To the Chairman of the
Scientific Jury, appointed by
Order No. I-116/4.08.2025 of the
Director of the Institute of Microbiology – BAS

R E V I E W

on the dissertation work for the educational and scientific degree of "Doctor" (PhD) in the field of 4.3 Natural Sciences, specility in "Microbiology" for Simeon Emilov Dimitrov, a full-time PhD student at the Institute of Microbiology at BAS, on the topic: "Testing the potential of new derivatives of ethambutol and isoniazid with the aim of studying their anti-tuberculosis activity", with Scientific Supervisors: Assoc. Prof. Dr. Violeta Valcheva Ruseva and Prof. Dr. Milka Milcheva Mileva.

Reviewer: Prof. Stefan Vacev Panaiotov, PhD, DSc

Scientific specialty: Microbiology

Institution: National Center of Infectious and Parasitic Diseases - Sofia

I declare that I have no conflicts of interest as defined in Art. 4, para. 5 of the Law on the Development of Academic Staff in the Republic of Bulgaria. I have no joint publications on the dissertation topic with Simeon Dimitrov. This review has been prepared in accordance with the requirements of the aforementioned law and the Institute of Microbiology's regulations for its implementation. I did not use artificial intelligence software to evaluate the dissertation.

I. General characteristics of the dissertation

The dissertation presented is in accordance with the instructions published in the Regulations for the Implementation of the Law on the Development of the Academic Staff in Bulgaria and the Institute of Microbiology-BAS's Internal Regulations. The dissertation

is 167 pages long. It comprises the following main sections: introduction; goal and objectives; materials and methods; results; discussion of the results; conclusions; and cited literature.

The dissertation is written in a professional style. It is easy to read. There is no unnecessary terminology, long, complex sentences or unclear expressions. The correct methodology has been chosen to assess the toxicity, antimycobacterial activity, and mechanisms of action of compounds that are active against *M. tuberculosis*. A wide range of innovative and classical research methods were applied in the development of the dissertation.

II. Literary awareness and theoretical preparation of the candidate

The presented text, the relevance of the set goals, and the methods used for planning, conducting, and describing the research provide a clear indication of the candidate's awareness and theoretical preparation on the topic of the dissertation. The goals of the planned and conducted research are theoretically very well justified based on the literary data.

III. Methodology of the research conducted

The methodology applied to study the activity of new compounds against *M. tuberculosis* is both innovative and appropriate. A wide range of methods were employed to determine the antituberculosis activity of the selected compounds, assess their in vitro cytotoxicity, determine their transmembrane permeability, assess their acute and subacute toxicity, evaluate the pathomorphology of tissue samples, determine their antioxidant activity and redox-modulating capacity, perform in vitro mutagenesis and isolate DNA from resistant mutants, and complete the genomic sequencing of the isolated resistant mutants.

The tasks set for implementation are adequate to achieve the dissertation's objectives.

The results are presented clearly.

IV. Significance and persuasiveness of the results, their interpretation and the conclusions drawn

The antimycobacterial properties of the newly synthesised aroylhydrazone and nitrofuroylamide compounds were studied and found to be effective against *M. tuberculosis* and *M. smegmatis*. These compounds demonstrate improved membrane permeability and stable binding to target enzymes, making them promising candidates for antimycobacterial agents. Pathological studies in animals demonstrate that these compounds have minimal toxic effects, suggesting their safety. Histological studies in mice treated with aroylhydrazones and nitrofuroylamides reveal minimal deviations from normal parameters in the liver, kidneys, and small intestine. Changes in biochemical markers of oxidative stress indicate that two aroylhydrazone derivatives (3a and 3d) suppress lipid peroxidation and restore the hepatic content of the low-molecular-weight endogenous antioxidant, glutathione. They also lead to compensatory changes in the activities of antioxidant enzymes associated with liver toxicity and exhibit antiradical activity in model chemical systems compared to INH.

Genome sequencing revealed mutations associated with resistance to the compounds under study, emphasising the importance of future studies to elucidate the mechanisms of drug resistance.

V. Evaluation of scientific contributions

The generalised conclusions drawn from the results obtained have original value for science and for the treatment of tuberculosis patients in general. The PhD student has deposited empirical data in international databases. I accept the scientific conclusions

made, most of which are original. A significant contribution of the PhD candidate is the pharmacological screening of new EMB and INH derivatives active against *M. tuberculosis*, which is being conducted for the first time in Bulgaria.

VI. Assessment of the quality of scientific papers reflecting the dissertation research

The five published and presented scientific papers fully correspond to the dissertation's topic, goals and objectives. The publications are in Q1 and Q2 journals and have a total impact factor of 23. The PhD student Simeon Dimitrov has successfully fulfilled all the set goals and objectives. The results obtained make a significant scientific contribution to the dissertation topic.

VII. Critical remarks on the dissertation:

I have no significant critics on the dissertation text, planning, performance of the research work, obtained results and conclusions. During the preliminary presentation of the dissertation, I drew the student's attention to the fact that it would be useful to comment on limitations related to the methodology of the research conducted, either in the text or in a separate point. I drew the student's attention to minor technical errors in the text. The corrections and additions made to the text correspond to these remarks.

Conclusion:

From the materials presented in the dissertation, it is evident that the PhD candidate Simeon Dimitrov has demonstrated diligence and organisation, and that the research is his own work. He is enrolled to defend a dissertation for the award of the educational and scientific degree of 'Doctor' and meets the minimum national requirements. The number of credit points collected exceeds the minimum required for dissertation defence. The dissertation contains scientific or applied scientific results that represent an original contribution to science. It demonstrates that the candidate possesses in-

depth theoretical knowledge of the relevant specialty and the ability to conduct independent scientific research. I give a **positive assessment** and recommend that the Scientific Jury award the educational and scientific degree of ''Doctor" to Simeon Emilov Dimitrov in the field of higher education 4. Natural Sciences, Mathematics and Informatics, specialising in Biological Sciences: Microbiology (code 01.06.12).

Prepared by:

Prof. Stefan Panaiotov, PhD, DSc