



SCIENTIFIC OPINION

by Prof. Dr. Petya Koycheva Hristova
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Topic of the dissertation: "In vitro and in vivo study of the antiviral activity of a series of new diaryl ethers and their analogues - promising chemotherapeutics in anti-enterovirus therapy" for the award of the scientific degree "Doctor of Science" in field 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences.

Author of the dissertation: Assoc. Prof. Dr. Ivanka Nikolova Nikolova

By order of the Director of the Institute of Microbiology at the Bulgarian Academy of Sciences No. I-46/28.03.2025, I have been appointed as a Member of the Scientific Jury for conducting a procedure for the defense of the dissertation of Assoc. Prof. Dr. Ivanka Nikolova Nikolova for the acquisition of the scientific degree "Doctor of Sciences". The submitted documents and materials meet the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

1. Brief biographical data

Ivanka Nikolova Nikolova graduated in 1995 with a Master's degree in Biology and Chemistry from the Faculty of Biology of Sofia University "St. Kliment Ohridski". The same year, she began her career as a specialist biologist at the Institute of Microbiology at the Bulgarian Academy of Sciences in the "Microbial Genetics" section. In the period 1998-2001, she was a full-time doctoral student in virology at the same institute, but in the Department of Virology. Ivanka Nikolova developed a dissertation on the topic: "Resistant and dependent mutants of Cocksackie B1 virus to the picornavirus inhibitor disoxaril", which she successfully defended in 2004. Dr. Nikolova successively went through the different stages of her scientific development in the Department of Virology, initially as a Research Associate III degree/Assistant (2004-2008), Chief Assistant (2008-2020) and Associate Professor (2020 - present). Since 2012, he has been the head of the laboratory "Experimental Chemotherapy of Enterovirus Infections", and since 2016 he has been the head of the Department of Virology.

2. Relevance and significance of the dissertation topic

Enteroviruses infect millions of people each year and have a variety of clinical manifestations, affecting mainly children and high-risk groups. The majority of enterovirus infections occur inapparently, i.e. these viruses circulate in the human population without being detected. In a number of cases, however, they cause clinically manifest diseases of varying severity, including those affecting the CNS, heart, endocrine pancreas, and others. The lack of effective vaccines (except polioviruses) due to the unusually large number of enterovirus serotypes, defines chemotherapy as the main means of controlling enterovirus infections. However, to date, there are no effective chemotherapeutic agents registered for the specific treatment of these infections due to toxicity or due to the induction of resistance. Therefore, one of the ways to combat resistance is the development of approaches for the combined application of new or already known substances with high inhibitory potential against key stages of the replicative cycle of enteroviruses. The present dissertation investigates the in vitro and in vivo antienterovirus efficacy of both reference and novel diaryl ether analogues as inhibitors of cellular phosphatidylinositol-4-kinase beta (PI4KB), with the aim of identifying more effective and safe chemotherapeutic agents. These compounds can be applied both in combination therapy regimens and in monotherapy courses for the etiologic treatment of enterovirus infections.

The presented facts clearly motivate the choice of the topic, its relevance and practical significance. The content of the dissertation fully covers the announced nomenclature specialty in professional direction 4.3 Biological Sciences, for the award of the National Academy of Sciences "Doctor of Sciences"

3. Assessment of the structure of the dissertation

The dissertation is written on 189 pages and contains 46 figures, 31 tables, 1 scheme and 351 literature sources. The scientific publications, the subject of which is included in this dissertation, are 26 (12 in Q1, 8 in Q2, 3 in Q3 and 3 in Q4). The results have been presented in 20 oral or poster reports at scientific conferences in Bulgaria and abroad. The results included in the dissertation were obtained during the implementation of research projects funded by the Scientific Research Fund: B02-11 (2014-2019); KP-06-CHINA/31 (2020-2022), KP-06-H49/2 and KP-06-H31/7 (2019-2025).

Literature review

The literature review is structured with a direct relationship to the aim of the dissertation and the tasks set. The review of the dissertation work presents three thematic axes: one presents the distribution of enteroviruses and their importance for human pathology. Moreover, the architecture of the enterovirus capsid and the organization of the genome are described in detail. The second

thematic axis examines the mechanisms in the replicative cycle of enteroviruses, paying particular attention to the role of viral proteins and host factors in membrane rearrangements in the cell. The third topic tracks the effectiveness of various inhibitors of enterovirus replication, disclosing both antivirals that have shown in vitro activity against enteroviruses, and chemotherapeutics that have reached clinical trials.

Purpose and tasks

The aim of the dissertation is formulated relatively clearly, and 10 tasks are formulated for its achievement. Solving these problems contributes to the development and testing of new, more effective compounds that may find application in solving this important health problem.

Materials and methods

This is an important part of the dissertation work, which shows the level of scientific research. Modern virological, cytological and molecular methods are presented. Their development and successful application alone is already an undisputed success in the development of the dissertation.

Results and discussion

The main results are presented according to the tasks set and the chosen methodological approaches and are essentially an in-depth characterization of the antiviral potential of reference and new analogues of diaryl ethers. The experimental part is very precisely designed and presented reliably. In the first part, the effect of triple combinations of Pleconaril/Guanidine-HCl/Oxoglaucin (PGO) and Pleconaril/MDL-860/Oxoglaucin (PMO) in sequential alternative alternation of the compounds (SAA course) was studied against experimental neuroinfection with coxsackievirus B1 in newborn mice. The effect of both combinations on the content of infectious virus in the brains of the treated laboratory models was assessed. The sensitivity of viral brain isolates to various chemoagents after treatment of the animals with the above triple combinations was monitored. The genetic basis for the development of resistance was studied. In the second part of the dissertation, studies on the effectiveness of synthesized diaryl ethers and their analogues on the replication of enteroviruses - coxsackievirus B1 (CVB1), coxsackievirus B3 (CVB3) and poliovirus 1 Sabin (PV1) are presented. Their in vitro and in vivo applicability is evaluated and highly effective compounds are selected.

Conclusions and recommendations

The vast experimental work is summarized in the Conclusion, but no specific conclusions are presented.

4. Scientific and applied contributions

The research conducted outlines both theoretical and original applied contributions, which prove the significance of the scientific work. The contributions that I consider to be particularly significant and of great application are as follows:

- The proposed new therapeutic scheme for combined sequential administration of antiviral compounds inhibits the development of resistance in enteroviruses.
- 10 compounds were selected that showed significant activity against 3 enteroviruses (poliovirus type 1, coxsackievirus B1, coxsackievirus B3).
- Six compounds were selected that, in in vivo tests in a mouse model of experimental Coxsackie B1 neuroinfection, had higher activity than the reference compound and are promising for further studies.
- Diaryl ethers have been identified with high in vitro antiviral activity against human coronavirus OS43 and human adenovirus type 5.

5. Publications related to the dissertation

A list of 26 publications (12 in Q1, 8 in Q2, 3 in Q3 and 3 in Q4) related to the dissertation is presented, which fully meet the requirements.

6. Conclusion

The presented dissertation is a completed study that provides valuable scientific information and reveals opportunities for practical application. I believe that the volume of this work is impressive, many analyses have been made, valuable results have been obtained, important conclusions and recommendations have been made, and the study has significant scientific and applied contributions.

Based on the analysis made, I propose to the esteemed members of the scientific jury, formed by order of the Director of the Institute of Microbiology and Biochemistry at the Bulgarian Academy of Sciences No. I-46/28.03.2025, to award Ivanka Nikolova Nikolova the scientific degree "Doctor of Sciences" in the professional field 4.3 Biological Sciences.

22.05.2025

Reviewer:

prof. Petya Hristova