STATEMENT

*by **Assoc. Dr. Rainichka Petrova Mihaylova-Garnizova**, Head of the Department of Infectious Diseases - MMA, Sofia*

dissertation for the degree of Doctor of Science in the professional field 4.3, Biological Sciences

Author.
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Head of the Department of Virology, Institute of Microbiology "Stefan Angelov", BAS, Head of the Laboratory "Experimental Chemotherapy of Enterovirus Infections" at the Department of Virology, Institute of Microbiology "Stefan Angelov",
BAS

Topic: **In vitro and in vivo study of the antiviral activity of a series of novel diaryl ethers and their analogues - promising chemotherapeutics in anti-enteroviral therapy**

1. General description of the procedure and the candidate

Brief notes on the procedure

By Order№ Order No. I-46/28.03.2025 of the Director of the Institute of Microbiology "Stefan Angelov" - BAS I was appointed as a member of the scientific jury in connection with the procedure for obtaining the degree of Doctor of Science in the professional field 4.3.

For this purpose I was provided with the following materials:

- Author's abstract:
    - **"In vitro and in vivo study of the antiviral activity of a series of novel diaryl ethers and their analogues - promising chemotherapeutics in anti-enteroviral therapy**"
    - 94 p., of which 3 p. "List of scientific publications related to the topic of the dissertation"

- In electronic version the following files:
    - the full text of the PhD, 189 pages
    - autobiography,
    - Diplomas,
    - the articles on which the doctorate is based,
    - list of 20 participations in conferences and symposia of Assoc. Nikolova
    - list of citations - 60 in total, not counting 2 self-citations

The presentation by Assoc. Nikolova's set of materials meets the criteria for obtaining the scientific degree "Doctor of Sciences".

The dissertation is a summary of 26 publications on the subject and contains 46 figures, 31 tables, 1 diagram and 351 references.

The results have been presented at 30 scientific conferences in Bulgaria and abroad.

Overall, I have no comments on the documents submitted.

Brief biographical data

As can be seen from the presented CV in European format she graduated in 1995 from Sofia University "St. Kliment Ohridski", majoring in biology and chemistry (master's degree). From 1998 to 2001 she was a regular PhD student in virology

at the Institute of Microbiology "Stefan Angelov", Department of Virology on the topic. A. C. A. A. Galabov. In 2004 she received the degree of Doctor of Education and Science in the specialty of Virology with the code 4.3 "Biological Sciences".

Assoc. Nikolova also has teaching activity: she has given classes in Molecular Virology at the Faculty of Biology, Sofia University. Kliment Ohridski" and New Bulgarian University. Since 2016 he has been lecturing on Bacterial and Viral Infections of the Eye at Sofia University "St. Kli Kliment University". Kliment Ohridski".

She has worked as a biologist-specialist, research assistant and senior assistant at the Department of Virology, Institute of Microbiology "Stefan Angelov", BAS, and since 2016 she has been the head of the same department.

2. Topical relevance

The dissertation deals with a problem that is relevant in both scientific and applied terms, as enteroviruses are one of the most common human pathogens that affect all age groups, but the most vulnerable are children and high-risk groups -
patients with compromised immune systems and newborns. Although there are more than 280 human enterovirus serotypes, only
against four of them there are licensed vaccines (the three types of poliovirus and enterovirus A-71). It is because of the unusually large number of enterovirus serotypes that the introduction of vaccination will not be possible in the near future.

3. Knowledge of the problem

The author is familiar with previous research in this area, as is evident from the comprehensive list of references, their analysis and commentary.

4. Research methodology

In general, the materials and methods chosen meet the aims and objectives of the study, namely:

Materials

- Reference inhibitors of enteroviral replication: Pleconaril, MDL-860 and Oxoglaucine, Guanidine hydrochloride
- Tested compounds - 114 newly synthesized series of diaryl ethers and their analogues
- In vitro viral models: Coxsackievirus B1, Connecticut strain (CVB1), Coxsackievirus B3, Nancy strain (CVB3), Poliovirus type 1, Sabin strain LSc-2ab (PV1), Human coronavirus OC43 (HCoV OC43) and Human Adenovirus type 5 (HAdV-5)
- Viral model for in vivo experiments: coxsackievirus B1 (CVB1) Connecticut neurotropic strain 5 (Conn-5).
- Cell cultures: HEp-2 and HCT-8
- Experimental animals: the mice used for the in vivo experiments were random-bred white neonatal mice from the ICR line

Methods:

- Determination of: the viral infectious titre; the individual cytotoxicity; the individual antiviral effect of the compounds studied; the in vitro antiviral effect of the dual combinations; the in vitro combined cytotoxicity of the dual combinations of the substances studied
- Experimental coxsackie B1 virus infection in experimental mice and viral titer determination in vivo
- In vivo testing of triple combinations of anti-enteroviral
- compounds
- Preparation of viral isolates from mouse brain and titer determination and plaque purification of brain isolates in vitro; determination of sensitivity of viral isolates to test compounds by plaque method
- RT-PCR and sequencing
- Statistical methods

5. Characteristics and evaluation of the thesis

This is a dissertation that is the result of many years of work and an established strong tradition in the Department and Institute of Microbiology. Some of the issues have been addressed within international and national projects, publications are often internationally involved. Bulgarian microbiology has long been a part of world microbiology and this work is no exception. Some of the contributions that I will comment on below may form the basis of future preclinical studies, which, in my opinion, not only as a scientist but also as a therapist, is correct and commendable.

6. Evaluation of the publications' record and author's contribution

Assessment of publications

It is difficult to assess the personal contribution of the PhD student in collective publications - practically all of them are co-authored. It makes a good impression that 8 of the publications are in quartile 1 and only one is categorized in quartile 4. This is a high enough score.

Assessment of contributions

In my opinion, the contributions of the thesis are scientifically applied
- a new therapeutic scheme for combined sequential administration of anti-enteroviral compounds in coxsackie B1 virus Neuroinfection in neonatal mice.
- ten compounds were selected that are comparable and even more effective than the reference compound against enterovirus (poliovirus type 1, coxsackievirus B1, coxsackievirus B3), making them suitable for further preclinical phases in future antiviral drug development.

- six of these in vitro tested compounds against coxsackievirus B1 have undergone further in vivo testing in a mouse model and are promising for further research
- Diaryl ethers with high in vitro antiviral activity against human coronavirus OS43 and human adenovirus type 5 were found.
antivirus agents, which suggests the possibility of developing broad-spectrum compounds against other viruses (except enteroviruses), including DNA viruses.

7. Abstract

The content of the abstract meets the requirements and reflects the main results of the thesis. An abstract in English would be a good style.

8. Recommendations for future use of the dissertation contributions and results

I have no special recommendations for the work and the candidate. As a scientist and therapist, I wonder about the appropriateness of using the term "placebo" when studying the effect of an intervention on mice.

CONCLUSION

As a member of the scientific jury, I consider the award of the scientific degree "Doctor of Sciences" on professional area 4.3 Biological science to the candidate Assoc. Prof. Ivanka Nikolova Nikolova to be well deserved. The dissertation, with its results and contributions, supported by the scientific data, meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria.

Prepared by.....

(Assoc. Prof. Dr. Rainichka Garnizova)

Sofia, 29.05. 2025.