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HR EXCELLENCE IN RESEARCH

Evaluation of the doctoral dissertation entitled

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

performed by
mgr Dawid Maliszewski

**in Department of Organic Chemistry, Faculty of Pharmacy
with the Division of Laboratory Medicine,
Medical University of Bialystok**

in medical and health sciences in the discipline of pharmaceutical sciences

In the development of medicinal chemistry, including the search for compounds with the multidirectional biological activity, the s-triazine ring (1,3,5-triazine), which is usually the core of the molecule, was found to exert a wide range of applications in cross-linking polymeric materials and combining the properties of various substituents. The s-triazine molecule, due to the possibility of substituting three substituents, gives the possibility of obtaining hydride compounds with various activities. Noteworthy is the wide spectrum of biological activity of s-triazine derivatives, which is multidirectional and includes, among others, antibacterial, antifungal, anticancer and anti-neurodegenerative activity. This fact is confirmed by numerous derivatives that have been introduced to the pharmaceutical market, including altretamine, decitabine or almitrine, and numerous conjugates in the phase of both preclinical and clinical research.

Population aging and the development of effective treatments and prevention of cardiovascular diseases cause changes in the structure of causes of mortality. Therefore, the most important challenges of modern medicine include cancer, neurodegenerative diseases and progressive antibiotic resistance. In this context, research aimed at finding new drugs seems to be extremely important, which is one of the overriding goals of pharmaceutical sciences. There is no doubt that this is a multi-stage process that requires the cooperation of many scientists and practitioners from several disciplines.

Therefore, the justified aim of the research, submitted for the review of Dawid Maliszewski's doctoral dissertation, was to design, synthesize and evaluate the biological activity of 1,3,5-triazine derivatives containing a dipeptide, 2-ethylpiperazine and a methoxy group as substituents. It is worth emphasizing that the implementation of the assumptions of the interdisciplinary project was possible thanks to the cooperation and coordination of research work by the doctoral dissertation supervisors Mrs. Dr. hab. Danuta Drozdowska and Mr. Prof. Dr. Rasime Demirel. In the chemical part, new hybrid compounds were designed and obtained, for which potential antibacterial, antifungal and anticancer activity was determined as part of biological tests. Moreover, the ability of 1,3,5-triazine derivatives to inhibit AChE and BACE1 enzymes was tested. The study was supplemented by the determination of the possibility of interaction of new conjugates with *E. coli* and *S. aureus* DNA gyrase using the molecular docking technique.

The title of the doctoral dissertation has been correctly formulated and fully reflects the purpose of the research. I consider the choice of the subject of doctoral thesis to be accurate, consistent with the direction of modern scientific research and the current state of knowledge. I consider the undertaken problem to be justified, extremely important both: i/ from the scientific point of view (including the development of medicinal chemistry), ii/ medical and iii/ social significance, due to the fact that civilization diseases are the main cause of death in the world. Therefore, one of the priorities of today's medicine is to search for active, selective drugs with limited cytotoxicity in relation to normal body cells.

The doctoral dissertation submitted for review includes 98 pages of text with references and an attached appendix - a review publication in which the PhD student is the first author. The text of the work contains chapters typical for scientific studies, with the right proportions. In addition, the work contains a list of abbreviations used in the text of the work, which greatly facilitates the assessment of the dissertation.

In the Introduction, the PhD student basing on extensive literature review, presented the current knowledge in the field of characterization of approved and researched s-triazine derivatives. It is worth emphasizing that the last subchapters of this part of the work were devoted by the author to discussing the benefits of designing innovative drugs with a multidirectional mechanism of action. The issues discussed on the 16 pages of the Introduction section have been additionally supplemented with 13 figures. The issues presented in the Introduction of the work testify to the very good preparation of the PhD student to undertake research, which is the basis of the doctoral dissertation, and also constitute an introduction to the set research goal.

The series of research presented by Dawid Maliszewski, MSc, is a carefully planned and well-thought-out experimental panel enabling the analysis of a complex research hypothesis. Achieving the set goal of the work, the PhD student synthesized new 1,3,5-triazine derivatives and conducted research using the in vitro model and i/ on pathogenic bacteria, yeast and filamentous fungi and ii/on breast cancer cell cultures (estrogen-dependent cells of the MCF-7 line and non-estrogen-dependent cells of the MDA-MB-231 line as a model of triple-negative breast cancer). In addition, Dawid Maliszewski, MSc, assessed the inhibitory potential of the tested compounds against AChE acetylcholinesterase (Ellman's colorimetric method) and BACE1 β -secretase (FRET method).

It is worth noting that the in vitro experiments using breast cancer cell cultures were carried out in cooperation with the Department of Drug Analysis and Bioanalysis and the Department of

Hematology Diagnostics of the Medical University of Białystok. In the case of in vitro studies on the model of pathogenic bacteria, fungi and yeast, the analyzes were performed in cooperation with the Department of Biology of the Technical University (Eskisehir) in Turkey.

A detailed description of the research methods used is included in the Materials and Methods chapter. The presented methods are, in principle, up-to-date and adequate to the scientific problems being solved and do not raise any doubts. However, there was an issue that I feel needs clarification or discussion:

i/ what was the selection criterion for the used in vitro cellular model?

ii/ did the PhD student participate in in vitro tests (microbiological panel) as part of the international character of the work?

The wording of the above question results only from the reviewer's privilege, which I have used, and in no way is it a critical remark. The above issue may, however, be a guide to improving the research workshop and an interesting subject of discussion in the scientific and academic field.

The research results obtained were edited on 19 pages of the work in the "Results" chapter and documented in detail in 8 summary tables and 4 figures. In the part devoted to the discussion, the author makes a basic discussion of the results as well as draws partial conclusions after each cycle of research. This chapter proves a very good knowledge of the subject and good preparation for the implementation of research. It is worth noting that the PhD student confronts the obtained results of his own work with numerous research results of the works of other authors.

The research carried out allowed for the formulation of justified conclusions, corresponding to the assumed purpose of the work and bringing new important information to the current state of knowledge. In my opinion, the most important results of the conducted research were the demonstration that:

i/ the applied methodology for the synthesis of 1,3,5-triazine derivatives is characterized by high efficiency and purity of the obtained compounds,

ii/ s-triazine derivatives containing a dipeptide, 2-ethylpiperazine and a methoxy group as a substituent are characterized by high antibacterial and antifungal activity,

iii/ new hybrid compounds show the ability to interact with bacterial DNA gyrase as a molecular target,

iv/ the new derivatives were characterized by high anticancer activity and the ability to modulate the activity of acetylcholinesterase and β -secretase, which confirms the multifunctional nature of the tested compounds as potentially new drugs, in which the 1,3,5-triazine core is an important structural element.

At this point, due to the fully justified scientific achievements of the PhD student and the application nature of the presented research results, let me ask a question about possible directions for further research in the subject of the synthesis and assessment of biological activity of new hybrid compounds containing the 1,3,5-triazine core. Are further experimental works planned with the use of normal cells in order to demonstrate the potential selectivity of their mode of action.

The 106 items of scientific literature cited by the PhD student reliably reflect the state of knowledge within the discussed topic. It is worth emphasizing that most items of all literature data are reports from the last 10 years, and of them, 52/106 items (about 50%) are works published in the last 5 years, which indicates the high topicality of the scientific, medical and social problem undertaken for

assessment by the PhD student. The doctoral dissertation was completed with an abstract in Polish and English.

The results obtained by the PhD student are original and presented in a reliable manner. The author uses professional vocabulary, which proves that he is well versed in the researched field. The content of the work is written carefully, concisely and is maintained in a logical sequence.

It is with great satisfaction that I would like to emphasize the cognitive value of the scientific studies submitted for evaluation and their importance for understanding the mechanisms and treatment options for civilization diseases. The high substantive value of the dissertation presented for review is confirmed by the publication of some of the research results in the form of original papers - in journals with a significant Impact Factor index (Pharmaceuticals - Impact Factor: 5.215; Score of the Ministry of Science and Higher Education: 100; Molecules - Impact Factor: 4.927 Score of the Ministry of Science and Higher Education: 140). It should also be emphasized that in the case of both publications, the PhD student is the first author. It is also worth noting that the results of the dissertation submitted for review have been presented at international scientific conferences (Swiss Chemical Society ONLINE, 2021, French Medicinal Chemistry Society: Caen, France, 2020).

To sum up, the doctoral dissertation of Mr. Dawid Maliszewski, M.Sc., submitted for evaluation is included in the research trend, which is extremely important both from the scientific and medical point of view, and is devoted to the search for potential new therapeutic forms of civilization diseases. In addition, I believe that the dissertation submitted by the PhD student, due to the presented scientific level and cognitive values, as well as basing the conclusions drawn on extensive experimental material, fully meets the statutory requirements for doctoral dissertations.

The high substantive value of the dissertation presented for review is confirmed by the publication of research results in the form of original papers in journals with a significant Impact Factor index.

At the same time, taking into account the new cognitive elements contained in the evaluated dissertation, the use of a complex experimental panel, as well as the very important practical significance of the presented research, I propose to distinguish the work.

In conclusion, with reference to the above, I express the opinion that the thesis submitted for review meets the requirements for doctoral dissertations, which in turn entitles me to apply to the Council of Pharmaceutical Sciences of the Faculty of Pharmacy with the Department of Laboratory Medicine of the Medical University of Białystok for admission doctoral dissertation and admitting Mr. Dawid Maliszewski to further stages of his doctoral dissertation.

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