

OPINION

from Assoc. Prof. Konstantin Borisov Simeonov, PhD,

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Regarding: Dissertation for the acquisition of the scientific degree “Doctor of Sciences” in scientific specialty 4.3 Biological Sciences

Author: Assoc. Prof. Ivanka Nikolova Nikolova, Institute of Microbiology, BAS

Topic of the dissertation: In vitro and in vivo study of the antiviral activity of a series of new diaryl ethers and their analogs – promising chemotherapeutics for anti-enteroviral therapy.

General presentation of the procedure. Based on Order No. I-46/28.03.2025 of the Director of the Institute of Microbiology, BAS, I was appointed as an external member of the scientific jury for the procedure for acquisition of the scientific degree “Doctor of Sciences” in the field of Natural Sciences, professional field 4.3 Biological Sciences. The order was issued on the basis of Art. 39, para. 3 of the Regulations on the conditions and procedure for acquiring scientific degrees and for occupying academic positions at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences and a decision of the Scientific Council of the Institute of Microbiology, Protocol No. 4 of 25.03.2025). I hereby declare that I have no conflict of interest with the candidate as defined in para. 1, items 3 and 5 of the Law for Development of the Academic Staff in the Republic of Bulgaria.

I was provided with the following materials: the full text of the dissertation, an abstract, a CV, a list of publications included in the dissertation, the publications themselves in PDF format, diplomas, a list of conferences where results from the dissertation were presented, a citation list, and a report on compliance with the minimum requirements for the academic degree "Doctor of Science". The candidate's participation in scientific projects is listed in the CV – 11 projects in total, including 3 international, 6 funded by the National Science Fund, and 2 financed by BAS. The submitted set of materials fully complies with the Regulations for Awarding Academic Degrees and Positions at IMicB–BAS and the requirements of Act of the Academic Staff Development.

Brief biographical data. According to the submitted CV, the entire scientific career of the candidate has developed at the “Stefan Angelov” Institute of Microbiology – BAS. After

graduating in biology and chemistry from Sofia University “St. Kliment Ohridski,” she was appointed as a PhD student in virology. Following the successful defense of her dissertation, her academic progression moved from research biologist through research associate III degree and senior assistant, ultimately reaching the academic position of associate professor, which she has held since 2020. She currently heads the Department of Virology at the Institute and has held various organizational roles, including Secretary of the “Acad. Prof. Dr. Stefan Angelov” Foundation, Secretary and now Chair of the Seminar on General Virology, and Chair of the Scientific Council at the Institute.

Relevance of the topic and general characteristics of the dissertation. The numerous representatives of the genus Enterovirus, their diversity (over 280 human serotypes), and the high variability of their RNA genome make vaccine development (except for polioviruses and enterovirus-A71) nearly impossible in the near future. Therefore, antiviral drug development is a key strategy. A major challenge, however, is the rapid emergence of drug-resistant viral mutants. This necessitates the synthesis and evaluation of new antiviral compounds, as well as the exploration of alternative therapeutic approaches, including combination therapy schemes. These issues are central to the presented dissertation, which makes the work both actual and of potential practical importance. The choice of topic is not accidental—it reflects Assoc. Prof. Nikolova’s scientific interests and is a continuation of the research tradition of the Virology Department at IMic-BAS, were an internationally known school, established by Acad. Galabov exists.

The dissertation comprises 189 pages and follows the standard structure: Literature Review, Aims and Objectives, Materials and Methods, Results and Discussion, Inference, Contributions, and References. The literature review (351 references) demonstrates a well knowing of the topic. However, it is not appropriate to include the author’s own publications both in the literature review and again in the list of dissertation-related papers. The review presents an in-depth analysis of enterovirus morphology and morphogenesis, their replication cycle, key structural targets for antiviral intervention, as well as the current range of antivirals, mechanisms of resistance, and strategies to overcome it. The literature section also includes data on the laboratory’s COVID-19 research efforts, such as testing compounds for anti-coronavirus activity and developing antibacterial textile materials—important applied research, though somewhat outside the main topic of the dissertation.

The formulated goal gives the main direction in which Assoc. Prof. Nikolova's scientific research should be implemented (study of the antienterovirus effectiveness of MDL-860 and its newly synthesized analogues). However, the actual experimental scope is broader and includes monotherapies and combinations not involving MDL-860, and evaluation of antiviral activity beyond enteroviruses. A more precise formulation of the aim would better reflect the work's breadth. Nonetheless, the specific experimental tasks are consistent with the conducted research.

The methodology reflects the candidate's intent to comprehensively address the issue, using a wide range of traditional and modern methods, including molecular-level approaches. The *in vitro* and *in vivo* experimental designs are well described, and the animal procedures followed national and EU regulations (European Communities Council Directives 86/609/EEC). The ability to apply bioinformatics for data analysis is commendable—for example, using the Prichard and Shipman 3D model to assess compound interactions or QSAR models to guide synthesis. Statistical analyses strengthen the validity of the findings. The “Results and Discussion” section of the dissertation can be seen as having two major focuses. First, it expands on earlier work of a research team, in which Assoc. Prof. Nikolova participate, validating the higher efficacy of triple combination therapies using the CAA (Consecutive Alternating Administration) approach compared to simultaneous application. This is convincingly demonstrated in experiments *in vivo*, including also MDL-860. The experimental work optimized treatment schemes and analyzed the resulting viral populations, identifying phenotypic markers and mechanisms of suppressed resistance. Second, the research encompasses broader screening of new diaryl ethers and their analogs, including QSAR-guided synthesis of more potent molecules. Some newly synthesized compounds showed antiviral activity comparable or superior to MDL-860. Their potential as broad-spectrum antivirals (effective also against human coronaviruses and adenoviruses) and other heterocyclic compounds (quinoline derivatives) shows the capacity of the laboratory to address emerging challenges, such as the COVID-19 pandemic. The conclusion summarizes and synthesizes the results well, emphasizing the preclinical and clinical potential of the studied compounds. The dissertation is written at a high scientific level, and minor issues (e.g., missing abbreviations such as QSAR, SiRMS in the abbreviation list) do not detract from the overall quality. The large number of figures (mainly chemical structures), graphs, and 3D models, as well as tables, aid in understanding the work. Overall, the dissertation is of both fundamental and applied

scientific importance, offering a solid foundation for new therapeutic approaches and rational drug design.

Evaluation of publications and personal contribution. Assoc. Prof. Nikolova has submitted a list of 26 publications related to the dissertation. However, 8 of them (No. 7, 8, 9, 11, 13, 21, 23, 26) concern topics outside the dissertation, and are therefore not considered. Among the remaining 18, 9 are published in Q1 journals (50%), which is very impressive. Two are in Q2, three in Q3, and two in Q4. Dissertation results have been presented at 20 scientific forums, many of them international, enhancing their visibility. The candidate presents a list of 103 references to her work by other authors (35 of which are accepted with reservation for reasons mentioned earlier), indicating interest and recognition from the scientific community. The author points out 5 contributions, the content of which reflects what has been achieved as a result of the research conducted, emphasizing the practical significance of the results obtained and the prospects for their further development.

Abstract of the dissertation. The submitted abstract outlines the key elements of the dissertation and meets the formal requirements. A minor note: the section titled "Review of the Literature Review" is redundant, as "review" already implies an overview, making the title tautological.

CONCLUSION

The dissertation submitted by Assoc. Prof. Ivanka Nikolova is based on a methodologically well-structured study and presents scientific and applied results that clearly contribute to the search for effective anti-enteroviral chemotherapeutics. The proposed treatment schemes and drug development approaches are not only significant findings but have potential for further theoretical and applied development. Even after reducing the number of accepted publications, the scientometric indicators meet and exceed the minimum national and BAS requirements for the Doctor of Science degree. Based on the above, I give my **positive assessment** for awarding the scientific degree "Doctor of Science" to Assoc. Prof. Dr. Ivanka Nikolova in professional field 4.3. Biological Sciences and I call on the other members of the esteemed scientific jury to support this opinion.

28.05.2024.

Prepared by:

/Assoc. Prof. Dr. Konstantin Simeonov, PhD/