REVIEW
by prof. Antoaneta Borissova Trendafilova, PhD,
Institute of Organic Chemistry with Centre of Phytochemistry
Bulgarian Academy of Sciences ((IOCCP-BAS)
on the Doctoral Thesis for acquiring the scientific degree "**Doctor of Sciences**" in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics";
professional area: 4.2. "Chemical Sciences";
scientific specialty "Organic Chemistry"

Author: Assoc. Prof. PhD Georgi Milchev Dobrikov,
Institute of Organic Chemistry with Centre of Phytochemistry, BAS

Dissertation topic: **New compounds as perspective antitubercular and antiviral agents**

1. Subject of review

The set of materials presented by Associate Professor Dr. Georgi Dobrikov is in accordance with the Regulations for the Development of the Academic Staff of the IOCCP and meets the criteria of the IOCCP-BAS for the acquisition of the scientific degree "Doctor of Sciences". Fulfillment of the required criteria is based on:

Indicator A: Received an educational and scientific degree "Doctor" - PhD (50 points).

Indicator B: Submitted dissertation work for the scientific degree "Doctor of Sciences" (100 points).

Indicator G: A list of 8 scientific papers that are publications in journals with an impact factor is presented. The total number of points for this indicator is 180 points out of a required 150 points.

Indicator D: A list of citations in scientific publications indexed in world-renowned databases is presented. The total number of citations (excluding self-citations by all authors) is 303, which equals 606 points out of the required 200 points.

The total number of points is 936 and exceeds the minimum requirements of 500 points. Assoc. Prof. G. Dobrikov has also attached a list of participations in scientific conferences and a list of participations in scientific projects directly related to the subject of the dissertation.

2. Brief biographical data

Assoc. Prof. Dr. Georgi Dobrikov graduated with a master's degree in the Faculty of Chemistry and Pharmacy of Sofia University "St. Kl. Ohridski" in 1998. In the period 1998-1999 he was appointed as a specialist chemist at the Institute of Polymers, BAS. From 1999 to 2001, he was involved in developing air quality analysis methods as a chemist at the National Center for Public Health and Analysis, Sofia. During the period 2001-2004, Georgi Dobrikov was a full time doctoral student in the lab. "Organic Synthesis and Stereochemistry", IOCCP-BAS under the guidance of Prof. DSc Vl. Dimitrov, where he prepared and defended a dissertation for obtaining a PhD on the topic "Preparation of chiral ferocene derivatives - absolute configuration and application in asymmetric synthesis" in 2006. In the period 2004-2016, he worked at the IOCCP-BAS, holding consecutive positions of Assistant and Assistant Professor, and from 2016 until now as an Associate Professor at the same institute after a successful habilitation. The overall scientific output of Assoc. Prof. Dr. Georgi Dobrikov includes 41 scientific articles. According to Scopus data, these works have been cited a total of 289 times (without self-citations by all authors), with h-index = 10. Assoc. Dr. Georgi Dobrikov took part in 14 scientific projects, 5 of which he was the leader of, and in a series of projects with industry - the companies Janssen and Johnson & Johnson (2005-2008) and IPSEN (2008). Assoc. Prof. Dr. Georgi Dobrikov is also a participant in 2 European scientific networks and is a member of the Management Board of COST ACTION CM1407.

3. Relevance of the topic and expediency of the set goals and tasks

The dissertation work's topic is related to human health and more specifically to the possibilities of treatment of tuberculosis and infections caused by enteroviruses. Tuberculosis is a chronic infectious disease affecting respiratory organs and is caused by the bacteria *Mycobacterium tuberculosis*. In its treatment, a combination of several drugs is used, which often leads to the emergence of drug-resistant bacteria, causing multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis, and side effects. Enteroviruses (intestinal) viruses are RNA viruses that affect humans and animals and most commonly cause nausea, vomiting and diarrhea. Enteroviruses, which include the polio virus, echo, coxsackie viruses (A and B group), etc., can also cause more serious diseases (meningitis, encephalitis, myocarditis, poliomyelitis, diabetes, etc.), which can later be life-threatening. They spread very quickly and are relatively durable in the environment. The treatment of infections caused by enteroviruses is non-specific and is made difficult by the frequent mutation of viruses leading to the rapid development of resistance to antiviral drugs, as well as by the limited number of biochemical processes in viruses that can be affected by antiviral agents. Therefore, screening multiple synthetic, semi-synthetic and natural compounds is one way to discover effective anti-tuberculosis agents. On the other hand, the discovery of new compounds that selectively inhibit viral RNA replication may be effective in fighting viruses.

The scientific topic is complex and requires the participation of scientists from various specialties - chemistry, biophysics, biology, biochemistry, microbiology, medicine, etc. The focus of Assoc. Prof. G. Dobrikov's dissertation work is on the synthesis of new compounds as potent *in vitro/in vivo* anti-tuberculosis and antiviral agents with reduced cytotoxicity and improved pharmacological properties. The aim of the dissertation and the planned tasks to achieve it define this work as truly relevant and with significant scientific and scientific-applied potential.

4. Knowledge of the problem

The introduction of the dissertation, as well as the discussion, give me reason to believe that Assoc. Prof. G. Dobrikov is well aware of the huge number of studies in the field of developing new anti-tuberculosis and anti-enterovirus drugs and has skillfully selected which of them to consider as most relevant and related to their research and results, described and discussed in the dissertation work.

5. Research methodology

The research methods in the dissertation work can be grouped into two main directions: synthesis of new R-2-aminobutan-1-ol and (+)-camphor derivatives, and analogues of the antiviral agent MDL-860 and *in vitro/in vivo* microbiological studies of the newly synthesized compounds. Special emphasis is placed on the synthesis and characterization of the new compounds obtained, without detailing the biological methods used. In some cases, QSAR analyzes were performed to elucidate the opportunities for further development of improved biologically active compounds. Methods described in the literature, as well as their modifications, are most often used for the synthesis of new compounds. Thin-layer chromatography was used to check the progress of the reactions, classic column chromatography - for the purification of the intermediate and final products, and NMR (Nuclear Magnetic Resonance), MS (Mass Spectrometry), optical rotation angle, elemental analysis, X-ray analysis, etc. - for the structure characterization of the synthesized compounds. The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation work.

6. Characterization and evaluation of the dissertation work

The dissertation is written in English on 184 pages, of which I. Introduction – 16 pages, II. Aim and tasks – 1 page, III. Results and Discussion – 66 pages, IV. Experimental part – 71 pages, V. Literature – 21 pages (340 literary sources), VI. Conclusion and Contributions – 2 pages and Appendices – 3 pages. The dissertation has 34 figures, 28 tables and 29 diagrams. The introduction presents a brief analysis of the nature and spread of tuberculosis and methods of its treatment,

the key stages of the life cycle of enteroviruses, the main classes of anti-enteroviral drugs, and methods for the synthesis of diaryl esters. Current trends in the development of anti-tuberculosis and anti-enteroviral drugs are shown. The dissertation's aim is clearly defined and the specific tasks for its achievement are outlined.

The "Results and Discussion" section describes the main schemes and methods for preparation of a wide range of new compounds, grouped by the type of starting product - 2-aminobutanol derivatives (N-acyl, N-alkyl/aryl, and heterocyclic derivatives, ureas, thioureas and acylthioureas), derivatives of (+)-camphor, (-)-fenchone, nitrofurans, diaryl ethers, etc. In each subgroup, Assoc. Prof. Dobrikov has made a brief literature survey about the known methods for the synthesis of anti-tuberculosis and anti-enteroviral compounds from the corresponding starting product. The number of synthesized compounds (> 300) is impressive, and their yields are mostly good to excellent. Most of them are new compounds not described in the literature. Some of the known synthesized compounds have been obtained in better yield and higher degree of purity. The results of the antibacterial (mainly anti-tuberculosis) and antiviral activities and cytotoxicity studies, which were carried out in collaboration with scientists from other institutions, mainly from the Institute of Microbiology, BAS are also presented. These data are discussed in the dissertation as evidence for the biological properties of the synthesized compounds and looking for a structure/activity relationship. Among the vast array of synthesized substances, only a few have shown incredibly good *in vitro* antituberculosis, antibacterial and antifungal activity and low cytotoxicity. With the participation of one of the active new nitrofuranoylamides, *in vitro* mutagenesis was carried out on *M. tuberculosis* reference strain H37Rv subcultures grown at increasing concentrations of the selected compound and six mutations in 6 genes were identified. A possible mechanism of action has also been proposed for some of the compounds obtained. More than 10 new MDL-860 diaryl ether analogues (out of a total of 137 compounds) tested against enteroviruses were determined to be highly active and non-toxic *in vitro*. Some active compounds have been selected for *in vivo* experiments showing a high percentage of surviving animals (mice). QSAR analyzes of some of the synthesized compounds were also performed to find additional variations in their molecules and based on the results obtained, compounds that were analogs of fenofibrate, a drug used to treat abnormal blood lipid levels, were purposefully synthesized.

In the "Experimental part" the materials used, apparatus, technique, and procedures for preparation of the compounds, their structural formula and spectral data are given. Literature sources of the methods used to determine the biological activity of the synthesized compounds are written down, since these experiments are not the work of the dissertation student but were used in the discussion.

After the cited references, the obtained results and the contributions of the dissertation work are summarized. The appendices contain a list of publications on the dissertation (8 items), personal participations at scientific conferences (26 items) and research projects on the dissertation's topic (8 items).

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

The main contributions received during the development of the dissertation work are expressed in:

- Synthesis of a new subclass analogues of classical antitubercular drug ethambutol. Some of these analogues showed higher activity and lower cytotoxicity, than ethambutol.
- Synthesis of a new class of antitubercular drug-like molecules, bearing fenchane skeleton.
- Synthesis of a new class of antitubercular drug-like molecules, bearing camphane skeleton, showing strong antitubercular and antibacterial activity.
- Synthesis of new drug-like nitrofuranoyl compounds and investigation of their possible mechanism of strong antitubercular activity by using *in vitro* provoked mutagenesis.
- Synthesis of different new analogues of known diaryl ether MDL-860 with stronger activity toward 6 enteroviruses than MDL-860 and establishment of the mechanism of action for MDL-860.
- Discovery of many promising bioactive compounds (so called “hit compounds) among above

mentioned groups which are suitable for further drug development in next preclinical phases.

The results obtained in the dissertation work of Assoc. Prof. Dobrikov hold new and original information for science. They can be evaluated as significant theoretical and experimental achievements that have a high potential for practical application.

8. Evaluation of the publications related to the dissertation thesis

The dissertation includes 8 scientific articles (4 - Q1 and 4 - Q2), published in internationally renowned scientific publications such as Bioorganic Chemistry, Pharmaceuticals, Biomedicines, ACS Infectious Diseases, etc. The total impact factor of the articles is 28.086. The interdisciplinary nature of the research requires the participation of scientists from different fields, a large part of which involves scientists from Russia, China, Portugal, and Spain, but the leading role and contribution of Assoc. Prof. Dobrikov is indisputable, as he is the corresponding author of 5 of the publications (2 Q1 and 3 Q2). Assoc. Prof. Dobrikov has declared that the articles included in the dissertation were not used for other competitions. All articles, except one, were published after the habilitation of Assoc. Prof. Dobrikov. The research results included in the dissertation were presented at 26 national and international scientific forums, 6 of which were scientific reports.

9. Personal participation of the author

The dissertation's main contributions are in design, synthesis, and structural determination of new compounds. It is noteworthy that Assoc. Prof. Dr. Dobrikov has mastered the specific microbiological terminology and skillfully analyzes the obtained results, both in the field of organic synthesis and in the field of microbiology and medicine. I am convinced that the results obtained, and the contributions formulated, are primarily the personal results of Assoc. Prof. Dobrikov.

10. Abstract

The abstract in Bulgarian and English is written according to the requirements of the regulations of IOCCP and BAS, follows the structure of the dissertation, without the experimental part, and faithfully reflects the main scientific results described in the dissertation and the conclusions drawn.

11. Critical remarks and recommendations

I have no critical notes and recommendations for the conducted research and the set of materials. All results have been published in peer-reviewed international journals and have passed the critical evaluation of experts in the respective fields.

12. Recommendations for future use of dissertation contributions and results

New more promising candidates for the treatment of tuberculosis and enterovirus infections could be synthesized based on the relationship/activity data obtained. The promising results obtained for some of the newly synthesized compounds are a good prerequisite for further studies in *in vivo* and clinical conditions with the aim of developing new medicinal products.

CONCLUSION

The dissertation has scientific, scientific-applied, and applied results, which 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 (ZRASRB), the Regulations for the Implementation of the ZRASRB and the Regulations for the Implementation of the ZRASRB of the Bulgarian Academy of Sciences. The presented materials and dissertation results fully follow the specific requirements of the Regulations of the IOCCP-BAS for the application of ZRASRB.

The dissertation work shows that Assoc. Prof. Dr. Georgi Dobrikov has in-depth theoretical knowledge and professional skills in the scientific specialty "Organic Chemistry" by showing qualities and skills for conducting research with obtaining original and significant scientific contributions.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury to award the scientific degree *'Doctor of Sciences*' to Assoc. Prof. Dr. Georgi Dobrikov in the field of higher education *4. Natural sciences, mathematics and informatics,* professional direction *4.2. Chemical sciences,* the scientific specialty "*Organic Chemistry*".

26.09. 2023 Reviewer: .....

(Prof. Dr Antoaneta Trendafilova)