

REVIEW

By Snezhanka Metodieva Bakalova, PhD

**Assoc. Prof. in the Institute of Organic Chemistry with Centre of Phytochemistry,
BAS**

Member of the Scientific Jury according to order No RD-09-189/09.12.2022 of the
Director of IOCCP-BAS

On a thesis for acquiring the scientific degree "Doctor of Philosophy" (PhD) in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics", Professional area: 4.2. "Chemical Sciences"; scientific specialty "Organic Chemistry"

Author: Maria Andreeva Argirova

TITLE: Synthesis of 1-H-benzimidazol-2-yl hydrazones and study of their antihelmintic, antineoplastic and radical-scavenging activity

Research supervisor: Prof. Dr. Denitsa Yancheva Pantaleeva (IOCCP – BAS)

1. General description of the presented documents

The author of the thesis is Maria Andreeva Argirova – a full-time PhD student at the Laboratory "Structural Organic Analysis" - IOCCP - BAS

Documents presented: 1. Autobiography 2. Diploma of higher education, educational and qualification degree Master. 3. Order No. RD-09-201/31.07.2018 for enrollment in full-time PhD studies. 4. Order No. RD-09-169/30.07.2021 for dismissal with right of defense. 5. Order No. RD-09-91/11.07.2022 for change of the title of the thesis. 6. Protocol of examination on a basic specialized subject in the professional area "Chemical Sciences", code 4.2, scientific specialty "Organic Chemistry". 7. Certificate No. 7/05.02.2019 for successfully completed English language course. 8. Protocol for a successfully completed Photoshop course. 9. Certificate for successful completion of a course on "Lipid oxidation and antioxidants". 10. Certificate for successful completion of a course on "Computer-aided drug design". 11. Thesis entitled "Synthesis of 1H-benzimidazol-2-yl hydrazones and study of their anthelmintic, antineoplastic and radical scavenging activities" 12. Abstract of the thesis in Bulgarian and English. 13. List and copies of scientific publications. 14. List of noticed citations of the publications. 15. List of participation in scientific conferences. 16. List of participations in scientific projects. 17. List of awards received. 18. List of received personal scholarships and grants. 19. List on the fulfillment of the criteria of IOCCP - BAS for acquiring the educational and scientific degree "Doctor of philosophy".

The set of materials delivered by PhD student Maria Andreeva Argirova as hard copy and/or on electronic media is in accordance with the Law of development of the academic staff in the Republic of Bulgaria, the Regulations for its implementation, the set of rules of development of the academic staff of IOCCP – BAS, and meets the criteria of IOCCP – BAS for acquiring the scientific and educational degree "Doctor of Philosophy,

PhD". The candidate has presented 2 publications to support the claim, both being in the Q1 quartile, as well as a list of 5 citations.

2. Brief biographic data

In the period 2012 to 2016 Maria Argirova was a student at the Faculty of Chemical Technologies, majoring in "Fine Organic Synthesis" at the University of Chemical Technology and Metallurgy - Sofia. In 2016, she graduated as a Bachelor of Chemical Engineering and continued her studies as a Master of Chemical Engineering in the same specialty until 30.11.2017. After defending her thesis in April 2018, she was appointed as a chemist at the Structural Organic Analysis Laboratory in IOCCP-BAS. During the period 01.08.2018 - 31.07.2021, she has been a full-time PhD student at IOCCP-BAS. Currently she is an assistant professor at IOCCP-BAS, starting on 01.08.2022.

3. Timeliness of the topic and appropriateness of the set goals and tasks

The choice of the problem developed in the present thesis is logically related in a scientific and scientific-applied sense to the previous experience of the PhD student. Diverse forms of biological activity of the group of benzimidazole derivatives have been reported related to their structurally similar vinca alkaloids, colchicine and combretastatin A4. This analogy leads to the potential for socially beneficial medical applications. The specific tasks chosen for design, synthesis and investigation of previously undescribed biologically active substances in the thesis support the relevance and actuality of the chosen topic.

4. Knowledge of the problem

The literature review covers 68 printed pages, and the total of cited sources is 293. The first 20 pages of the review deal with the types of biological activity of 2-amino benzimidazole derivatives. Since the specific molecules under study have been known since 1988, the volume of literature data available on their biological implications is enormous. The diversity of the studied biological effects also implies the use of numerous methodologies for their registration.

The literature review in the thesis, as well as the discussion, give me a reason to believe that the author is well aware of a large part of the vast amount of research in the field and has skillfully selected which of it to consider as most relevant to her research and results described and discussed in the thesis.

5. Research methodology

The chosen research methodology is to a certain extent traditional for chemical science. The presented work begins with the synthesis of new representatives of a selected group of molecules with known physiological effects. A sought effect is finding molecules with combined physiological properties. I cannot decide how useful it would be to combine diverse properties in a broad-spectrum approach to medical problems. In relation to the fact that benzimidazole derivatives are related to their influence on tubulin and its function in cells, their research requires the application of a wide variety of generally speaking chemical methodologies, which the candidate applies with specific help from specialists. On the other hand, the study of biological properties was also carried out with

the help of other specialists. In this sense, the presented thesis is a collective effort to obtain adequate answers to the tasks set before it, to which the contribution of M. Argirova is significant. This is also stated in the author's contributions to the publications presented by the candidate.

6. Characterization and evaluation of the dissertation work

The thesis has the impressive volume of 215 pages and consists of eight chapters – (i) Introduction, (ii) Literature Survey, (iii) Aims and Objectives, (iv) Materials and Methods, (v) Results and Discussion, (vi) Conclusions, (vii) Contributions, (viii), References. Finally there is a section that contains appendices (list of publications, information on noticed citations, participations in scientific fora). 57 figures, 20 tables and 68 diagrams that illustrate the results obtained are included. The thesis is written in good professional language.

The objectives of the dissertation and the tasks to achieve them are briefly and clearly stated. The main goal is the synthesis of new, not previously described in the literature 1H-benzimidazol-2-yl hydrazones and their metal complexes, the study of their structure with modern methods, the study of biological and radical-trapping activities, as well as the study of probable mechanisms of action with quantum chemical calculations. An impressive amount of work has been done.

The synthesis of precursors of the studied compounds by known methodologies, along with their characterization, is described on 15 pages of the dissertation. Their pharmacological studies are further described on as many pages. Another 45 pages are devoted to the synthesis and characterization of selected target benzimidazolyl-hydrazones and metal complexes. At the same time, their biological activity was also examined. The synthesis of hydrazones is probably also the source of the relatively mechanistic unification (eclectic idea) to include also compounds with potential antioxidant properties in the present study, *i.e.* hydrazones of hydroxyl aromatic aldehydes. Nevertheless, the contribution with the most prominent pharmacological features is the copper complex of benzimidazolyl-hydrazone of 2-hydroxy-4-methoxy benzaldehyde. It is not clear whether the latter complex is cupro- or cupri- and how its structure was determined, which I think would be of interest.

I find no reason to doubt the reliability of the presented scientific data.

7. Contributions and significance of the development for science and practice

The biological activity of the compounds studied in the thesis gives reason to expect their applicability. The main achievement of the presented study is the synthesis of 40 derivatives of benzimidazolyl-2-hydrazone, of which 37 have not been previously described in the literature. The compounds exhibit significant biological activity as antitumor agents, antihelmintic agents. The ones possessing a hydroxyl group in the aldehyde fragment, supplemented by additional oxygen-containing phenolic substituents, also exhibit antioxidant properties. On the educational side of the work on her

dissertation, assistant professor M. Argirova got acquainted with a significant number of modern instrumental and theoretical research methods. A continuation of research in the field of biological activity may lead to the formulation of new antitumor and anthelmintic compounds.

M. Argirova is a member of the team of 8 projects, 3 of which directly related to the topic of the thesis.

8. Evaluation of publications on the thesis. Personal participation of the PhD student.

Results included in the thesis are published in two papers in high level international journals, which are referenced by both WoS and Scopus, have a high impact factor/rank and are in quartile Q1. Publications include synthesis, computational modeling and biological activity and are markedly multidisciplinary in nature. The first of them is in *Chemico-biological Interactions* and has 10 authors, M. Argirova being the second one. The second is in *RSC Advances*, authored by 7 authors, M. Argirova being the first one. I noticed that after the submission of the thesis, another paper in the field of the thesis was published in the prestigious journal *Molecules*, with M. Argirova being again the first author. This shows her active role and undoubted contribution to the research. The authors' contribution statements required by the publishers mention the participation of this PhD student in the synthesis and theoretical calculations, as well as in the determination of the inhibition of tubulin polymerization and the writing of the publications. So far, 6 citations from foreign authors of the publications included in the thesis have been noticed.

M. Argirova actively promotes the results of her research. 10 posters were presented at international scientific conferences and schools, based on studies from the thesis, 3 of which abroad. 5 reports were presented in proceedings of international scientific conferences in Bulgaria, as well as 2 reports in proceedings of international scientific conferences. She presented one independent report at an international conference in Sofia, and in the others she has from 2 to 6 co-authors. Some of the participations in conferences were financed by her personal scholarships and grants.

There is no doubt regarding the active participation of the PhD student in the conducted syntheses in the presented thesis. The obtained results using quantum chemical calculations to a significant extent are also her personal contribution.

9. Abstract

The abstract submitted by M. Argirova is correctly written according to the requirements of the relevant regulations, essentially reflecting the main results achieved in the thesis.

10. Critical remarks and recommendations

I believe that a single-point calculation of energies of molecules with a given density functional method using a geometry optimized with another density functional method is incorrect. Stationary points located with each method and basis set have different geometries. Moreover, the equilibrium study results reported in the present thesis consider molecules small enough to be fully optimized directly with the chosen method

using the computational facilities available to the PhD student both in our country and abroad.

I would recommend that M. Argirova to provides more detailed explanations in her future research and publications on the design of the new compounds intended for synthesis and research.

11. Personal impressions

I have personal impressions of M. Argirova's work, as she is a member of the team of an ongoing project with the Scientific Research Fund, of which I am the coordinator, which started last year. Regardless of her busy schedule in preparing her thesis, she found time to start being acquainted with the relatively new for her topic, actively participating in group discussions, and performing the necessary quantum-chemical calculations.

12. Recommendations for future use of dissertation contributions and results

I think that getting to know and mastering the many methods used in her thesis is a sufficient recommendation for M. Argirova to continue mastering them in further research.

Future use of the scientific and scientific-applied contributions of the present thesis is possible in view of their quality as biologically active substances, preparations and/or in the best cases, drugs. There are procedures and requirements for the latter, which may need to be met in favorable cases.

CONCLUSION

The thesis contains fundamental and applied scientific results, which represent an original contribution to science and meet all requirements of the Law of Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the same law, and the Regulations of BAS for the Implementation of this Law. The presented materials and thesis results fully comply with the specific requirements of the Regulations of IOCCP-BAS for the application of the mentioned law.

The thesis shows that PhD student Maria Argirova possesses sufficient theoretical knowledge and professional skills in the scientific specialty Organic Chemistry, demonstrating qualities and skills for independent conduct of scientific research.

On the basis of the above considerations, I confidently give my positive assessment of the conducted research, presented by the above-reviewed thesis work and propose to the Honorable scientific jury to award the educational and scientific degree "Doctor of Philosophy" to Maria Andreeva Argirova in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics", Professional area: 4.2. "Chemical Sciences"; scientific specialty "Organic Chemistry"

March 05, 2023

Reviewer:

Assoc. Prof. Snezhanka Bakalova, PhD