SCIENTIFIC OPINION

by Assoc. Prof. Lyubomira Dimitrova Yocheva, PhD, Sofia University "St. Kliment Ohridski", Faculty of Medicine, Department of Biology, Medical Genetics and Microbiology, member of the Scientific Jury, formed by order No. I-116/04.08.2025 of the Director of the Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences

on PhD Thesis on the topic: **"Investigation on the potential of new derivatives of ethambutol and isoniazid with the aim of studying their anti-tuberculosis activity"**

for the acquisition the educational and scientific degree PhD in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional direction 4.3. Biological Sciences, scientific specialty: Microbiology - 01.06.12.

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Relevance of the topic and general data of the dissertation work

The treatment of tuberculosis still represents a serious challenge for medicine. Many factors influence the impossibility of eradicating this infectious disease worldwide. A leading factor is the increasing spread of *Mycobacterium tuberculosis* resistant strains: multidrug-resistant (MDR-TB), extensively drug-resistant (XDR-TB) and "totally drug-resistant" (TDR-TB). They cause new forms of tuberculosis that are difficult to treat or untreatable with the drugs used in medical practice at present. According to WHO data, Bulgaria is one of the 18 countries with a high incidence of tuberculosis in Europe. In this regard, a National Program for the Prevention and Control of Tuberculosis in the Republic of Bulgaria 2021-2025 has been established. Undoubtedly, the topic of the dissertation is extremely relevant and in line with the goals of the Program, as it contributes to the development of new anti-tuberculosis agents - newly synthesized derivatives of ethambutol and isoniazid. Many requirements are placed on newly synthesized chemical compounds: to possess high antimycobacterial activity, low toxicity, good efficiency and minimal risk of adverse effects. This requires conducting a thorough biological and pharmacological screening for the selection of substances with antimycobacterial potential, which is an essential stage of research for their further application in medical practice.

The PhD thesis contains 151 pages (excluding the Title Page and Acknowledgements Page) and includes the necessary sections: Table of Contents (3 pages), List of Abbreviations (2 pages), Introduction (2 pages), Literature Review (40 pages), Aim and Objectives (1 page), Materials and Methods (13 pages), Results and Discussion (53 pages), Conclusions (2 pages), Contributions (1 page), References (30 pages). The PhD thesis is illustrated by 18 tables and 39 high-quality color or black-and-white figures. At the end of the dissertation, lists of publications, participation in scientific forums in the field of the presented work and citations (3 pages) are included.

The **Introduction** reveals the need for the development of the presented dissertation work as a stage of the study of newly synthesized derivatives of ethambutol and isoniazid, establishing their efficacy against different strains of *M. tuberculosis* and clarifying the mechanisms of their action.

The **Literature Review** presents current data on the spread of drug-resistant tuberculosis around the world and in Bulgaria. The pathogenesis of the infection is described, as well as the role of oxidative stress for the macroorganism and mycobacteria in the development of the disease and the possibility of using therapeutic approaches aimed at antioxidant balance in tuberculosis. The review contains a

description of the cell wall and the factors of pathogenicity and virulence of *M. tuberculosis*. The mechanisms of antibiotic resistance are presented. The anti-tuberculosis drugs used in practice, their mechanism of action and possible undesirable side reactions, as well as new substances with antimycobacterial potential are described. Some modern technologies for preclinical testing of drug candidates are presented, such as molecular docking, transport of molecules through the cell membrane, complete genome sequencing. The literature review is extremely informative, written in a professional language, but at the same time understandable and interesting. It shows the excellent awareness of the Ph.D. student on the topic of the thesis and his ability to analyze and summarize information.

The aim of PhD thesis is clearly formulated. To achieve the goal, 6 objectives have been outlined.

The materials and methods used are precisely selected and described in detail and may be useful to anyone who plans to work in this area. They fully meet the aim and the tasks.

Results and scientific contributions

A screening of 15 newly synthesized aroylhydrazones (structural analogues of isoniazid) and 6 nitrofuranamides (derivatives of ethambutol) was carried out and two aroylhydrazones (3a and 3d) and two nitrofuranamides (DO190 and DO209) with the highest activity against *M. tuberculosis* H37Rv and *M. smegmatis* were selected. The studied compounds demonstrate better membrane permeability and more stable binding to target enzymes than isoniazid, making them promising antimycobacterial candidates. At the same time, the compounds exhibit weak cytotoxicity and minimal toxic effects in mice, suggesting their good biocompatibility and safety. Histological studies in mice treated with the four compounds showed weak deviations from the normal structure of the liver, kidneys and small intestines and therefore minimal risk of drug damage. Monitoring of biochemical markers of oxidative stress in the liver showed that aroylhydrazones 3a and 3d suppress lipid peroxidation, restore glutathione content, lead to compensatory changes in the activities of antioxidant enzymes and exhibit better antiradical activity in model chemical systems, compared to isoniazid. Whole genome sequencing revealed a mutation in the Rv3755c gene leading to enhanced efflux of the studied compounds and outlines the need for future studies to elucidate the mechanisms of drug resistance.

A huge amount of experimental work has been carried out, and the results obtained are convincing and definitely deserve attention. The section is illustrated with 30 excellently crafted figures.

Ten conclusions have been formulated, which I fully accept, as they clearly express the achieved results. I also agree with the **four contributions** defined, which are of fundamental importance.

The list of cited literature includes 346 titles in Latin, 46% of which (161 issues) have been published since 2015.

Dissertation related publications and participation in scientific events

Three publications and a number of presentations at scientific forums distribute the results of PhD thesis. The articles have been published in international journals with an impact factor: *Pharmaceutics* (IF: 5.500, Q1); *Biomolecules*, (IF: 6.525, Q1) and *Bulletin of Experimental Biology and Medicine*, (IF: 0.9; Q3). In one of them, Simeon Dimitrov is the first author. At the time of submitting the documents, the publication in the journal *Biomolecules* is already cited in 5 articles, which shows excellent receiving the results among the scientific community.

The new knowledge obtained and original results have been presented at 7 scientific forums in Bulgaria and abroad (Singapore, Indonesia, Italy, France, Spain).

Critical notes and comments, recommendations, questions to the PhD student

I have no comments on the work. Minor technical errors have been made (e.g. misspelled or missing letters or prepositions; repetitions of sentences, confusion in the numbering of figures), which in no way reduce the quality of the dissertation.

Conclusion

The presented PhD thesis is dedicated to a current and significant topic, the aim and the objectives have been fully achieved at a high scientific level, and the original results obtained are an undeniable contribution to the field of microbiology, pharmacology and experimental medicine.

During the experimental work, the PhD student Simeon Dimitrov has expanded and upgraded his biomedical culture, mastered a wide range of modern microbiological, biochemical, histological and genetic methods and techniques, and has grown as a capable and promising scientist. He has actively participated in the successful implementation of two national projects at the Ministry of Education and Science and two international research projects under the European COST Cooperation Program, which, with their innovative ideas and professional implementation, are not inferior to the best world examples.

I believe that the most important contribution of this dissertation work is that for the first time in Bulgaria, a pharmacological screening of selected derivatives of isoniazid and ethambutol has been carried out. For the first time, an attempt to create an algorithm for preclinical testing of compounds with antimycobacterial potential has been made, as a result of which these newly synthesized compounds can be unequivocally considered as promising drug candidates for the treatment of tuberculosis, especially in patients with impaired liver function or when long-term therapy is required. Because of this, the efforts made by the PhD student Simeon Dimitrov in the preparation of the dissertation work and the final product obtained deserve high praise.

In conclusion, the PhD student fulfills the criteria for awarding the educational and scientific PhD degree according to the Development of Academic Staff in the Republic of Bulgaria Act, as well as the Regulations for its application at the Institute of Microbiology "Stefan Angelov" - BAS. I confidently give my **positive assessment** and I propose to the members of the Honorable Scientific Jury to award Simeon Emilov Dimitrov the educational and scientific degree “Doctor” in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional direction 4.3 Biological Sciences (Microbiology - 01.06.12.).

28.09.2025 Prepared the opinion:

/assoc. prof. Lyubomira Yocheva, PhD/