Review

by Prof. Reni Emil Kalfin, PhD
Head of Department "Biological Effects of Natural and Synthetic Substances"
at the Institute of Neurobiology – Bulgarian Academy of Sciences
on the PhD thesis submitted for the award of the Educational and Scientific Degree
"Doctor"
Professional field 4.3. "Biological Sciences"
Doctoral programme "Virology"

Author: MSc Hristo Kanchev Hristov
Form of doctoral training: Full-time doctoral studies
Department: "Pathology" at the Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences
Topic: "Antitumor and Antiviral Activity of Newly Synthesized Metal Complexes of Schiff Bases and Salinomycin and of 1,8-Naphthalimide-Modified Glucosamines"
Scientific supervisor: Professor Radostina Ivaylova Alexandrova, PhD – IEMPAM-BAS

1. Brief biographical data of the doctoral candidate

Hristo Hristov was born on January 1, 1980 in the town of Polski Trambesh, Veliko Tarnovo region. In 2009 he completed the specialty "Electrical Engineering" at the Professional Technical School "Karol Sverchevski" in Sofia. He obtained the educational and qualification degree "Master" in the specialty "Virology" in 2020 from Sofia University "St. Kliment Ohridski" with excellent grades. After completing his higher education and passing a competition, Hristo Hristov was enrolled in full-time doctoral studies in "Virology" at the Institute of Experimental Morphology, Pathology and Anthropology with Museum – BAS, and as of January 1, 2024 he was discharged with the right to defend. Since 2019 and up to the present, MSc Hristo Hristov has been a member of the Bulgarian Anatomical Society.

2. Relevance of the topic

The PhD thesis of full-time doctoral student Hristo Kanchev Hristov is devoted to an important problem in contemporary medical-biological science – the search for new agents with potent antitumor activity, high selectivity and good biological tolerability. Despite significant progress in the treatment and diagnosis of malignant neoplasms, they continue to represent one of the major challenges not only for the medical and scientific communities but for society as a whole. It is sufficient to mention the continuously increasing incidence of cancer worldwide. It is worth noting that a significant proportion of malignant neoplasms are directly or indirectly related to virus-induced carcinogenesis, in which oncogenic viruses and virus-cell interactions contribute to malignant transformation and tumor progression. It has been established that 10–15% of all human cancers are caused by viruses. Viral infections can contribute to malignant cell transformation through a combination of mechanisms, including integration of the viral genome, expression of oncogenes, induction and maintenance of chronic inflammation, epigenetic changes, and suppression of the immune response.

The main reason for treatment failure in malignant neoplasms remains multidrug resistance and, to some extent, the unwanted side effects of chemotherapy. This justifies the efforts of scientists to synthesize, characterize, investigate and apply new compounds with potential antitumor properties. It is well known that platinum-based chemotherapy is an effective method for treating oncological diseases. Platinum-based drugs (cisplatin, oxaliplatin and carboplatin) are routinely used – alone or in combination with other agents – for the treatment of various malignancies. Ruthenium compounds, which are one of the objects of study in the present dissertation, possess a number of advantages over platinum preparations, including lower toxicity and a reduced ability to induce resistance in tumor cells. In this context, the investigation of newly synthesized metal complexes of Schiff bases and salinomycin, as well as 1,8-naphthalimide-modified glucosamines, represents a scientifically justified and promising approach. Everything stated above makes the topic of the doctoral dissertation relevant in both scientific and applied scientific terms and justifies the need for the conducted research.

3. Knowledge of the problem

The extensive literature review shows that Hristo Hristov is excellently familiar with the state of the research problem addressed in his doctoral dissertation. The literature overview is up-to-date and indicates an author with solid knowledge who understands what has been done so far and the future perspectives. Overall, the cited sources demonstrate in-depth knowledge of the scientific literature and the issues related to the developed doctoral thesis. The used literature includes contemporary sources and covers a wide range of publications in the fields of virology, oncology and medicinal chemistry. The cited data are current and aligned with the present state of scientific research.

The literature review is structured into six main thematic subsections with a clearly expressed logical sequence. There is a very well-constructed thematic framework that integrates oncology, virology, molecular biology and bioorganic chemistry. The interdisciplinary nature of the review makes a particularly positive impression – it combines epidemiological data, molecular mechanisms of carcinogenesis and pharmacological strategies.

The literature review begins with a presentation of cancer diseases as a global health and social problem, using current epidemiological data and prognostic estimates of morbidity trends worldwide. In this way, the author convincingly argues for the societal significance of the researched problem.

Subsequent sections focus on virus-induced carcinogenesis and examine both the historical aspects of oncoviroloy (discovery of Rous sarcoma virus and identification of viral oncogenes) and modern views on the role of human oncogenic viruses. The mechanisms of action of viral oncoproteins (E6/E7 in HPV), integration of the viral genome and regulation of the cell cycle are analyzed in detail.

A clear conceptual line runs through the review, consistently leading from the fundamental mechanisms of carcinogenesis to the specific experimental models used in the dissertation – cell lines transformed with HPV18, as well as models based on avian leukosis and sarcoma viruses (RSV and Mc29).

The sections devoted to metals in medicine, Schiff bases, salinomycin and nanomedical approaches logically continue the exposition of the molecular mechanisms of carcinogenesis. The author correctly traces the successful therapy with platinum complexes and justifies the need to develop new metal compounds with an improved selectivity and lower toxicity profile. Potential mechanisms of action of metal complexes are presented – interaction with nucleic acids, induction of oxidative stress, influence on mitochondrial function and signaling pathways. The possibilities for enhancing biological activity through coordination with Schiff bases and salinomycin, as well as the role of modified carrier systems, are discussed.

These sections convincingly prepare the experimental part of the dissertation and clearly delineate the scientific niche in which the study is positioned.

Particularly positive is the integration of virology with molecular oncology, as well as the linkage of theoretical propositions with the specific experimental approaches in the doctoral work.

In conclusion, the literature review demonstrates a high level of theoretical preparation. The author shows good knowledge of the molecular mechanisms of oncogene activation, including the MYC family; understanding of the differences between viral and cellular oncogenes (v-onc and c-onc); the role of chronic inflammation and genetic instability in the process of malignization; clear distinction between the various mechanisms of virus-induced transformation.

4. METHODOLOGY OF THE STUDY

The objects of the present dissertation are newly synthesized metal complexes of Schiff bases, metal complexes of salinomycin with ions of essential metals and ruthenium, as well as 1,8-naphthalimide-modified glucosamines (nanoparticles). The conducted experiments aim to investigate the antitumor activity and mechanisms of action of these compounds on cellular models of cancer cells transformed with HPV18, MC29 and RSV, and expressing viral oncogenes (v-onc, myc-onc). To study them, modern and classical methods were successfully applied: molecular-biological, cell-culture, biochemical, cytomorphological, and others.

The synthesis and physicochemical characterization of the investigated metal complexes were carried out by colleagues from the Romanian Academy of Sciences (Schiff base complexes) and the Medical Faculty of Sofia University "St. Kliment Ohridski" (salinomycin complexes and 1,8-naphthalimide-modified glucosamines).

One of the main requirements for antitumor drugs is that their action should be directed toward malignantly transformed cells and not toward normal cells of the organism. Therefore, the doctoral candidate included in his experiments the non-tumor human cell line Lep3, derived from embryonic lung fibroblasts.

Hristo Hristov has demonstrated his skills in working with cell cultures, acquired under the guidance of Prof. Radostina Alexandrova and the colleagues from the team.

5. CHARACTERISTICS OF THE DISSERTATION AND OBTAINED RESULTS

The doctoral dissertation is written on 221 pages according to the standard scheme and includes all sections recommended for obtaining the educational and scientific degree "Doctor", with the appropriate ratio between the individual parts maintained. The work is richly illustrated with 73 figures and 58 tables. The bibliography consists of 318 literature sources.

The aim of the dissertation is clearly formulated – to investigate the cytotoxic activity of newly synthesized complexes of metals with various ligands (Schiff bases, salinomycin) and 1,8-naphthalimide-modified glucosamines (nanoparticles) on tumor cells transformed with viruses (avian retroviruses, human papillomaviruses) and the influence of the tested compounds on the replication of human herpesviruses (HSV-1/HHV-1 and HSV-2/HHV-2).

The doctoral candidate also aims to develop an in vitro cellular system for infection with hepatitis E virus (HEV) and maintenance of viral replication.

The set tasks are specific, logically follow from the objectives of the doctoral thesis and include:

- Assessment of cytotoxicity;
- Analysis of antiproliferative effect;
- Investigation of apoptosis;
- Study of impact on viral models;
- Comparative evaluation of different complexes.

The experimental part of the PhD thesis provides a clear idea of the scientific value of the conducted research. The obtained results are systematically presented, methodologically justified and logically structured in accordance with the set goals and objectives. The author uses established quantitative methods for assessing cell viability (MTT, NR, CV tests), which allows an objective and reproducible evaluation of the effect. The obtained data show that some of the metal complexes exhibit significant cytotoxic activity, comparable or in certain cases superior to reference compounds used in clinical practice. This represents a real contribution to the search for new antitumor agents.

An important achievement of the dissertation is the study of the mechanisms of cell death. Through fluorescent methods (AO/PI) and morphological analysis, typical signs of apoptosis were established. The data support the hypothesis that some of the metal complexes induce programmed cell death rather than nonspecific necrosis. This is essential, as apoptotic induction is a preferred mechanism for antitumor agents.

A particularly valuable part of the dissertation is the investigation of the activity of the compounds on virally transformed cellular systems (HPV18, RSV, Mc29).

In preparation of the dissertation, an *in vitro* cellular system for infection with hepatitis E virus and maintenance of viral replication was developed. It will contribute to studies on the possible oncogenic potential of the hepatitis virus and the search for antiviral agents directed against it.

The obtained experimental data represent a substantial and original contribution to the study of the antitumor activity of new metal complexes. They expand knowledge regarding the potential of coordination compounds as therapeutic agents and create a basis for future preclinical developments.

The choice of virally transformed cells is particularly valuable, as it allows evaluation not only of cytotoxicity but also of the potential impact on oncogene-dependent signaling pathways (E6/E7–p53/pRb axis, v-src tyrosine kinase signaling, v-myc transcriptional regulation).

The discussion of the experimental results demonstrates the doctoral candidate's in-depth and contemporary knowledge of virology, biochemistry and experimental chemotherapy. The used literature is rich, showing thorough familiarity with the subject matter Hristo Hristov works on. The dissertation is excellently written – intelligently and with a great deal of knowledge, extremely richly illustrated with color figures and tables. A wide range of foreign authors are cited, including the most recent developments. The dissertation is written in excellent Bulgarian, which unfortunately is no longer common in contemporary dissertations, with minimal use of foreign words.

Everything stated above is evidence of the large volume of material processed and described in detail by Hristo Hristov, which gives me grounds to give a high evaluation of the author's ability to consider the obtained results in the context of known facts from the literature, to discover causal relationships between the studied parameters and to formulate precise conclusions, exceeding the expected level for a doctoral candidate.

In conclusion, the doctoral dissertation demonstrates the ability to think at the level of molecular pathogenesis and drug design. The presented results go beyond purely descriptive level and allow the formulation of hypotheses of high scientific value.

6. Significance of the thesis for science and practice

The obtained results have potential significance for medical practice, which allows them to be given a high evaluation. Six main conclusions are formulated in the dissertation, which I fully accept. They reflect the most essential results and findings obtained during the development, both regarding the observed effects after application of the investigated metal complexes and regarding the probable mechanisms of their antitumor action.

The complex studies conducted and the results obtained in the investigation of 16 newly synthesized metal complexes (CuII, ZnII, CoII, RuII, NiII, MnII) with Schiff bases (13 complexes) or salinomycin (3 complexes) on the survival and proliferative activity of human carcinoma cells of the HeLa line and tumor cells transformed with human papillomaviruses or avian retroviruses cultured under laboratory conditions, as well as on non-tumor human cells, can be systematized as contributions of original, methodological and applied character as follows:

1. Original scientific contributions
   - It was established that salinomycin complexes (SalCo > SalCu > SalZn) most strongly reduce the survival and 2D/3D growth of human carcinoma cells of the HeLa line cultured under laboratory conditions, with their activity surpassing that of established clinical antitumor drugs cisplatin, oxaliplatin and epirubicin

- For the first time, information was obtained on the cytotoxic, genotoxic and mitotoxic effects of four 1,8-naphthalimide-modified glucosamines (nanoparticles)

2. Methodological contributions
   - Oncoviral models were used as a platform for evaluating the antitumor and potentially antiviral activity of new compounds
   - An interdisciplinary complex approach was applied to study the cytotoxic activity of 1,8-naphthalimide-modified glucosamines on tumor cells transformed with avian retroviruses and human papillomaviruses

3. Scientific-applied contributions
   - A cellular model system was developed for long-term cultivation of hepatitis E virus, which can be used in studies on the potential oncogenic effect of this virus and testing of antiviral agents directed against it.

7. Publications and conference presentations on the topic of the PhD thesis

In accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria and its Implementing Regulations, for the defense of a doctoral dissertation in professional field 4.3. “Biological Sciences”, a minimum of 30 points is required from group indicators 5 to 10. On the topic of the doctoral dissertation, two scientific articles have been published – one review published in a Q2 quartile journal, which brings 20 points, and one experimental article published in a Q3 quartile journal, from which the doctoral candidate receives 15 points. Hence, with a total of 35 points, Hristo Hristov exceeds the minimum requirements for defense of a PhD thesis. Another experimental article on the topic of the dissertation has been submitted for publication and is under review in the international journal “Results in Chemistry” with a Q2 quartile.

It makes a very good impression that the published articles have been cited a total of 25 times. In connection with the developed PhD thesis, the doctoral candidate has 10 participations in scientific forums in the country, in 80% of which he is the first author. The doctoral candidate is also a co-author in 17 presentations of results at scientific events abroad or at international scientific forums in Bulgaria, in 35% of which he is the first author. The above facts unequivocally show that Hristo Hristov has acquired skills to present his work to the scientific community, which is an essential part of doctoral training.

8. Personal participation of the PhD student

Hristo Hristov personally participated in the conducted research. The contributions of the doctoral dissertation noted by the doctoral candidate are his own work, obtained with the support and assistance of the scientific supervisor Professor Radostina Alexandrova.

9. Abstract of the PhD thesis

The abstract is written on 60 standard pages and corresponds to the content of the dissertation. From the abstract one can get a very good idea of the scientific research carried out. The rich illustration of the abstract with 25 tables and 21 figures, the vast majority of which are in color, contributes to the easy perception of the results by the reader. The abstract includes the aim and objectives of the study, all main results of the doctoral dissertation and their discussion, the conclusions and contributions. In conclusion, the abstract fulfills its main function – to present in a synthesized but sufficiently detailed manner the scientific idea, experimental approach and obtained results, without going into excessive details characteristic of the full text of the dissertation.

10. Critical remarks and recommendations

I have no critical remarks toward the doctoral candidate. The obtained experimental data are numerous and their significance will continue to grow. My recommendation is that they be summarized in scientific articles and submitted for publication in journals with high impact factor, because they deserve to become accessible to the international scientific community.

Questions to the PhD student:

1. What are the main advantages of metal complexes over classical cytostatics?
2. Are there indications of mitochondrial involvement in the mechanism of action of the investigated metal complexes?
3. What is the significance of the obtained results for clinical practice?

CONCLUSION

The PhD thesis developed by the doctoral candidate Hristov is a complex and labor-intensive study on an extremely relevant problem of experimental oncology and oncopharmacology. Knowledge has been expanded regarding the interaction between newly synthesized metal complexes and virus-induced tumor systems. Original data have been obtained which have not only scientific value but are also of interest for diagnostic and therapeutic medical practice.

The doctoral dissertation shows that the full-time PhD student Hristo Hristov possesses in-depth knowledge and professional skills in the scientific specialty “Virology”, demonstrating qualities and abilities for independent conduct of scientific investigations. The dissertation is well conceived, methodologically justified, precisely executed and fully meets, and in some aspects exceeds, the scientific and educational criteria for the doctoral degree set out in the Law on the Development of the Academic Staff in the Republic of Bulgaria, its Implementing Regulations and the internal regulations of IEMPAM-BAS.

In view of the above, I give my positive evaluation of the scientific research presented by the reviewed doctoral dissertation, abstract, achieved results and contributions, and with conviction I recommend to the honorable members of the Scientific Jury to vote with “YES” for awarding the educational and scientific degree “Doctor” to MSc-virologist Hristo Hristov in Professional field 4.3. “Biological Sciences”, scientific specialty “Virology”.

March 4, 2026

Reviewer:

Prof. Reni Kalfin, PhD