REVIEW

from Prof. DSc Sonia Varbanova Ilieva,

Faculty of Chemistry and Pharmacy, Sofia University “St. Kl. Ohridski"

on a dissertation for awarding the educational and scientific degree "**Doctor**"

in Professional field 4.2. Chemical sciences (Organic chemistry)

Author: Maria Andreeva Argirova

Topic of the dissertation: *Synthesis of 1H-benzimidazol-2-yl hydrazones and study of their anthelmintic, antineoplastic, and radical scavenging activities*

Supervisor: Prof. Dr Denitsa Pantaleeva

General presentation of the materials deposited

The author of the dissertation is Maria Andreeva Argirova - full-time PhD at the laboratory "Structural Organic Analysis", IOCCP, BAS, enrolled by order No РД -09-20 / 31.07.2018. The supervisor is Prof. Denitsa Pantaleeva. Maria Argirova was unenrolled with the right of defense on August 1, 2021. She has successfully passed exams in three specialized disciplines, an exam in language training, and a Photoshop course.

Maria Argirova has graduated with a Master of Chemical Engineering, majoring in Fine Organic Synthesis at the University of Chemical Technology and Metallurgy, Sofia. In 2018 she entered a full-time PhD at IOCCP, BAS, and since 2022 she has been an assistant at IOCCP.

The materials presented by Maria Argirova meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the relevant regulations for its implementation, and meet the criteria for obtaining the scientific and educational degree "Doctor". Two publications in reputed international scientific journals are included in the dissertation: *RSC Advances*, IF 4.036, quartile Q1 и *Chemico-Biological Interactions*, IF 5.168, quartile Q1. Five citations are noted on the article in *RSC Advances*, 2021. Therefore, the minimum criteria for the Doctoral degree are met and exceeded: M. Argirova has achieved 50 points (according to the indicator Г 30 points are required).

The thesis is dedicated to the synthesis of new benzimidazolyl hydrazones and their metal complexes and the study of their biological activity in three main areas - anthelmintic, antineoplastic, and radical scavenging action. One of the hypotheses underlying the research is that the presence of more than one pharmacophore in a given molecule could cause synergism of biological activity. A range of spectral methods has been used to prove the newly synthesized compounds. Theoretical quantum-chemical calculations have been applied to investigate the structure and properties of compounds. The biological activity of the synthesized benzimidazolyl hydrazones has been investigated, and through a combined analysis of the conducted in vitro experiments and molecular docking, conclusions are made on the mechanism of action.

The dissertation (215 pages) consists of 3 main parts – Bibliographic review (75 pages), Materials and methods (20 pages), Results and discussion (80 pages). At the end of the dissertation a summary of the results is made, the main conclusions and the scientific contributions of the conducted research are formulated. Some of the materials included in the dissertation have been presented by posters at 10 scientific forums in the country and abroad. Maria Argirova has participated with oral presentations in 7 national scientific conferences/seminars.

The subject of the dissertation is topical and especially interesting from a scientific point of view as related to a versatile study of the properties and biological activity of newly synthesized substances in order to examine a synergism in their biological action. An interesting question is whether studies of similar synergism in the biological activity of benzimidazole hydrazones have been published in the literature.

In the literature review the biological activity of 2-aminobenzimidazoles is examined, paying special attention to the influence of benzimidazole derivatives on the polymerization of tubulin. The latter is related to one of the goals set - to examine newly synthesized benzimidazole derivatives as potential inhibitors of tubulin polymerization. The biological activity of different classes of compounds is presented mainly in terms of the results from molecular docking studies and most of the figures in this part (e.g. L13-L21) present published results from molecular docking studies. I believe that the main contributions of the PhD student in the two presented publications, which have a pronounced multidisciplinary character, are in the field of organic synthesis and theoretical quantum chemical calculations. These two fields are not included in the bibliographic review.

The Material and Methods section presents the organic synthesis of the new compounds and their metal complexes (synthetic procedures, yields, spectroscopic characteristics). The methodology for measuring the three types of biological activity (antineoplastic, antiparasitic, antioxidant and radical-scavenging) is presented, as well as the methodology for estimating the effect of hydrazones on the polymerization of tubulin. The theoretical methods for quantum-chemical calculations and molecular docking are briefly introduced. It is stated on p. 99 that *"for a better description of geometry, calculations with functional M062X - SP calculations have been carried out for computing frequencies on the basis of geometry, optimized with the B3LYP functional"* (the same sentence is repeated on page 117). SP calculations do not change geometry and structure and therefore SP calculations cannot be used *for a better description of geometry*. The second part of the sentence is unclear. The "*second derivative matrix*" is quoted on the same page - it is not clear what is this matrix. I would like to ask for an explanation.

Scientific contributions

(i) 40 1H-Benzimidazole-2-yl hydrazones have been synthesized, of which 37 new compounds, and 4 metal complexes with Cu(II) and Fe(II), of which 2 new - not published in the literature.

The synthesized benzimidazole hydrazones are grouped into 3 series: Series A - with varying substitutes in benzimidazole heterocycle and in the phenyl ring, Series B - with hydroxy and methoxy substituents only in the phenyl moiety, and compounds of Series C, containing the hydrazones of series B with a methyl group substituent in the benzimidazole heterocycle. I would like to ask for clarification – what is the basis for the decision to include a methyl group in the benzimidazole heterocycle?

The structure of the synthesized hydrazones has been proven by IR and NMR spectroscopy. Particular attention is paid to the possibility of the formation of carcinogenic impurities - nitrosamines, with an analysis of problems found in existing medicines and the issue is referred to the synthetic approach applied in the study.

For the IR spectra, presented in the figures, it would be clearer if some bands discussed in the text are marked in the respective figures as well: for instance, the IR bands discussed on page 107 for NH bending and C=N stretching vibrations (Fig. 3). In my opinion, when a vibrational band is used to prove the insertion of a substituent, a comparison between the spectra before and after the introduction of the functionality should be made for clarity. Another similar example is the discussion of the IR spectra of the complexes on page 115 of the thesis: ν_{NH}, $\nu_{C=N}$, ν_{C-O} bands exist in the spectrum of the ligands and therefore the question arises, as how they could be used as evidence for complexes?

(ii) Quantum chemical calculations have been carried out by applying the Density Functional Theory and an extended triple zeta basis set in order to characterize tautomeric and conformational equilibria for the studied compounds. Based on the calculated Gibbs free energies, the lowest energy structures have been determined.

(iii) The antitrichinella and antineoplastic activity of the hydrazones from the three groups and the antineoplastic activity of the synthesized metal complexes have been investigated.

It has been established that the larvicidal biological activity of all compounds is higher than the effect of the established drugs (albendazole and ivermectin) used for comparison. Therefore, the compounds possess a high application potential. It is concluded that the presence of OH groups in the phenyl residue causes an increased larvicidal effect and an OH group in the third position in the phenyl ring is essential for the anthelmintic effect.

It is important to note that cytotoxic activity has been studied on two cell lines of cancer cells and one of normal cells, which made it possible to determine the selectivity of the action. It is concluded that the studied compounds exhibit significantly lower cytotoxicity toward normal cells.

(iv) The influence of the synthesized benzimidazoles on tubulin polymerization has been investigated spectrophotometrically and by molecular docking.

(v) The radical scavenging ability of the compounds has been investigated experimentally and theoretically. The experimental results allow the activity to be compared with that of reference compounds. A detailed and in-depth study of the possible mechanisms of

radical catching has been carried out through theoretical quantum chemical calculations, considering the possible reaction paths, intermediate products, transition states, and energy barriers.

The conducted research and the published results have fundamental scientific contributions, which can be formulated as proving with new means of significant new aspects of existing scientific fields, problems, theories, hypotheses, and obtaining new facts. The results obtained are a very good basis for further studies of some of the newly synthesized compounds with a potential for practical application.

The thesis and abstract are accurately written. I would make the following remark: in many places in the dissertation, tables are carried over to the next page, or the header is on a previous page, or the caption under the figure is on the next page - details that should have been cleaned up.

I have the following questions:

1) It is known that the presence of a 3,4,5-trimethoxyphenyl fragment in the molecular structure is crucial for influencing the polymerization of tubulin. Do the studies conducted and the results obtained contradict this statement?

2) The following approach has been applied for performing the quantum chemical calculations: optimization at B3LYP/6-311++G(d,p) level and subsequent SP (single point) calculations at M062X/6-311++G(d, p) level. What do SP calculations improve? This procedure is usually applied when performing MP2, and MP4 calculations, requiring much more processing time and computer power. Why a full optimization with the M062X functional has not been applied?

3) At the end of the literature review, the following interesting task is formulated: to investigate whether the binding of different pharmacophores in one molecule would lead to synergism in biological activity. What is the answer to this question based on the results obtained?

The research results are summarized in the Conclusions section. It is clear from the exhibition, the scientific research conducted, the conclusions drawn, as well as from the publications, that the research has a multidisciplinary character. This is confirmed both by the co-authorship in the publications and by the colleagues with whom the PhD student has collaborated. I believe that the synthesis, spectral studies, and theoretical quantum chemical calculations, their analysis, and the achieved results are largely the personal work of M. Argirova. It is possible that she has also been involved in pharmacological studies, but even if she has not, the fact that they are detailed and analyzed in the dissertation means that she has successfully entered this field of scientific knowledge. I believe that her overall performance demonstrates excellent knowledge in chemistry and related fields, persistence, and consistency.

CONCLUSION

According to the submitted materials and scientific papers, the above analysis of their importance, and scientific contributions, I confidently give my **positive assessment** and vote **'yes'** for awarding the educational and scientific degree "**Doctor**" to **Maria Andreeva Argirova**, in the professional field 4.2. Chemical Sciences (Organic chemistry).

02/03/2023 Reviewer: Sonia Ilieva

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