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Sosnowiec, November 14, 2022

Review of the doctoral dissertation of Mr. Dawid Maliszewski, M.Sc.

Title:

„Synthesis and investigation of biological activities of the new 1,3,5-triazine derivatives"

The evaluation was prepared in response to a letter from Prof. Wojciech Miltyk, the Dean of the College of Pharmaceutical Sciences, Medical University of Bialystok (the letter 3.11.2022r).

General information

Mr. Dawid Maliszewski, M.Sc. is a graduate of chemistry, University of Bialystok, specializing in organic chemistry. He completed his first degree in 2015 and his second degree in 2017. He began his doctoral studies in 2018 at the Medical University of Bialystok. During his education, he obtained a teaching license. Since 2018, he has been working as a chemistry teacher and educator at Elementary School No. 30 in Bialystok. While carrying out research work during his doctoral studies, he completed a one-month research internship at the Institute of Organic Chemistry of the Technical University of Lodz. In 2019 - 2021, he carried out two research projects on s-triazines (in the role of project leader) and was a co-principal investigator of the statutory project N/ST/ZB/18/001/2204.

PhD student has 5 scientific publications with a total IF = 21.053. The papers were published in the journals: *Pharmaceuticals, Molecules, Investigational New Drugs and Journal of*

Antibiotic. He is co-author of 12 presentations at national and international scientific conferences. The publication: Maliszewski Dawid, Drozdowska Danuta, "Recent advances in the biological activity of s-Triazine core compounds" presented in the journal *Pharmaceuticals* is a review paper and was included in the dissertation. The work presented is a very significant and impressive.

Evaluation of the dissertation

The doctoral dissertation of Mr. Dawid Maliszewski, M.Sc. was prepared in English. The dissertation was supervised by Assoc. Prof. Dr. Danuta Drozdowska and Prof. Dr. Rasime Damirel of Eskisehir Technical University, Turkey.

The synthetic part of the research was carried out at the Department of Organic Chemistry, Medical University of Bialystok and the Institute of Organic Chemistry, Technical University of Lodz, while the biological part was performed at: Eskisehir Technical University, Turkey (microbiological research), the Department of Pharmaceutical and Biopharmaceutical Analysis and the Department of Hematology Diagnostics (anticancer research), Medical University of Bialystok. The dissertation does not indicate the information where biological studies of acetylcholinesterase and beta-secretase inhibition were performed.

The PhD student's research addresses current, applied and important scientific topics. They concern the synthesis and analysis of the activity of: antibacterial, antifungal, anticancer, antineurodegenerative new nitrogen-rich s-triazine derivatives. These topics are well in line with modern trends in medicinal chemistry, focusing attention on the search for new biologically active compounds effective in the treatment of diseases of civilization.

The Author has divided the dissertation into XIII chapters: Introduction, Purpose of the work, Materials and methods, Results, Discussion, Conclusions, and Abstracts: English and Polish, References and a list of figures, diagrams and tables. The dissertation is 97 pages long, in which the last 19 pages are Annex 1 (Chapter XIII, a review publication from *Pharmaceuticals* 2022). In the literature section, the author shows the method of synthesis of the 1,3,5-triazine (s-triazines) system and various perspectives of biological activities of these derivatives as multi-target therapeutic substances. He shows compounds that are already drugs, new derivatives at various stages of biological research, and performs preliminary SAR analyses. For this part of the dissertation, Mr. Maliszewski used 74 literature reports, mostly related to recent worldwide publications.

Next, the aims and scopes for the dissertation are shown. In this chapter, the Author indicates that the topic of the doctoral dissertation is a continuation of the previously started research in the area of s-triazines that was conducted at the Department of Organic Chemistry of Medical University of Bialystok. The PhD student carried out a several-step synthesis leading to efficient preparation of final 1,3,5-triazines with substituents: 2-chloroethylpiperazine, methoxy and dipeptide groupings. The Author obtained 8 new, previously undescribed s-triazine derivatives **4a-4h**, the structure of which was confirmed by ^{1}H NMR, ^{13}C NMR and HR MS spectroscopic analyses. These compounds and their substrates **3a-3h** are summarized in Table 1. At this point, I would like to ask why the synthesis was limited to only such eight derivatives, and why HR MS mass spectrometry analyses (confirming purity) were not performed for compounds **3a-3h**. The Reviewer also points out that it would be good to assign individual chemical shifts of the carbon atoms, which can be done using available 2D NMR techniques. Attention should also be paid to the correct notation of: the ^{1}H NMR and ^{13}C NMR expressions, as there were inaccuracies on pages 27, 64, the name of the substituent 2-chloroethylpiperazine rather than 2-ethylpiperazine (page 64), and the notation of dichloromethane as DCM (page 27).

The final new s-triazine **4a-4h** derivatives obtained were subjected to antibacterial activity (including gyrase inhibition analysis), antifungal activity, anticancer activity and acetylcholinesterase, β-secretase inhibition analysis. Molecular modeling of bacterial gyrase inhibition and preliminary *in silico* structural studies were also carried out (Table 9).

For the study of antibacterial and antifungal activity, the Author included compounds **5a-7c**, which were described in 2016 by the team of Frączyk at al., and were previously obtained in the team of Professor Drozdowska. At this point, my question arises as to what purpose the Author included these compounds in the bioassays as this was not clearly specified in the Aims section. During the study of antibacterial and antifungal activity, the most active derivatives **4c** and **4d** were selected. A molecular target was identified in the bacterial gyrase inhibition assay, which was further confirmed and visualized by molecular modeling.

In the next step, the anticancer activity against breast cancer cells was tested: MCF-7 and MDA-MB-231 for compounds **4a-4h**. For comparison of anticancer activity, the IC_{50} literature activity of chlorambucil as a reference substance was recalled. Among the derivatives, derivative **4a** was found to be the most active with IC_{50} = 18.99 μM, while the other derivatives

showed generally lower activity. Derivatives **4b** and **4d** showed comparable activity to chlorambucil against MDA-MB-231 cells. As a reviewer, I would like to ask why normal human cells were not used for the panel of this study to test full cytotoxicity, additionally why only two types of cancer cells were selected, and whether it would not be worthwhile to test the activity of these derivatives against other cancer cell lines. It also merits consideration to investigate the mechanism of anticancer activity for the most promising derivative. To the results of these studies, it is also necessary to attach a statistical analysis, which is missing in this part.

Next the Author checked the effect of the obtained compounds **4a-4h** on the inhibition of acetylcholinesterase and β-secretase, as enzymes that play an important role in the neurodegenerative process. However, the author did not indicate the place where such tests were performed. Is it implicitly understood that they were performed in the Department of Organic Chemistry? Among the derivatives obtained, compound **4a** showed inhibition comparable to the reference substance donepezil. The PhD student also conducted a preliminary analysis of molecular descriptors as well as checked compatibility with the Lipinski rule of five with the BIOVIA program. The results are summarized in table 9 in the discussion chapter. I think that table 9 should be included in the results chapter along with a clear indication of the web server, which should also be noted in the references.

In the final part of the dissertation, Mr. Maliszewski specifies the conclusions of the research work performed and confirms the positive verification of the research hypothesis.

As a Reviewer, I appreciate the synthetic work in the organic chemistry laboratory, the ability to perform difficult structural analyses and a wide panel of biological research. Out of the Reviewer's duty, I have to mention some editorial errors that appeared in the dissertation: references are cited in parentheses but also as a superscript (p. 42), the compound numbers on p. 52 were not marked in bold, or the 2-chloroethylpiperazine substituent was mistakenly called 2-ethylpiperazine (pp. 62,64). These inadequacies do not detract from the value of the results presented.

Summary

The conducted research appropriately fits into the current trend related to the design of new bioactive substances that can be used in the future to treat diseases of civilization. The previously presented scientific achievements of the PhD student are significant and highly

promising. The results gathered in the doctoral dissertation can certainly become the subject of several scientific publications in prominent journals in medicinal chemistry. Mr. Maliszewski's doctoral dissertation fully demonstrates general theoretical knowledge as well as the ability to conduct scientific work independently.

In conclusion, I state that the doctoral dissertation of Mr. Dawid Maliszewski, significantly contributes to the development of the discipline of pharmaceutical sciences and meets all the requirements for doctoral dissertations. I request the College of Pharmaceutical Sciences, Medical University of Bialystok to admit the dissertation to further stages of the procedure in applying for doctoral degree.

Sosnowiec, November 14, 2022

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