# **STATEMENT**

from Associate Professor Dr. Metodi Antonov Kunchev, PhD Head of the Department of Microbiology, Virology, Clinical Laboratory and Immunology at the Military Medical Academy, Sofia

on thesis for awarding the degree Doctor of Sciences (DSc)

Thesis Author: Associated Professor Ivanka Nikolova Nikolova, PhD

**Title of the thesis:** In vitro and in vivo study of the antiviral activity of a series of new diaryl ethers and their analogues – promising chemotherapeutics in antienterovirus therapy

Field of higher education: 4. "Natural sciences, mathematics and informatics " Professional area: 4.3. Biological Science

The present statement is prepared in agreement with an order (I-46 from March 28, 2025) of the Head of Stephan Angeloff Institute of Microbiology – BAS (IMicB), following a decision of the Scientific Council of the IMicB with Protocol  $N_{2}$  4 from March 25, 2025.

#### 1. General description of the procedure

The presented set of materials and documents from Associated Professor Ivanka Nikolova Nikolova, PhD on an electronic medium complies with the requirements of the act on the development of the academic staff in the Republic of Bulgaria and the regulations for its implementation, as well as complies with the requirements of Section III: conditions and procedure for acquiring the scientific degree "Doctor of Sciences" of the regulations on the conditions and procedure for acquiring scientific degrees and holding academic positions in of Stephan Angeloff Institute of Microbiology – BAS (IMicB).

The thesis is presented at the National seminar of General microbiology on March 18, 2025 and further addressed for initiating a procedure of its official defense.

## 2. Candidate information

Ivanka Nikolova Nikolova joined IMicB-BAS as a Biologist-specialist in the Microbial Genetics Section. After successful defense (PhD diploma from 2003), she went through all levels of research scientist III to chief assistant (2004-2020), while at 2020 she was elected as an Associated Professor at IMicB-BAS, a position that she occupies at present.

## 3. Relevance of the topics

One of the greatest challenges in modern medicine is the prevention and treatment of widespread, socially significant diseases caused by infectious pathogens with high mutagenic potential. RNA viruses, and in particular Enteroviruses (EVs), are among the most variable microorganisms, which make their treatment difficult because their mutation rate is high and they exhibit rapid resistance to the chemotherapeutic agents used against them. EVs have a wide geographical distribution and cause a wide spectrum of diseases in humans, ranging from the common cold to severe involvement of the CNS, heart, pancreatic beta cells, skeletal muscles, etc. Newborns, children and patients with compromised immune systems are particularly vulnerable. Infection in late pregnancy can lead to perinatal transmission and cause severe disseminated neonatal infection and sepsis with system dysfunction, including hepatitis, meningoencephalitis, multiorgan myocarditis and pneumonia, and is associated with high mortality. The relevance of the dissertation work is determined by the goal to develop and test in vitro and in vivo, the antiviral activity of a series of new diaryl ethers and technical analyses promising chemotherapeutics in anti-enterovirus therapy. The great challenge in the application of new drugs for the treatment of EV infection is mainly the balance on the one hand of high bioavailability and antiviral efficacy, combined with low toxicity to the body, and on the other hand, using monotherapy or combination therapy of several antiviral compounds that target different targeted proteins.

#### 4. Characteristic and evaluation of the DSc thesis

The statement was prepared based on the thesis and author's summary of the thesis, which are well structured and meet all criteria. The thesis is written on 189 pages, illustrated with 31 tables, 46 figures and one diagram, which are informative and clearly present the results of the work. The References section covers 351 sources 351 in latin. The individual sections of the thesis are well balanced. the Literature survey is sufficiently extensive, detailed and well-structured at 36 pages. The comprehensive reading of the materials presented by Assoc. Prof. Ivanka Nikolova shows a complete and competently written thesis. The aim is specific, clearly formulated, and responsive to the problem at hand – to conduct in vitro and

in vivo studies of the antienterovirus efficacy of the cellular phosphatidylinositol-4kinase beta (PI4KB) inhibitor MDL-860, as well as a library of its newly synthesized analogues, in order to identify more effective and safe chemotherapeutic agents that could be applied both in combination therapy regimens and in monotherapy courses for the treatment of enterovirus infections. There are 10 main tasks that are well structured and focused on the aim. The materials and methods section, occupying 10 pages, lists in detail the used reference inhibitors of enterovirus replication, 114 newly synthesized series of diaryl ethers and their analogues, the viral models in vitro and in vivo, cell cultures and experimental animals, as well as molecular biological diagnostic methods (RT-PCR, Sequencing), microbiological methods and statistical methods and analyses. The results obtained, presented on 67 pages, are correctly described, analyzed and well illustrated. The discussion accurately and professionally discusses the own results with those of the authors cited in the literature review. The most significant results of the dissertation work, reflected in the discussion, define nitrobenzonitrile derivatives as a promising class of antiviral agents, which have high in vitro activity and selectivity towards PV1, CVB1 and CVB3 viruses, with their efficiency and cytotoxicity depending on the specific chemical structure and functional groups, while in vivo they show great therapeutic efficacy towards Human coronavirus OC43 (HCoV OC43) and Human Adenovirus type 5 (HAdV-5). The newly synthesized diaryl ethers are a good basis for obtaining highly effective and safe antiviral preparations by improving their structure.

The scientific and practical value of the work is determined by the following contributions:

- A new therapeutic regimen for combined sequential administration of antienterovirus compounds in coxsackie B1 viral neuroinfection in newborn mice has been introduced, preventing the development of resistant enterovirus mutants.
- After a large-scale in vitro screening of 114 analogues of the reference diaryl ether MDL-860, 10 compounds with higher antiviral activity than the reference compound against 3 enteroviruses (poliovirus type 1, coxsackievirus B1, coxsackievirus B3) were selected.
- The six most active in vitro compounds against coxsackievirus B1, subjected to additional in vivo testing in a mouse model of experimental coxsackie B1 neuroinfection, showed that some of them were superior to the reference compound.
- The study identifies diaryl ethers with high in vitro antiviral activity against human coronavirus OS43 and human adenovirus type 5, as a basis for future development

of broad-spectrum compounds against other viruses (besides enteroviruses), including DNA viruses.

# 5. Evaluation of the publications' record

The results of this thesis are summarized in 26 scientific publications (12 in Q1, 8 in Q2, 3 in Q3 and 3 in Q4) and presented in 20 oral or poster reports at scientific conferences in Bulgaria and abroad. Her scientific activity can be highly assessed, based on the high and scientometric indicators: Total number of publications – 51 (of which 38 in Scopus, 10 in proceedings of national congresses and 3 in non-indexed journals). Total IF - 95.353; Total number of citations (excluding self-citations) – 339 (of which 276 in Scopus). Participation in scientific conferences – 39 (25 posters and 14 reports, of which 36 at the international level and 3 at the national level). The scientometric indicators of Assoc. Prof. Nikolova are in accordance with the requirements of the act on the development of the academic staff in the Republic of Bulgaria and those of the Institute of Microbiology at the Bulgarian Academy of Sciences for the scientific degree "Doctor of Sciences", significantly exceeding some of them.

### 6. Author's summary of the thesis

The work complies with the content of the thesis and the requirements for an author's summary of the thesis. It reflects the main points of the study, the most demonstrative figures and tables from the dissertation are included. Lists of publications and participation in scientific forums, as well as the main scientific contributions, are also presented.

## **CONCLUSION:**

Based on my familiarization with the presented thesis, author's summary of the thesis and scientific works, after assessing their relevance and significance, as well as the scientific and applied contributions contained in them, I find it reasonable to state that **my statement** regarding the acquisition of the scientific degree "Doctor of Sciences" in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics", professional direction: 4.3. Biological Sciences by Assoc. Prof. Ivanka Nikolova, PhD **is positive**. The presented results and the achieved contributions, together with the scientometric data, meet the legal requirements according to the act on the development of the academic staff in the Republic of Bulgaria (ADAS)

and the Rules for ADAS application, as well as the terms and conditions for acquiring scientific titles and occupying academic positions at the Bulgarian Academy of Sciences.

March 29, 2025

Prepared by:

Sofia

/ Assoc. Prof. Dr. Metodi A. Kunchev, PhD /