

OPINION

by Assoc. Prof. Zhivko Asenov Velkov, Department of Chemistry, Southwest University
"Neofit Rilski", Blagoevgrad

on a thesis for awarding the educational and scientific degree "doctor" in the field of higher
education 4. *Natural sciences, mathematics and informatics*, professional field 4.2. "Chemical
Sciences", scientific specialty: "Organic Chemistry"

Author: Eng. Maria Andreeva Argirova

**Topic: "Synthesis of 1H-benzimidazol-2-yl hydrazones and investigation of their
antihelmintic, antineoplastic and radical scavenging activity"**

Supervisor: Prof. Dr. Denitsa Pantaleeva - INSTITUTE OF ORGANIC CHEMISTRY
WITH CENTRE OF PHYTOCHEMISTRY, "Structural Organic Analysis" Laboratory

1. Presentation of the procedure and the PhD student

The set of materials presented by Maria Argirova is in full compliance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the regulations for its implementation, as well as with the regulations on the terms and conditions for acquiring scientific degrees and holding academic positions in the Institute of Organic Chemistry with Center of Phytochemistry, BAS.

In addition to the mandatory documents, the PhD student submitted also certificates for completed courses in lipid oxidation, drug design and Adobe Photoshop – qualifications that she needed in the preparation of her dissertation.

2 publications in international journals with a high impact factor (5.194 and 3.361) are included in the thesis. Both articles are from the first quartile (Q1) of the Scimago Journal Rank classification. The results collected in the dissertation have been reported at 17 scientific forums at home and abroad and have already been cited.

Maria Argirova completed her master's program at HTMU-Sofia with excellent results.

2. Timeliness of the topic

The topicality of the dissertation subject is indisputable. Finding new compounds with antineoplastic and antihelmintic activity is a relevant synthetic task more than ever.

3. Familiarity with the problem

A complete overview of the literature on drugs containing benzimidazoles, 2-amino- and 2-carboxamidobenzimidazoles, nucleosides in which the 2-aminobenzimidazoles are aglycones, 1,2-substituted, 1,3,5-substituted benzimidazol-2-imines, etc., is compiled.

The possibility the 2-aminobenzimidazoles to exhibit inhibitory activity against cyclooxygenases, acetylcholine- and butylcholinesterases, as well as their neuroprotective activity, was investigated.

The literature describing the effect of benzimidazole derivatives on tubulin polymerization, as well as drugs that target microtubules, is also reviewed. The preparation of transition metal complexes with these compounds as ligands is discussed, as well as their antibacterial activity.

Throughout the review, attention is paid to the structural reasons underpinning the appearance of drug activity.

Maria Argirova demonstrates a very good knowledge of the literature on the topic of the dissertation. The thesis contains 219 pages and consists of a literature survey (Introduction) - on 76 pages, a "Materials and methods" section on 23 pages, and a "Results and discussion" chapter on 92 pages. The bibliography contains 293 references, 199 of which are related to the literature overview.

4. Methodology

The aim of the dissertation is the synthesis of new 1H-benzimidazol-2-yl hydrazones and investigation of their antihelminthic, antineoplastic and radical-trapping action.

To achieve these goals, Ms. Argirova has mastered the methods of modern organic synthesis, physical methods for proving the structure of the obtained compounds, methods for studying antineoplastic, antihelminthic and radical-trapping activity, as well as methods for studying the effect of the obtained compounds on the polymerization of tubulin.

In addition, Maria Argirova has also used various theoretical methods - quantum-chemical methods, docking-procedures and calculation of molecular descriptors, utilizing various parametric methods.

The three-parameter hybrid functional of Becke, Lee, Yang and Parr - B3LYP was employed for the optimization procedures, and the reparameterized functional - M06-2X in its unrestricted version was used to calculate the Hess matrix and to find the transition states.

5. Evaluation of the dissertation and contributions

The dissertation was developed in the Structural Organic Analysis Laboratory of IOC, where both experimental and purely theoretical studies are conducted. Both approaches are used in the dissertation.

Experimental

The preparation of 40 1H-benzimidazol-2-yl hydrazones and 4 complexes with Cu(II) and Fe(II) is included in the dissertation work. IR and Raman spectroscopy, ^1H and ^{13}C NMR were used to identify these compounds.

The *in vitro* antitrichinella and antineoplastic activity of the synthesized 1H-benzimidazol-2-yl hydrazones, as well as the antineoplastic activity of the metal complexes, was investigated. The effect of benzimidazoles on tubulin polymerization was also studied *in vitro*. Spectrophotometric determination of the radical-scavenging activity of 1H-benzimidazol-2-yl hydrazones from the second series was carried out and the level of iron-induced peroxidation in biologically relevant systems was determined.

Theory

A series of quantum-chemical studies using DFT-functionals and high-orbital basis sets have been performed.

To elucidate the interactions of different ligands with tubulin, molecular docking was implemented and a theoretical study of the possible mechanisms of radical-scavenging action was also carried out.

I give a positive assessment of the thesis.

6. Assessment of the PhD student's publications and personal contributions

As already mentioned, Maria Argirova has included in her dissertation 2 publications in specialized journals with a very high impact factor, which in itself is an assessment of the authors' work. In her presentation before the Approbation Committee, the PhD student showed that she is well-acquainted with the terminology of both the fields of synthesis and spectroscopic methods, as well as in the domain of theoretical chemistry. In addition, her position as first author in one and second author in the other article convinces me that her contribution as a co-author is substantial.

7. Abstract

The abstract is formatted according to the generally accepted requirements and adequately reflects the main scientific contributions of the dissertation.

8. Recommendations for future use of dissertation contributions and results

The topic of the thesis is significant and should be pursued with the same enthusiasm and diligence in future.

I would recommend to Maria Argirova in her forthcoming theoretical studies of the organic compounds' reactivity and even of their drug action, to use primarily DFT or perturbation methods, the reliability of which is significantly higher than that of purely parametric methods.

CONCLUSION

The dissertation contains scientific and applicable results, which represent an original contribution and the thesis meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of the Law and that of the Institute of Organic Chemistry with Centre of Phytochemistry at the Bulgarian Academy of Sciences.

The dissertation shows that the PhD student Maria Andreeva Argirova possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Organic Chemistry".

Despite the presence of minor remarks on the thesis and the synopsis, I assuredly give my positive assessment of the conducted research and the achieved results, and I will vote for awarding the educational and scientific degree "doctor" to Maria Andreeva Argirova.

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Blagoevgrad

Opinion author:
Assoc. Prof. Zhivko Velkov