TO
THE CHAIRMAN OF THE SCIENTIFIC JURY,
IN ACCORDANCE WITH
ORDER No. NO-05-05 – 19/18.12.2025
OF THE DIRECTOR OF IEMPAM-BANAS

REVIEW

By Prof. Galina Kurteva, MD, PhD

Regarding: PhD thesis for the award of the educational and scientific degree "DOCTOR" in Professional field: 4.3. "Biological Sciences", Doctoral program in "Virology", presented to the Scientific jury, determined by order No. NO-05-05 – 19/18.12.2025 of the Director of IEMPAM-BANAS, Sofia.

PhD topic:
"ANTITUMORAL AND ANTIVIRAL ACTIVITY OF NEWLY SYNTHESIZED METAL COMPLEXES OF SCHIFF BASES AND SALINOMICIN AND OF 1,8-NAPHTHALAMIDE-MODIFIED GLUCOSAMINES"

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1. Relevance and scientific significance

The PhD thesis fits into one of the most dynamically developing areas of modern biomedicine – the search for new antitumor agents with improved selectivity and reduced toxicity. The focus on virus-induced carcinogenesis gives additional weight to the study, since oncogenic viruses are an indisputably established etiological factor in a number of malignant diseases.

The dissertation work logically continues and builds on the research line of the team of Prof. Radostina Alexandrova, related to the identification of metal complex compounds with potential antitumor activity. The topic combines classical virological methods with an

interdisciplinary approach, including coordination chemistry, cell biology and experimental oncology.

2. General characteristics and structure of the dissertation work

The dissertation is presented in a volume of 218 pages and follows the established structure:

- Introduction – 2 pages
- Literature review – 52 pages
- Aim and objectives – 1 page
- Materials and methods – 25 pages
- Results and discussion – 104 pages
- Conclusions – 1 page
- Contributions – 1 page

The bibliography includes 302 literary sources. 73 figures and 58 tables are presented, which indicates a significant experimental volume and good visualization of the results.

3. Evaluation of the literature review

The review part of the work demonstrates a thorough and systematic knowledge of the current state of the scientific problem under consideration. It consistently analyzes the molecular mechanisms of virus-induced carcinogenesis, outlines the characteristics of the main oncogenic viruses, and traces the relationship between chronic viral infections and tumor progression. It also examines modern therapeutic approaches (and the challenges facing them), as well as the role of metals in medicine in the context of antitumor strategies.

Particular attention is paid to the hepatitis E virus and its potential role in the development of liver pathology and hepatocellular carcinoma, which testifies to the relevance of the selected issues and to an analytical approach to aspects that are less studied and discussed in the scientific literature.

In its entirety, the review builds a conceptually and logically justified basis for the formulated goals and objectives of the study.

4. Aim and objectives

Two main goals and six specific objectives are formulated. They are clearly structured, consistent and directly derived from the literature analysis. The tasks set cover both cytotoxicity assessment and analysis of effects on 3D cell growth and various experimental models.

5. Materials and methods

The methodological part of the dissertation work is developed in detail and precisely, as the presented description of the used approaches and procedures provides the opportunity for full reproducibility of the experimental studies. The work includes both tumor and non-tumor cell lines of different biological origin - human, rat, chicken and cattle, which expands the scope and comparative value of the obtained results.

The experimental work covers three groups of compounds - metal complexes of Schiff bases, metal complexes with salinomycin and glucosamines modified with 1,8-naphthalimides. Their synthesis and physicochemical characterization were carried out by scientific teams from Bulgaria and Romania, which clearly emphasizes the interdisciplinary and international nature of the work.

In the preparation of the dissertation work, a wide range of methods were applied, including cytotoxic tests, biochemical, molecular biological and virological methods. Monolayer cell cultures, but also 3D colony-forming cell models, were used, which allow a more adequate approximation to in vivo conditions and a more in-depth assessment of the antitumor potential of the studied compounds. In addition, the determination of the content of essential metals in eggs from different production systems was performed, which expands the applied aspect of the study.

6. Results and Discussion

The results are presented systematically and analytically. The cytotoxic effect of complexes of Ru, Zn, Co, Cu and Ni was studied at different exposure intervals (3, 6, 24, 48 and 72 hours). The effective cytotoxic concentrations were determined and the time-dose-effect relationship was analyzed. Compounds with promising cytotoxic activity were identified - the metal complexes of salinomycin deserve special attention due to the ability of this ionophore antibiotic to attack cancer stem cells and to be effective in drug-resistant tumor cells, as well as due to the expectations of specialists that its metal complexes will show advantages compared to its independent application. The optimized system for long-term cultivation of hepatitis E virus-infected cells allows for further studies on the biological behavior of this virus, including those aimed at clarifying its potential carcinogenicity and the search for successful antiviral therapies.

The discussion demonstrates the ability to critically compare one's own results with data from international literature.

7. Conclusions and contributions

6 conclusions and 5 contributions have been formulated, which I accept:

- 3 of original scientific nature
- 2 of scientific-applied nature

The conclusions correctly reflect the obtained results and demonstrate scientific maturity.

8. Publication activity

On the topic of the dissertation, the following have been published:

• 2 articles in refereed publications (Q2 and Q3), at the time of submission of the dissertation work, 25 of their citations were found.

• 1 publication has been proposed for publication in a scientific journal.

The results have been presented at 27 scientific forums (10 national and 17 international).

9. Abstract

The presented abstract has been prepared in accordance with the regulatory requirements and adequately presents the main results, structure and scientific contributions of the dissertation work. In a volume of 60 pages, it correctly summarizes the theoretical framework, the methods used, the experimental data obtained and the formulated conclusions and contributions.

The text is clearly structured and analytically sound, providing sufficient information about the goals, objectives and significance of the research conducted. The illustration with 21 figures and 25 tables contributes to the better visualization and comprehensibility of the presented results, without disturbing the balance between synthesis and scientific detail.

In this form, the abstract fulfills its function as an independent and full-fledged document, reflecting the content and contributions of the dissertation work.

The indicated indicators fully cover the regulatory requirements and testify to active and sustainable scientific activity.

9. Funding

The research is financially supported by:

• two projects of the Scientific Research Fund

• a bilateral cooperation program between the Bulgarian Academy of Sciences and the Romanian Academy

• a program for supporting doctoral students at the Bulgarian Academy of Sciences

• the European Union ("Next Generation EU")

This confirms the scientific significance and institutional support of the development.

Final assessment

The dissertation work of Hristo Kanchev Hristov represents an independent, in-depth and interdisciplinary scientific study, performed with a high degree of methodological correctness and experimental precision. The development is distinguished by clearly formulated goals, logical consistency of the research approach and convincingly interpreted results, which contribute to expanding knowledge in the field of virus-induced carcinogenesis and oncovirology, tumor biology and experimental oncopharmacology.

The obtained data have both fundamental scientific significance, related to the clarification of the biological activity of newly synthesized metal complexes, and an applied perspective with regard to future therapeutic strategies. The presented scientific contributions are clearly formulated, argued and supported by sufficient experimental material.

The publication activity, international cooperation and participation in scientific forums further confirm the active scientific research activity of the doctoral student and the good approbation of the obtained results.

Based on the above, I believe that the dissertation fully meets the requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria and the regulations for its implementation.

I confidently give a positive assessment and propose to the esteemed scientific jury to award Hristo Kanchev Hristov the educational and scientific degree "Doctor" in professional field 4.3. "Biological Sciences", doctoral program "Virology".

Signature: