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Review

by Prof. Dimitar Kadiysky, MD, PhD, DSci

Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) – Bulgarian Academy of Sciences

Regarding the dissertation work, for obtaining the educational and scientific degree "doctor" in the Field of higher education: 4. Natural Sciences, Mathematics and Informatics, Professional Field: 4.3 Biological Sciences, Scientific Specialty: "Virology".

Author: Hristo Kanchev Hristov – full-time doctoral student at IEMPAM

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

Scientific supervisor: Prof. Radostina Ivaylova Aleksandrova, PhD (IEMPAM-BAS)

Procedure for the defending of a dissertation.

By order No. NO-05-05-19/ 18.12.2025 of the Director of the Institute of Experimental Morphology, Pathology and Anthropology with a Museum (IEMPAM) - BAS I have been appointed as a member of the Scientific Jury in connection with the procedure for the defense of a dissertation for the acquisition of the educational and scientific degree "Doctor" - Field of higher education: 4. Natural sciences, mathematics and informatics, Professional field: 4.3 Biological sciences, Scientific Specialty "Virology". The author of the dissertation is Hristo Kanchev Hristov, a full-time doctoral student at the "Pathology" Section of IEMPAM, BAS. The doctoral student has been discharged with the right to defense. For the preparation of the review, I received in electronic form all materials required according to the Academic Staff Development Act (ASDA) of the Republic of Bulgaria and in accordance with the rules for the Application of the Academic Staff Development Act in the Republic of Bulgaria, BAS and IEMPAM. PhD candidate Hristo Hristov was a student at the Faculty of Biology of Sofia University "St. Kliment Ohridski", where he successfully graduated in 2020 with a Master's degree in Virology. His professional growth includes scientific work at IEMPAM - BAS since 22.11.2021. To date, he holds the position of specialist biologist at the same institute. Almost the entire growth of the doctoral student is related to the research and educational activities carried out at the institute, specifically in the Pathology Section. He was discharged from doctoral studies with the right to defense after successfully passing internal approval - the dissertation project was reviewed, approved and directed for defense at an extended meeting of the Pathology Section, IEMPAM-BAS, held by order No. NO -05-05-19 of 18.12.2025 of the Director of the Institute.

Biomedical significance of the study

The dissertation topic covers problems related to improving approaches to diagnosis and therapy in carcinogenesis (virus-induced and not only!). The subject of a large part of the content is the manifestation of cytotoxic and potential antitumor activity of newly synthesized metal complexes - Schiff bases, metal complexes of salinomycin with ions of essential metals and ruthenium, as well as nanoscale modifications of chitosan. The emphasis of the study is the search for effects caused by the action of these complexes on the course of virus-induced carcinogenesis (a process in which oncogenic viruses and specific virus-cell interactions trigger malignant transformation of cells and cause tumor pathology in the living organism).

The screening of the effects of the above-described substances was implemented through an experimental approach on human and animal tumor cells cultured in laboratory conditions, transformed with HPV18, Mc29 (containing the v-myc gene) and RSV (containing the v-src gene).

The chosen topic of the dissertation, divided by the doctoral student into 2 research aims and 6 specific tasks, has made it possible to obtain original scientific contributions and scientific-applied developments.

Evaluation of the dissertation

The presented dissertation is written on 218 pages and contains 73 figures and 58 tables. The list of cited literature includes 302 titles. It is constructed in accordance with the requirements of the ADAS and its Regulations, and an abstract is presented to it, satisfactorily reflecting all sections. The structure complies with the generally accepted requirements - introduction, literature review, goal and tasks, material and methods, results and discussion, conclusions, contributions and literature and finally summarized newly obtained data related to the cytotoxic activity of newly synthesized metal complexes with different ligands (Schiff bases, salinomycin, glucosamines modified with 1,8-naphthalimides, nanoparticles, etc.). Data on the influence of the tested compounds on the replication of human herpes viruses (HSV-1/HHV-1 and HSV-2/HHV-2) are indirectly related again to carcinogenesis.

With a minimum of 30 points required for the defense of a dissertation in the professional direction 4.3. "Biological Sciences" (according to the ADAS in the Republic of Bulgaria and the Regulations for its application), in the table with the sections with indicators from 5th to 10th, the doctoral student collects 35 points. In connection with the topic of the dissertation, two scientific publications are presented - a detailed review with quartile Q2 and an article with quartile Q3. To date, there is one more scientific article edited abroad in a journal with an impact factor with the participation of the doctoral student.

My personal impressions of the doctoral student's work under the supervision of the scientific supervisor give me reason not to doubt that Hristo Hristov has a major contribution to obtaining the results included in the dissertation.

The 49-page **literature review**, through its subdivisions (cancer diseases, viral carcinogenesis, metals in medicine, nanotechnologies, nanomedicine and nanooncology, Schiff bases, salinomycin) reviews over 300 literary sources and covers the essential part of the scientific knowledge and techniques in these areas. The review of oncological therapeutic approaches is extremely complete, loaded with modern data and well illustrated. On this basis, the doctoral student and his scientific supervisor select experimental schemes for research. The literature interpretation of the existing available data provoked the conducted study of the sensitivity of different lines of tumor and non-tumor cells to the effects of tested compounds - metal complexes, modified glucosamines, etc.

The available studies on metal complexes and their antitumor potential do not provide comprehensive data on the effects of newly synthesized complexes of Schiff bases, salinomycin-metal ions and chitosan-based systems based on essential metals (copper, zinc, cobalt, manganese, nickel) and ruthenium on oncovirally transformed cell models. Where such data are available, there are no studies on spectrum of cytotoxicity and antiproliferative activity. In-depth studies on potential mechanisms such as induction of apoptosis, changes in the expression of viral oncogenes (v-myc, v-src) and effects on cell signaling pathways associated with virally induced malignant transformation are simply lacking.

The experimental material for the study is a complex of selected diverse substances, possibly related to therapeutic approaches in oncology and allowing for real statistical processing of the results. These are: metal complexes of Schiff bases - group I (Schiff base SALTIAZ), metal complexes of Schiff bases - group II (Schiff base V – V complexes), metal complexes of Schiff bases - group III (Schiff base L – L complexes), metal complexes with salinomycin, glucosamines modified with 1,8-naphthalimides. When asked why this particular set of substances is being studied, the answer could be that there is evidence that metal complexes of Schiff bases modify the redox balance of cells and interact with nucleic acids and proteins, as well as that they affect mitochondrial function, which leads to effects of suppressing cell development and cytotoxicity, which is an indirectly expected antitumor activity. On the other hand, metal-salinomycin complexes are of interest due to the ability of salinomycin to attack tumor stem cells, disrupting ion homeostasis. The application of chitosan and its modifications allows the creation of biocompatible carrier systems that increase the cellular uptake of extracellular products.

The aims of the dissertation work, as well as the implementation of the tasks set for solution, are related to research approaches in relation to the experimental material described above. The subject of study in my presented dissertation is the cytotoxic and 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 nanosized modifications of chitosan, studied on human and animal tumor cells cultured in laboratory conditions, transformed with HPV18, Mc29 (containing the *v-myc* gene) and RSV (containing the *v-src* gene).

The research approach of the author of the dissertation includes sufficient in number, volume and representativeness methods used in virology - cell biology, immunology, general biochemical, morphological, including cell modeling (e.g., the development of an *in vitro* cell system for HEV infection and maintenance of viral replication).

The entire volume of the **Results and Discussion** section occupies a total of 104 pages of the presentation (from 84 to 188 pages), with almost everywhere the described results of the experiments being accompanied by a discussion on the spot immediately. The importance of this part of the dissertation work is emphasized by the fact that the results of the experiments performed allow for the derivation of biomedical conclusions and conclusions related to therapeutic oncology. For example, the result that metal complexes with Schiff bases or salinomycin *in vitro* reduce the survival and 2D/3D growth of virus-transformed tumor cells can be transferred with considerable reliability as an effect *in vivo*. The enhancement of this effect with increasing concentration and exposure time complements the result and is well illustrated graphically in the dissertation. Again in this sense, the weaker cytotoxic effect of glucosamines modified with 1,8-naphthalimides established in vitro does not allow for the expectation of a possible therapeutic effect when applied *in vivo*. The results obtained in the study of chitosan and modified chitosan are controversial as to their effect on carcinogenesis. There, the doctoral student's research approach shows us that they do not affect the replication of HSV-1 (strain F) and HSV-2 (strain BA).

The comparison made during the experiments of the sensitivity of tumor and non-tumor human embryonic cells of the Lep-3 line leads to the final conclusions in the dissertation. The cytotoxic effect of the tested metal complexes with Schiff bases and salinomycin and glucosamines modified with 1,8-naphthalimides was further analyzed with their effects on three antitumor drugs used in clinical practice – cisplatin, oxaliplatin and epirubicin. As a result, there is an important summary (clearly shown in tabular form in the relevant section of the dissertation) highlighting a significant result of the experiments.

Another section of the dissertation treats highly pronounced cytotoxic and, accordingly, potential antitumor properties on the HeLa cell line of the complexes of salinomycin with copper, zinc and cobalt. This fact suggests a possible additive therapeutic effect of these products in the complex treatment of carcinoma of the cervix in humans, especially since their cytotoxic activity exceeds that of the established antitumor drugs cisplatin, oxaliplatin and epirubicin in analogous conditions.

Two separate experimental tasks and research objectives were implemented by the doctoral student and included in the work submitted to me for review. These are tasks related to the *in vitro* development of a cell system for HEV infection and maintenance of viral replication in laboratory conditions, as well as the determination of the content of metals in chicken eggs from different territorial zones and production systems. The results of this experimental activity, carried out during the period of formation of the dissertation work and included in it to a certain extent, expand it.

The issue of the heterogeneity of tumor cell lines and their connection with the etiopathogenesis of the tumors from which they originate is discussed. It is considered from an immunological and morphological point of view based on the established clearly expressed differences in their characteristics.

The abstract includes the main results and contributions of the dissertation.

My recommendations are that in future research activities of the doctoral student, work should be done to expand the study with new substances of a similar category, suspected of potential antitumor activity.

CONCLUSION

The dissertation work of Hristo Kanchev Hristov includes the results of several years of virological research, relevant to the medicobiological therapy regimens in oncology. The continuous optimization of therapeutic behavior in oncology is an exceptional task of modern medicine, pharmacology, virology, cell biology and molecular pathology, and this makes the dissertation valuable with its practical utility.

The participation of the doctoral student in 10 national and 17 international scientific forums has allowed the scientific community to become familiar with and evaluate the achieved results. The three scientific articles with the participation of the doctoral student in journals providing the necessary points according to the ADAS RB for the defense of a dissertation work, have received a good scientific response, as evidenced by the more than 25 citations of these works available to date.

The doctoral student possesses the knowledge and skills for successful scientific research work in the scientific specialty of virology. The materials and documents of the procedure meet the requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of this Act and the Regulations for the Acquisition of the degree "Doctor" at IEMPAM - BAS.

I give a positive assessment of the conducted scientific research, reflected in a completed dissertation, and I propose to the esteemed Scientific Jury to award Hristo Kanchev Hristov the educational and scientific degree "Doctor" in the Field of higher education 4. Natural Sciences, Mathematics and Informatics, Professional field: 4.3 Biological Sciences, Scientific specialty "Virology".

Prepared by:

Prof. Dimitar Kadiysky, MD, PhD, DSci

Sofia, 19.02.2026