"

Context and scope of the thesis

Marcin Drop's thesis concentrates on the search for new ligands of the 5-HT$_6$ receptor in the group of 2-arylpyrroles obtained with the use of flow chemistry methods. The 5-HT$_6$ receptor belongs to the G-protein-coupled receptor (GPCR) family. There is considerable evidence for this receptor subtype playing a role in learning and memory. Hence, 5-HT$_6$ receptors are considered new targets for cognitive enhancement. However, so far, selective 5-HT$_6$ ligands have not been successful in clinical trials that would confirm the therapeutic utility of molecules with such a pharmacodynamics profile. M.Sc. Marcin Drop used the methods of flow chemistry to design and synthesize novel selective 5-HT$_6$R antagonists built on a 2-arylpyrrole central core. Continuous flow processing was invented to be inherently safer for many reasons, including lower reaction volume, better temperature control and ability to accommodate higher pressures without risk. Flow processing offers better selectivity, can be more environmentally friendly, and can offer an accelerated scale-up route. Flow chemistry can potentially reduce the number of steps in a synthesis, thereby resulting in a greener process. In addition, the increased robustness associated with a flow process means that should the molecule prepared prove to be a viable candidate, a repeatable protocol already exists for the materials preparation at the g to kg scale – reducing the time taken to obtain material for further assessment and clinical trials. Consequently, the topic of the Ph.D. thesis is current and relevant in the context of up-to-date research in medicinal chemistry and organic synthesis. I can therefore conclude that the choice of the topic of the Ph.D. thesis is scientifically interesting, with great practical value.

General description of the thesis

The thesis is well structures and in accordance with the international accepted scientific frame for this purpose. It begins with abstracts (in English, Polish and French) and an introduction to the subject, which directly leads to a list of several main goals of the thesis. The Ph.D. thesis is divided into 13 chapters. It is worth noting that the main part of the thesis is based on three papers, which were coauthored by the Ph.D. candidate and which were published in highly ranked scientific journals: Green Chemistry, ACS Chemical Neuroscience and Bioorganic Chemistry with a total IF = 19.88. The published papers are attached to the main text of the thesis as a supplement. I would like to emphasize that the Ph.D. candidate was in all cases the first author, which indicates his major contribution to these papers.

I appreciate that the thesis includes an introductory chapter providing a broader context of the work and one chapter clearly stating objectives of the Ph.D. thesis. All information needed for the comprehensive evaluation of the scientific values of the here presented work is available and appropriately described. The experimental part of the thesis consists of six chapters (Chapters 4-9), each addressing a specific scientific problem. The advantage of such a structure is that the chapters, consisting of integral parts, can be read and analyzed separately. Marcin Drop's Ph.D. thesis consists of 82 pages of text, with 13 figures, 15 scheme and 13 tables. Color figures and graphs presented throughout the work clearly convey information to a reader. The number and the time-span of references show that the author possesses a good comprehension of the current status of the field and its recent development. Tables and figures are included in the text, which makes it much easier to use them while reading. Great graphic and editorial diligence of the work is noticeable. The theoretical principles as well as the research part were validated with 107 valuable references. It is worth mentioning here, that most of the references are from the last decade, showing the current interest in the subject.

The introduction to the thesis presents information that illustrates the thematic scope of the doctorate. In these chapters, the author discusses the social importance of green chemistry and the need for sustainable development of the pharmaceutical industry. He emphasizes the role of continuous flow chemistry in the development of process chemistry and its role in the drug discovery process. The 5-HT_6 receptor as a molecular target of potential drugs is also characterized. The material in this part of the work is based on an in-depth analysis of research works from recent years and, according to the Reviewer, constitutes a comprehensive introduction to the experimental part.

The main aim of Ph.D. thesis was therefore to design and synthesize new selective 5-HT_6R antagonists built on the central 2-arylpyrrole core and to optimize the key synthesis steps in the green chemistry concept using a continuous flow chemistry approach. The biological studies presented in the dissertation were aimed at assessing the relationship between the structure of the synthesized compounds and their activity in signaling pathways operated by 5-HT_6R, while the pharmacological assessment was to verify the effectiveness of selected compounds in preclinical models of cognitive disorders and neuropathic pain.

The following chapters describing the experimental part of the dissertation have been developed in detail, but at the same time clearly. Figures, tables and charts are legible and clear. A large part of these chapters is a critical and comprehensive discussion of the results of own research. They are written very competently, and contain all the information needed to draw final conclusions from the research. The results are presented in an orderly manner, in accordance with the structure presented in the chapter on research methodology, which facilitates their evaluation.

Ph.D. thesis presents the synthesis of three classes of compounds. Series 1 was built using the 2-phenyl-1H-pyrrole-3-carboxamide scaffold. The structural modification comprised varying the alicyclic amines in the 3-carboxamide fragment, different electron-withdrawing and donating substituents at the 2-phenyl ring and functionalizing N1 pyrrole with aryl- or heteroarylsulfonyl moieties. The second series included the replacement of the 2-phenyl fragment with naphth-2-yl or heteroaryl moieties and the modification of the linkers between the pharmacophore fragments. Series 3 was derived from the simpler 2-phenyl-1H-pyrrole scaffold. The structural modifications involved the displacement of the alicyclic amines from the C3 position of the pyrrole ring to the C3 position of the 2-phenyl fragment or C3 position of the N1-phenylsulfonyl moiety. In total, 75 final compounds were synthesized using several multistep procedures. A significant scientific achievement of the M.Sc. Marcin Drop is development and optimization a highly efficient continuous flow protocol allowing the ring-closing metathesis of dienes to obtain 2,5-dihydro-1*H*-pyrrole-3-carboxylates. The scope of the optimization included selecting a green solvent, screening available homogeneous ruthenium catalysts and determining the suitable conditions: time, temperature, and flow rate. The Ph.D. candidate well documents his ability to conduct an independent research.

Structure-activity relationship studies showed that degradation of the central core of 1H-pyrrolo[3,2-c]quinoline to 2-phenyl-1H-pyrrole-3-carboxamide reduced the affinity for 5-HT_6R by about 10-fold. However, the compounds of series 1 showed high selectivity for 5-HT_6R over 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 receptors. Low affinity for the 5-HT_{2A} receptor is

particularly advantageous as it can avoid side effects associated with 5-HT_{2A} modulation such as psychosis, hallucinations, fear, headaches and dizziness. In addition, the compounds unlike other 5-HT_6R ligands, do not bind to α1A adrenergic receptors, M1 muscarinic, histamine H3, dopamine D2, dopamine D3, serotonin 5-HT_{2C} or 5-HT_3 receptors. They also had no affinity for the serotonin transporter (SERT) or the human ether-a-go-go gene channel (hERG). This may be beneficial in reducing the spectrum of side effects associated with the activity of these specified enzymes.

Using molecular docking methods the influence of individual groups of substituents on the activity of the 2-aryl-1H-pyrrole ring was discussed. SAR studies in series 1 showed that selected structural moieties were favorable for the preparation of 5-HT_6R ligands with high affinity for 2-phenyl-1H-pyrrole-3-carboxamide arylsulfonamide derivatives: pyrrolidin-3-yl or piperidin-4-yl moieties in the 3-carboxamide fragment, fluorine atom at the *C*3 or *C*4 position of the 2-phenyl ring and 3-chlorophenylsulfonyl moiety at the *N*1 position. SAR studies on the second series highlighted 1-(phenylsulfonyl)-2-phenyl-1H-pyrrole as the principal fragment of the 5-HT_6R ligands. Among the compounds of series 3, a preference was observed for the piperazinyl moiety directly substituted at the C3 position of the N-phenylsulfonyl moiety.

Another significant scientific achievement presented in Ph.D. thesis is the identification the compound (R)-2-(4-fluorophenyl)-1-[(3-chlorophenyl)sulfonyl]-N-(pyrrolidin-3-yl)-1H-pyrrole-3-carboxamide (110) as a 5-HT_6R inverse agonist at Gs and Cdk5 signaling and the compound 2-(4-fluorophenyl)-1-[(3-chlorophenyl)sulfonyl]-N-(piperidine)-4-yl)-1H-pyrrole-3-carboxamide (126), as 5-HT_6R inverse agonist in mTOR signaling. The replacement 1H-pyrrolo[3,2-c]quinoline scaffold in 2-phenyl-1H-pyrrole-3-carboxamide (Series 1) shifted the functional activity at 5-HT_6R-operated Gs signaling from neutral antagonists to inverse agonists. Subsequent studies showed that further degradation of the 3-carboxamide moiety and simultaneous shift of the basic center at the C3 position of the 2-phenyl moiety or at the C3 position of the N1-phenylsulfonyl fragment provide compounds with neutral Gs signaling antagonistic properties (Series 3). Moreover, the most promising 5-HT_6R inverse agonists showed high metabolic stability, no cytotoxicity and good brain penetration. Finally, the compound 126 showed a rapid and potent effect against in vivo allodynia in SNL-induced neuropathy in rats. Given the limitations of currently available therapies, the ability of 5-HT_6R inverse agonists to alleviate painful symptoms in various models of traumatic neuropathy as well as neuropathies caused by metabolic disorders (e.g. diabetes) and

chemotherapy (e.g. anti-cancer drugs) certainly prompts the continuation of research in this scope.

At the end of the dissertation, in Chapter 9, M.Sc. Marcin Drop reverts to optimizing the synthetic pathway for the compound 124, which has been shown in preclinical studies to alleviate painful symptoms associated with neuropathies of various etiologies and to improve concomitant cognitive deficits in rats. Taking into account the concept of green chemistry, it was developed a new method for the preparation of the compound 124 limiting the number of synthesis steps in order to maximize the incorporation of the starting materials into the final product. The currently used 4-step synthetic route for the preparation of methyl 2-(3-fluorophenyl)-1H-pyrrole-3-carboxylate has been replaced with palladium-catalyzed, single-pot, nucleophilic addition of 3-fluorophenylboronic acid to ethyl 2-cyano-4,4-diethoxybutyrate, followed by a cyclodehydration using continuous flow approach. The new pathway has been also designed to reduce the amount of solvents and chemicals used for both synthesis and purification that are toxic to humans and the environment. It is an important scientific achievement as keep in mind that if the technology selected then forms parts of a scalable flow chemistry platform, the researcher has the ability to directly apply the conditions identified at the laboratory-scale for production of the given material with the confidence that there will not be the risk of a change in product quality or the failure to scale while maintaining productivity at the projected costs.

The section "Conclusions and Perspectives" lists the key findings from this study. However, the reader may feel a certain insufficiency related to the lack of confrontation of own research with the achievements of other research teams. The concluding chapter includes also some perspectives for future work.

Specific comments

I have only few comments and questions, which should be answered during the Ph.D. thesis defense:

1. Ph.D. thesis presents a new optimized method for the synthesis of the compound 124, but we find no answer as to whether this method can be used to synthesize any compound of series 1. What are the limitations of this method in terms of the choice of substrates and reaction conditions?
2. Pharmacokinetics is of great significance in the selection of drug candidates, and estimation of pharmacokinetic parameter in the early stage of drug development has become the trend of drug research owing to its time- and cost- savings advantages. In

Ph.D. thesis pharmacokinetics evaluation was only performed for 4 compounds. However, they turned out to be crucial for excluding the compound 111 - the enantiomer of the compound 110 from further studies. Is it not worth considering ADME/Tox and pharmacokinetics assessments at an earlier stage in biological testing for more compounds, for example by using rapid in silico or in vivo methods?

3. Looking to the future, what are the key challenges facing flow and scale-up chemistry with respect to drug discovery?
4. Microwave-promoted synthesis has become a growing area of interest for opening up new avenues of drug development, while providing a greener alternative to traditional batch processes. How will using this tool of synthesis advance the principles of green chemistry?

Final evaluation statement

To sum up, the doctoral dissertation of M.Sc. Marcin Drop, presented for assessment, entitled "Flow chemistry methods in the search for new 2-arylpyrrole derivatives as 5-HT_6 receptor ligands" are characterized by significant advantages, which include methodology adequate to the tasks set, topicality and practical value of the obtained results as well as valuable discussion. Ph.D. thesis highlights very interesting results obtained in the framework of a consistent experimental work and well-designed objectives. I would like to emphasize the comprehensive interdisciplinary character of the Ph.D. thesis covering the research fields of medicinal chemistry, organic synthesis, pharmacology and biochemistry. The presented research contributes to our knowledge on structure and synthesis of 2-arylpyrrole derivatives as new 5-HT_6 receptor ligands, and brings new, important and original findings. It is an independent scientific output, fully meets the requirements for Ph.D. thesis. Finally, I recommend to the scientific committee to award the Ph.D. degree to Marcin Drop.

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