REVIEW
by prof. Rositca Dimitrova Nikolova, PhD,
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member of the scientific jury according to the
Order of the Director of IOCCP-BAS RD 09-102/04.07.2023
of a dissertation for the awarding the scientific degree "**Doctor of Sciences**" in the field of higher education *4. Natural sciences, mathematics and informatics*,
professional direction *4.2 Chemical sciences*,
scientific specialty "*Organic chemistry*"

Author: Assoc. Prof. Dr. Georgi Milchev Dobrikov,
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Topic: New compounds as perspective antitubercular and antiviral agents

1. Subject of review

The set of materials presented by Assoc. Prof. Georgi Dobrikov is in accordance with the Regulations for the Development of the Academic Staff of IOCCP-BAS and meets the criteria of IOCCP-BAS for the acquisition of the scientific degree "Doctor of Sciences". Attached are: a European-style curriculum vitae, a dissertation (in English), an abstract (in Bulgarian and English), a certificate of compliance with the minimum criteria for obtaining the scientific degree "Doctor of Sciences", a copy of the PhD diplom, a list of publications in refereed scientific journals on the competition and a copy of 8 publications, a list of participations in scientific conferences and a copy of 26 summaries of the presented results, a list of citations (303 in number) and information on projects related to the subject of the dissertation (8). From the presented materials, it can be seen that Associate Professor Dobrikov not only meets, but also in certain categories exceeds the minimum requirements for obtaining the scientific degree "Doctor of Sciences", specified in the Law on the development of the academic staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the development of the academic staff of IOCCP-BAS. The obtained results of the dissertation were published in 8 scientific publications (4 of them in Q1 and 4 in Q2) and until the submission of the documents, 33 were cited in other authors' research.

2. Brief biographical data

Assoc. Prof. Georgi Dobrikov graduated from the Faculty of Chemistry of the Sofia University "St. Kliment Ohridski" in 1998 with a master's degree. In the same year, he started working as a chemist at the Institute of Polymers, BAS, and in 1999 in the same position at the National Center for Public Health and Analyses. Since 2001, he has been a full-time doctoral student at IOCCP-BAS with scientific supervisor Prof. Vladimir Dimitrov. His doctoral dissertation on the topic "Preparation of chiral ferrocene derivatives - absolute configuration and application in asymmetric synthesis" was successfully defended in 2006. In 2004, after a competitive exam, he was appointed as an assistant, in 2007 he was promoted to chief assistant, and since 2016, he has been an associate professor of organic chemistry at IOCCP-BAS.

3. Relevance of the topic and expediency of objectives and tasks

The dissertation of Assoc. Prof. Georgi Dobrikov is aimed at obtaining new compounds with antituberculosis activity and new compounds with antiviral activity. In the course of establishing their biological activity, other properties such as cytotoxicity, genetics, etc. were also investigated. Based on the QSAR analysis of the synthesized compounds, additional possibilities for their modification were sought, in order to obtain more active analogues. The research has a multidisciplinary nature and includes current guidelines in the field of organic synthesis, spectral and computational techniques, as well as proof of biological activity. The results of the research

are related to improving the quality of life in terms of developing antiviral preparations and strengthening human health - tuberculosis is still a deadly disease nowadays. All this gives me a reason to define the dissertation research as socially significant, relevant and a substantial contribution to the scientific community. Adequate, modern and reliable methods were used to achieve the set goals.

4. Knowledge of the problem

The Introduction of the dissertation presents a thorough and analytical review of the state of research on antitumor and antienteroviral drugs and demonstrates a good knowledge of the topic. The drugs used so far are examined in detail with an emphasis on their chemical structure and the advantages and disadvantages of their pharmacological behavior. In view of the objectives of the dissertation, attention is also paid to the methods for the synthesis of diaryl ethers as antiviral drugs.

5. Research methodology

The research methodology includes the synthesis of a series of structurally different compounds, including (*R*)- and (*S*)-2-amino-1-butanol residue as *N*-acylated, alkylated and arylated derivatives of enantiomerically pure amino alcohols; ureas, thioureas and acylthioureas containing an (*R*)-2-amino-1-butanol moiety; *N*-acyl and cinnamamide derivatives of (-)-fenchone, arylmethylidene ketones and pyrimidines with a camphor skeleton. The synthetic procedures used are described in the literature, as well as some of the newly obtained substances. All compounds were purified by recrystallization or column chromatography to 99% purity and characterized by spectral and analytical methods. X-ray structural analysis was also used to prove the structure of more complex systems.

Their antimycobacterial activity was investigated, and a comparison of pairs of enantiomers of the substituted 2-amino-1-butanols in terms of their activity was also provided. For representatives that showed good activity, their cytotoxicity was also assessed. A qualitative assessment of the antibacterial and antifungal activity was made.

A series of new analogues of the diaryl ether MDL-860 were studied for antiviral activity, and their preparation and characterization was carried out according to the described methodology. All compounds were tested against enteroviruses, and certain samples against human corona virus, herpes and human adenovirus. QSAR analysis was also performed to identify more active analogues for further research.

6. Characterization and evaluation of dissertation

The dissertation is written in English and includes 177 pages, 28 tables, 34 figures, 29 schemes and 340 references.

The Introduction reviews the current trends in the development of anti-tuberculosis and antienteroviral drugs, as well as the most promising drugs in clinical research, and serves as a very good starting point in setting the goals and objectives of the dissertation.

In Aims and objectives, the three main research problems are presented and the main tasks for solving them are planned.

In the Results and Discussion chapter (spanning 61 pages) the series of novel compounds with potential antitumor and antiviral activity are comprehensively presented. Since the synthesis of the new substances is carried out according to known procedures, they are presented briefly, the emphasis is placed on the in vitro / in vivo studies of the individual series. In the first part, the possibilities for the synthesis of new anti-tuberculosis candidates are considered.

A series of new analogues of ethambutol were obtained, and the mechanism of its action and structure/activity relationship studies were taken into account when planning. The fact that there is not enough research on (*R*)-2-amino-1-butanol derivatives is also recognized and efforts are put into that direction. A series of *N*-acylated, alkylated and arylated derivatives of enantiomerically pure (*R*)-2-amino-1-butanol were obtained (50 representatives); ureas, thioureas and acylthioureas containing (*R*)-2-amino-1-butanol residue (22 representatives). Tests for in vitro

activity against the reference strain *Mycobacterium tuberculosis* H37Rv showed very good results – 5 of the compounds showed high activity combined with low cytotoxicity.

In the series of (-)-fenchone amides and cinnamamides with a fenchone skeleton, representatives with moderate activity and low cytotoxicity have also been reported.

Interesting results were also obtained with the series of arylmethylidene ketones and pyrimidines with a camphane skeleton. Two of the investigated compounds showed very good antituberculosis activity and metabolic stability. During *in vitro* testing of the antibacterial and antifungal activity of these series of compounds, it was found that one of them has the qualities of a broad-spectrum antibiotic.

Excellent antituberculosis activity was also shown by 3 of the series of 6 new nitrofuranoylamides. For one of the representatives, *in vitro* mutagenesis was carried out and mutations in 6 genes were proven.

The second part is devoted to the development and research of new compounds with antiviral activity. Several series of new analogs of the diaryl ether MDL-860 (137 compounds) were synthesized. All compounds were tested against enteroviruses Coxsackie 1 and 3 and poliovirus. Selected representatives were also tested against human corona virus OC43, herpes simplex virus type 1 and human adenovirus C serotype 5. Over 10 structures showed very good activity and were nontoxic in *in vitro* experiments. In the in vivo experiments, some of them showed a high percentage of surviving animals. QSAR analysis was also performed to identify more active analogues for further research.

The data presented in the Experimental part comprehensively and accurately present the synthetic procedures and the spectral and analytical data for the newly obtained substances, as well as the methods for studying antitumor and antibacterial activity.

In Conclusion and Contributions, the original scientific results of the dissertation work are correctly presented.

7. Contribution and significance of the work for science and practice

The dissertation of Assoc. Prof. Georgi Diobrikov is a thorough and comprehensive study on the possibilities of obtaining new antitumor and antiviral drugs, which covers both the synthesis and in vitro and in vivo studies of the obtained compounds. The obtained results have an interdisciplinary character and although they are strictly fundamental, they have a good potential for practical application. As the most significant contributions, I can point out:

- Several new classes of compounds with promising antituberculosis activity have been synthesized:

- a large number of analogues of the anti-tuberculosis drug ethambutol, some of which show higher activity and lower toxicity than ethambutol;
- compounds with a fenchan skeleton, some of which exhibit moderate antituberculosis activity;
- compounds with a camphane skeleton showing high antituberculosis and antibacterial activity;
- new nitrofuranoylamides whose possible mechanism of high antituberculosis activity was investigated using *in vitro* mutagenesis.

- New analogs of diarylether MDL-860 were synthesized, representatives of which showed better activity against 6 viruses.
- The mechanism of action of MDL-860 has been established.
- As a result of the research, a large number of promising bioactive compounds were found in each of the series suitable for drug development.

8. Evaluation of publication and personal contribution of the author

The total number of publications presented in the competition is 8. All have been published in refereed international editions, 4 of them in Q1 and 4 in Q2, and until the submission of the documents, 33 have been cited in other authors' research. In 5 of them Assoc. Prof. Georgi Dobrikov is the corresponding/first author.

9. Abstract of the thesis

The abstract (in Bulgarian and English) exactly follows the dissertation and correctly reflects the results of the scientific research. It has been prepared in accordance with the requirements of the Regulations for the conditions and procedures for acquiring scientific degrees at IOCCP-BAS.

10. Critical remarks and recommendation

I have no critical comments on the materials of the competition and on the conducted studies, which have been published in refereed and reputable journals in the relevant field.

11. Personal impression

I know Assoc. Prof. Dr. Georgi Dobrikov as a responsible and serious scientist who over the years has shown opportunities for development, a desire to work with young colleagues and to pass on his knowledge and skills.

CONCLUSION

The dissertation of Assoc. Prof. Georgi Dobrikov presents scientific results that represent an original scientific contribution and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law and the Regulations for the Implementation of the Law of the Bulgarian Academy of Sciences. The results of the scientific research, the publication activity and the presented materials fully correspond to the specific requirements of the Rules of the IOCCP-BAS for the application of the Law. The presented results and the dissertation show Assoc. Prof. Georgi Dobrikov as a well established scientist with a clear profile, in-depth theoretical knowledge and professional skills and a proven presence in the field of the scientific specialty "Organic Chemistry". Guidelines for its development and opportunities for obtaining new original and significant scientific results are clearly outlined in the dissertation work.

All of the above gives me the reason to give a positive assessment of the dissertation work submitted for review, the achieved results and scientific contributions. With conviction, I propose to the honorable scientific jury to award the scientific degree "Doctor of Sciences" to Assoc. Dr. Georgi Milchev Dobrikov in the field of higher education *4. Natural sciences, mathematics and informatics*, professional direction *4.2 Chemical sciences*, scientific specialty "*Organic chemistry*".

29.09.2023 Reviewer: ……..…….

(Prof. Dr. Rositca Nikolova)