To the Chairman of the Scientific Jury,
appointed by Order № HO-05-05 – 19, 18.12.2025 of
the Director of the IEMPAM–BAS, Sofia

Statement

By Assoc. Prof. Stefka Krumova Ivanova, PhD

Head of NRL Measles, Mumps, Rubella, Department of Virology at the National Center of Infectious and Parasitic Diseases (NCIPD), Sofia

On a dissertation presented to a scientific jury formed by order № HO-05-05 – 19, 18.12.2025 of the Director of the IEMPAM–BAS, Sofia, for awarding the educational and scientific degree "DOCTOR" in the Professional field: 4.3. "Biological Sciences", Doctoral program in "Virology"

Dissertation topic: **"ANTI-TUMOR AND ANTIVIRAL ACTIVITY OF NEWLY SYNTHESIZED METAL COMPLEXES OF SCHIFF BASES AND SALINOMYCIN AND OF GLUCOSAMINE MODIFIED WITH 1,8-NAPHTHALAMIDES."**

Dissertation author: **Hristo Kanchev Hristov**

Scientific supervisor: **Prof. Radostina Ivailova Alexandrova, PhD**

I declare that I have no common publications or any other conflict of interest with the candidate, as defined in Article 4, paragraph 5 of The Law on Academic Staff Development in the Republic of Bulgaria.

The documents of Hristo Kanchev Hristov submitted to me for consideration fully meet the requirements of the LPASB and the Regulations for its implementation, for the award of the educational and scientific degree "Doctor"; therefore, I can formulate the following opinion with full responsibility.

1. Relevance and significance of the dissertation topic

The topic considered in this dissertation is extremely relevant, in view of the global public health problem related to the diagnosis and therapy of malignant neoplasms and, in particular, virus-induced carcinogenesis. The influence of the group of oncogenic viruses on the malignant transformation of cells and tumor progression has been proven worldwide, and

this theory is no longer just a hypothesis, but a proven medical fact. It is a natural continuation of previous developments by the team of Prof. R. Alexandrova in terms of studying new antitumor preparations (complex compounds of metals with biologically active ligands), with potential for therapeutic potential, higher selectivity towards tumor cells, a more favorable toxicological profile, and avoidance of drug resistance. The dissertation aims to study the potential antitumor activity of newly synthesized metal complexes of Schiff bases, metal complexes of salinomycin with ions of essential metals and ruthenium, as well as nanoscale modifications of chitosan. It combines classical virological approaches with an innovative therapeutic strategy for virus-induced tumors.

2. Evaluation of the structure and content of the dissertation

The dissertation submitted to me for review by Hristo Kanchev Hristov, is written on 218 standard pages. The distribution of the content is in accordance with national requirements, as follows: introduction – 2 pages, literature review – 52 pages (23.9%), goals and objectives – 1 page (0.5%), materials and methods – 25 pages (11.5%), results and discussion 104 pages (47.7%), conclusions – 1 page (0.5%) and contributions 1 page (0.5%). The literature review covers 302 sources. The illustrative material includes 73 figures and 58 tables.

The laboratory research and practical work on the preparation of this dissertation work were carried out in the laboratories of the Institute of Experimental Morphology, Pathology and Anthropology with a Museum – Bulgarian Academy of Sciences (IEMPAM- BAS). Part of the research was conducted at the Institute of Molecular Biology "Acad. Rumen Tsanev" – Bulgarian Academy of Sciences. The metal complexes with Schiff bases were synthesized and chemically/physicochemically characterized by Prof. Dr. Gabriela Marinescu and Assoc. Prof. Dr. Daniela-Cristina Kulitsa, Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania; Eng. Dr. Ildiko Buta and Prof. Otilia Costișor from the Institute of Chemistry "Coriolan Dragulescu", Romanian Academy, Timișoara, Romania. The metal complexes with salinomycin were synthesized by Assoc. Prof. Dr. Juliana Ivanova, Faculty of Medicine, Sofia University "St. Kliment Ohridski". The glucosamines modified with 1,8-naphthalamides were prepared by Prof. Dr. Ivo Grabchev, Faculty of Medicine, Sofia University "St. Kliment Ohridski".

2.1. Literature review

The literature review is written competently, in a high scientific style, skillfully handling terminology, and includes a rich literature reference. It introduces the reader to the main problems and challenges arising from the timely diagnosis and therapy of cancer. The text logically focuses on virus-induced carcinogenesis, the types of oncogenic (tumor) viruses, their mechanism of action and the cancers they cause. The topic of hepatitis E virus (HEV) and its influence on the development of liver fibrosis, cirrhosis and hepatocellular carcinoma in patients with chronic HEV is interesting. Special attention is paid to therapeutic approaches in controlling tumor activation and the application of metals in medicine.

Subsequently, the goals and objectives are logically derived.

2.2. Purpose and Objectives

The formulated goal and tasks fully correspond to the topic and are arranged in a logical hierarchy, depending on the sequence of actions in the course of the experiments. The doctoral student summarizes two goals and sets six tasks for their implementation.

2.1. Materials and Methods

The "Materials and Methods" section is designed in accordance with the requirements and is presented with all the necessary details for conducting the experiments. The research protocols and their optimization in the work process are described in extremely detailed and precise terms.

The dissertation work includes the study of three groups of metal complexes of Schiff bases, metal complexes with salinomycin, and glucosamines modified with 1,8-naphthalimides. Experimental models are based on the used tumor and non-tumor cell cultures from humans, rats, chickens and cattle; determination of cell viability by MTT test, neutral red and staining with crystal violet; studies on the ability of substances to induce cytopathological changes in treated cells; study of the influence of substances on the 3D colony-forming ability of tumor cells; determination of metal content in eggs from different production systems.

2.2. Results and Discussion

The results are divided based on the experimental setup used, and the obtained data are discussed with available works on the topic, which allows the reader to delve into the problem in even more detail. The cytotoxic effect of metal complexes (Ru, Zn, Co, Cu, and Ni) with groups of schist bases on different groups of cell cultures is discussed, and the determined cytotoxic concentrations are compared with the exposure period: 3 h, 6., 24 h, 48 h, and 72 h. The influence of metal complexes with schist bases on the 3D growth of tumor cells is studied.

2.3. Conclusions and Contributions

At the end of the dissertation, the graduate formulates 6 conclusions and 5 contributions (of which 3 are of an original scientific and 2 of a scientifically applied nature), which I fully support.

3. Publications related to the dissertation

The results of the dissertation work have been published in 2 publications in refereed journals (with Q2 and Q3). The publications have been cited in 25 refereed journals, which further confirms the relevance of the topic. In addition, the results of the studies have been presented at 10 national and 17 scientific forums abroad.

The presented abstract complies with the requirements, fully reflecting the content of the dissertation work. It is written on 60 pages. It is illustrated with 21 figures and 25 tables.

4. Conclusion

In conclusion, I would like to summarize that the work provided to me is dissertable. The discussed topic is of an innovative and scientifically applied nature, and has the potential for subsequent enrichment and expansion of the focus of the experiments. The research was funded by two research projects from a competitive session of the Scientific Research Fund, by the

Program for Bilateral Cooperation between the Bulgarian Academy of Sciences and related organizations abroad (Romanian Academy), and by the European Union "Next Generation EU" through the National Recovery and Resilience Plan of the Republic of Bulgaria, which confirms their scientific value.

Given the above, I believe that the dissertation fully meets the requirements of The Law on Academic Staff Development in the Republic of Bulgaria, the Regulations for its implementation, and the Regulations of the BAS for the Award of the Educational and Scientific Degree "Doctor". All required scientometric indicators have been met, which indisputably confirms the candidate's academic potential.

I give my positive assessment of Hristo Kanchev Hristov's award of the educational and scientific degree "Doctor" in the scientific specialty „Virology."

Signature:

Assoc. Prof. Stefka Krumova Ivanova, PhD