OPINION

by Dr. Antoaneta Borissova Trendafilova, Professor at the Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences

on the PhD Thesis for awarding the educational and scientific degree "doctor" in higher education field: 4. Natural sciences, mathematics and informatics, professional field: 4.2. Chemical Sciences, PhD Program: Organic Chemistry

Author: Maria Andreeva Argirova
Title: Synthesis of 1H-benzimidazol-2-yl hydrazones and study of their anthelmintic, antineoplastic and radical scavenging effects
Research supervisor: Prof. Dr. Denitsa Pantaleeva - Institute of Organic Chemistry with Centre of Phytochemistry – BAS

1. General presentation of the procedure and the PhD student

According to order РД 09 189/09.12.2022 of the Director of IOCCP - BAS, I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation on the topic "Synthesis of 1H-benzimidazol-2-yl hydrazones and study of their anthelmintic, antineoplastic and their radical-trapping action" for the acquisition of the educational and scientific degree "doctor" with author assistant Maria Argirova.

The set of materials presented by Maria Argirova on paper and electronic media is in accordance with the Regulations for the Development of the Academic Staff of IOCCP - BAS, meets the criteria and includes all necessary documents. The PhD student has also attached 2 publications on the topic of the dissertation, a list of noted citations to the scientific works included in the dissertation, a list of participation in scientific events, a list of participation in the implementation of scientific projects, awards and the relevant evidentiary material.

Assistant Maria Argirova was born in 1993. During the period 2012 - 2016, she was a student in the bachelor's program of the Chemical Technology and Metallurgical University (CTMU) - Sofia, and in 2017 she graduated from the master's program at the CTMU as a master in fine organic synthesis with a focus on design of biologically active substances and pharmaceutical agents of plant and animal origin. She was enrolled as a full-time PhD student in the "Structural Organic Analysis" laboratory of IOCCP- BAS on 01.08.2018 and was awarded with the right to defense on 31.07.2021. The topic of the dissertation was updated on 11.07.2022. From August 2022, she is an assistant in the "Structural Organic Analysis" laboratory of IOCCP - BAS. In the framework of his scientific research activities so far, she has participated in a total of 8 projects. She has won 4 grants and scholarships, as well as three awards - for the best thesis of the Union of Chemists in Bulgaria, for the best report at the Third Interdisciplinary Doctoral Forum and the best poster at the XIX Scientific Poster Session for students, doctoral students and young people scientists of CTMU.

2. Relevance of the topic

It is known that cancer cells very easily develop resistance to the drugs used, so that the development and testing of new drug candidates are objects of numerous studies. In recent years, research in this area has been aimed to find agents that exhibit a combined effect on cancer cells - to reduce their proliferation and growth, as well as to limit their formation by blocking the formation of reactive oxygen species. A number of benzimidazole compounds have shown

strong cytotoxicity *in vitro* against a wide range of human tumor cell lines, and their appropriate modification would lead to compounds with combined antioxidant and antineoplastic activity as agents for new effective anticancer therapy. Therefore, the topic of the dissertation work is extremely relevant and important, both in a scientific and in a scientific-applied aspect.

3. Knowledge of the problem

A total of 293 references are cited in the dissertation, a significant part of which were published in the last 5 years. The literature review includes detailed information on the synthesis and biological activity of 2-aminobenzimidazole compounds exhibiting antiviral, antibacterial, anti-inflammatory, neuroprotective, anticonvulsant, antiparasitic and antiproliferative activities. Another important part represented in the literature review is the information on the *in vitro* effect of benzimidazole compounds on tubulin polymerization. The literature review is well structured and shows that assistant Maria Argirova has a thorough and excellent knowledge of the state of research in several scientific fields and the current problems on the topic of the dissertation. The aim and tasks are clearly formulated and fully correspond to the topic of the dissertation.

4. Research methodology

The methodology used for the preparation of the series of compounds containing a benzimidazole heterocycle and an arylhydrazone fragment with various combinations of functional groups did not require specific reagents or catalysts and resulted in the preparation of the compounds in good to very good yields. Spectral methods, mainly IR and NMR, were used to identify the synthesized compounds, established methods such as MTT, spectrophotometry, etc. were applied for *in vitro* assessment of biological activity and specialized software was used for quantum mechanical calculations and molecular docking. The applied instrumental and theoretical methods are up-to-date and modern.

5. Characterization and evaluation of PhD thesis and its contributions

The dissertation is constructed in a classic style with a good balance between the individual parts (introduction, aims and objectives, literature review, experimental part, results and discussion, conclusions and contributions) and is written in 219 pages with 20 tables, 56 figures, 69 diagrams and 293 references. The obtained results are described and discussed in detail in the "Results and Discussion" section (93 pages). A total of 40 compounds were synthesized, of which 37 were new, not previously described in the literature. Their pharmacological activity was determined, as well as their effect on tubulin polymerization. The contributions are mainly scientific and can be systematized as follows:

• New benzimidazole derivatives - 1*H*-benzimidazol-2-yl hydrazones containing phenyl ring with halogen atoms, hydroxy and methoxy groups, which exhibit combined antioxidant, antineoplastic and/or anthelmintic action, were obtained.

• Several compounds among them were selected, which suppress the tumor cell proliferation at very low micromolar concentrations, as well as possess a remarkable larvicidal effect.

• By *in vitro* experiments, the putative common mechanism of antineoplastic and anthelmintic action - modulation of tubulin polymerization has been demonstrated.

• The mechanisms of antioxidant activity, interaction with tubulin, drug likeness, oral bioavailability, gastrointestinal absorption and blood brain barrier penetration were studied by theoretical methods.

6. Evaluation of the publications and personal contribution of the PhD student

The research, included in the PhD thesis is summarized in 2 publications in scientific journals from Q1 quartile and with high impact factor: RSC Advances (JCR-IF 3.36) and Chemico-biological interactions (JCR-IF 5.19). The PhD student is respectively the first and second author in these publications, which confirms her personal contribution to achieving the published results. There are 5 citations from foreign authors in renowned publications, which is proof of the relevance of the topic.

7. Abstract

The presented abstract is prepared according to the requirements and it fully presents the results, discussions and conclusions included in the dissertation work. The English version of the abstract corresponds to the Bulgarian text.

8. Recommendations for future use of dissertation contributions and results

I have no critical comments on the thesis except of a minor remark regarding the use of the symbol "C-50" in the assays of DPPH and ABTS radical scavenging activity. There is a lacking of information what this symbol means and how it is defined. I assume that this is the concentration of the test compound at which 50% of the respective radicals are inhibited. If so, it is more correct to use the conventional symbol "IC50".

I would recommend the PhD student to continue the research in *in vivo* conditions as the results obtained show promising candidates.

CONCLUSION

The PhD thesis contains scientific results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations for the Implementation of the LDASRB of the Bulgarian Academy of Sciences. The presented materials and results fully comply with the specific requirements of the Rules of the IOCCP-BAS for the application of LDASRB. The PhD thesis shows that the PhD student Maria Argirova possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Organic Chemistry" by demonstrating qualities and skills for independent scientific research.

Due to the above, I confidently give my positive assessment of the conducted research and propose to the honorable scientific jury to award the educational and scientific degree "doctor" to Maria Argirova in higher education field: 4. Natural sciences, mathematics and informatics, professional field: 4.2. Chemical Sciences, PhD Program: Organic Chemistry.

27.02.2023 Reviewer: .....

(Prof. Dr. Antoaneta Trendafilova)