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# The Review on PhD Dissertation of Mr. Dawid Maliszewski, M.Sc., entitled "Synthesis and investigation of biological activities of the new 1,3,5-triazine derivatives"

## Introduction

The PhD thesis of Mr. Dawid Maliszewski was performed under supervisions of Dr hab. Danuta Drozdowska and Prof. Dr. Rasime Demirel at Department of Organic Chemistry, Faculty of Pharmacy with the Division of Laboratory Medicine, Medical University of Bialystok, Poland and at Department of Biology, Eskisehir Technical University, Turkey, respectively. The dissertation has been prepared in a traditional way, and the theoretical background presented in *Introduction* has been extended in the review article of this PhD-Student, published in *Pharmaceuticals*, the prestigious JCR journal. In addition, Mr. Maliszewski, is the first author of one original paper published in *Molecules* journal and the co-author of three other scientific articles, which translates into a total impact factor >21 (MNiSW=550) as well as he performed 12 congress presentations, including 4 international conferences. He took part in three Polish scientific grants, playing the role of principal investigator in two ones.

At the outset, therefore, I would like to praise the comprehensive scientific activity of this PhD Student, which from the formal point of view fully entitles him to apply for a doctoral degree.

## The scientific and editorial value of the Dissertation

The main subject of the dissertation is the synthesis and evaluation of the biological activity of compounds containing the 1,3,5-triazine core (named s-triazine) in the search for a new drug, taking into account three important therapeutic directions, i.e., these against: microbial infections, cancers and neurodegenerative diseases - in particular Alzheimer's disease, respectively.

A justification of such a direction of research in the discipline of pharmaceutical sciences is indisputable both in the light of the greatest therapeutic challenges and the current state of the pharmaceutical market. Additionally, this choose of research direction can be aided with the wide range of biological activity and the still inexhaustible possibilities for further pharmacomodulation in the group of 1,3,5-

triazine derivatives. The PhD-candidate presents the essence of such argumentation in his 16-page *Introductio*n, analyzing the achievements of world research in medicinal chemistry on 1,3,5-triazine derivatives, representing the three aforementioned directions of the pharmacological action, including drugs available on the pharmaceutical market, as well as those in the preclinical studies. In addition to the pharmacological characteristics of the chemical group under consideration, he smuggled the methods of their synthesis, also referring to broader data in this field, contained in his review article, which he attached at the end of the dissertation. In *Introduction*, he also emphasized the importance of introducing an polypeptide substitution into the triazine system, thus justifying the direction of chemical modifications taken in his dissertation.

The main area of the research tasks described in the dissertation is the chemical synthesis of s-triazine derivatives, substituted with both a differentiated dipeptide fragment and the piperazine with the terminal chloroethyl group, conditioning alkylating actions useful for the desired cytotoxic activity against cancer, bacterial or fungal cells, respectively.

The obtained compounds were then tested in an extensive *in vitro* biological screening in order to assess the antibacterial, antifungal, anticancer and inhibitory effects of enzymes involved in the pathophysiology of Alzheimer's disease. The tests also included the assessment of potential cell mechanisms at the molecular level - in particular, experimental studies and computer-aided theoretical assessment of the effect of this series of compounds on gyrases A and B.

Although the experimental chemical works concerned the synthesis of only 8 final compounds, their high chemical level is worthy of recognition. The scope of chemical works included ambitious, multi-step syntheses, preceded by the preparation of appropriate dipeptide intermediates, which involved various methods of protection and deprotection, or chlorine substitution at the triazine ring, i.e. reactions that are not easy to control, and which require high analytical skills to interpret spectral results in order to assess the purity and identity of the obtained structures.

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In addition, the PhD student's experience in conducting comprehensive biological screening, i.e.: experimental microbiological tests and all *in vitro* studies on molecular mechanisms at the cellular level, as well as molecular modeling taking into account the optimization of crystallographic structures of protein targets (gyrases) available in the PDB, an accurate determination of the appropriate binding pockets and docking simulations with the correct assessment of favorable energy states, more than meets the requirements for a young scientist applying for the PhD degree in the field of pharmaceutical sciences.

Therefore, the comprehensive scientific expertise and research tools that Mr. Maliszewski mastered during his doctoral studies deserve my great appreciation, especially if adding to this the ability to elaborate and publish results, as evidenced by his first authorship in two scientific articles in the renowned MDPI journals.

The undoubted scientific achievements of the PhD-thesis, which are part of the global research in organic and pharmaceutical chemistry, are the design and successful synthesis of 8 new dipeptide s-triazine derivatives, which turned out to be active gyrase inhibitors in the fight against drug-resistant microorganisms, as well as the pro-apoptotic compounds in breast cancer cells - useful for potential cancer therapies, also showing moderate AChE and BACE1 inhibitor actions - promising for innovative approaches in the treatment of neurodegenerative diseases. Adequately to the performed biological tests, the qualitative analysis of the structure-activity relationship based on the obtained results, enables an election of new lead structures, for the next modifications in the search for a drug with a stronger and selective direction of pharmacological action, which contributes significantly to the development of Polish and global pharmaceutical sciences.

Thus, I highly appreciate the scientific value of this dissertation and its contribution to the development of the pharmaceutical sciences discipline.

The dissertation book has a total of 98 pages, which consists of 13 chapters, including 8 main and 5 additional, i.e. the lists and attachment. The main chapters begin with a 16-page *Introduction*, followed by the *Aims and Scope*. *Materials and Methods* chapter presents experimental details of the conducted research, the results of

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which have been collected in the next one, named *Results*, and additionally discussed in the following *Discussion* chapter, while the conclusions drawn from them were collected in the form of nine points in *Conclusions* chapter. The PhD student referred to 108 scientific literature items, in most of the JCR journals published in the last twenty years. The dissertation is characterized by the clarity of descriptions supported by an elegant graphic design, composed of 5 Figures, 18 Schemes and 9 Tables, where a particularly aesthetic impression is made by carefully selected figures of the results coming from the molecular modeling performed.

## Questions and critical comments

1. On the other hand, I should complain the Scheme 13 (p. 24), to which no referring descriptions in the text are found, and the laconic title without explanations, requires a lot of intuition from the reader to understand its idea and role in this dissertation. I would like the PhD-student's to explain this to me during the defense.
2. Another ambiguity for me, it is the study of biological activity for the group of s-triazines described by Frączyk et al. in 2016. On page 42 it says:
   "The antimicrobial activity of all the compounds was tested *in vitro* on pathogenic bacteria, yeast, and filamentous fungi […] and compared with 11 s-triazine derivatives (Table 2) descripted by Frączyk et al. [80]".

From further context, however, it follows that the group of these 11 compounds is not a comparison, but rather an extension of the tested series (**4a**-**4h**) - tested and analyzed in terms of results in an equal way. What, then, decided that this series was included in the dissertation? Was the biological screening for compounds **5a**-**7c** the subject of the PhD Student's own research?
The dissertation does not show these issues and, at all, there is no information about which experimental and theoretical works were the subject of the PhD Student's own research, in which he partially participated, and which were entirely carried out by collaborators (and who was it by name). The lack of any acknowledgments suggests that all research tasks were exclusively performed by the PhD-candidate. Was it so? Please address and explain these issues during the defense.

3. The following mistakes should also be noticed and corrected prior further presentations of the PhD-thesis results:

3.1. *M. luteus* (NRRL B-4375) is missing in „Materials and methods ", but results for it occur in *Results* paragraph;

3.2. pp. 43 and 44 - inconsistency in the description of the effects of compounds on *E. coli* gyrase, i.e:

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"In turn, substances **4c**, **4d** and **7b** (100 nM) and **4a**, **4d**, **4e**, **6f** and **6c** (300 nM) inhibited *E. coli* gyrase action on supercoiled kDNA (Figure 2)" is writen in the first part of the description, then (at the end):

"Triazine analogues: **4a**, **4e** and **6c** not inhibited *E. coli* gyrase at neither concentration"

The PhD-thesis is written in correct English, I found only minor and few language mistakes and the so-called. typos, e.g.:

- page 9 – it is „ChemSpaider", it should be „ChemSpider"
- pages 17 and 18 – incorrect strain nomenclature (incoherent with the Linnean rules), i.e.:
*S. Aureus*
Staphylococcus aureus, Bacillus subtilis, Bacillus cereus
Candida albicans, Candida glabrata, Cryptococcus neoformans and Aspergillus niger
*A. Baumannii*
*C. Neoformans*
- page 21 – it is „Lorak et al.", it should be „Lolak et al."
- page 21 **–** "Maqbool et al. designed and synthesized derivative **20**" should be "Maqbool et al. designed and synthesized derivative **21**".
- page 43 "Triazine analogues: **4a**, **4e** and **6c** not inhibited *E. coli* gyrase" ("did" missing)

## Conclusions

However, the aforementioned minor shortcomings do not diminish my overall positive opinion for this PhD-dissertation. The PhD-thesis represents the high level of interdisciplinary research in the areas of: organic chemistry, microbiology, pharmacology, molecular biology and molecular modeling - in line with the broadly understood pharmaceutical sciences.

**Thus, I recommend this PhD-thesis to the high Senate of the Medical University of Bialystok with a request to admit Mr. Dawid Maliszewski, M.Sc. to the final stages of the doctoral procedure.**

Krakow, 16 November 2022

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