Attitude of Reviewer

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Re: defense of dissertation for acquisition of PhD degree of virology, in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", Professional direction 4.3. "Biological Sciences" at the Department of Pathology, IEMPAM-BAS, Sofia

Author: Hristo Hristov

Title: Antitumor and antiviral activity of newly synthesized metal complexes of Schiff bases and Salinomycin and 1,8-naphthalamide glucosamines.

The topic of Hristo Kanchev Hristov's dissertation work "Antitumor and antiviral activity of newly synthesized metal complexes of Schiff bases and salinomycin and of glucosamines modified with 1,8-naphthalimides" is distinguished by its high relevance, due to its health, social and demographic importance, resulting from the increase in morbidity and mortality from malignant neoplasms, a significant part of which are directly or indirectly related to virus-induced carcinogenesis. Development of highly effective and specific antitumor preparations is the subject of extensive research in the field of modern oncopharmacology and, more specifically, of complex compounds of metals with biologically active ligands. Among them, essential elements such as copper, zinc, cobalt, manganese, ruthenium stand out, and as biologically active ligands, interest is directed to Schiff bases and salinomycin, whose complexes have high potential for application as antitumor and antiviral agents. Along with them, chitosan and its modifications stand out with high reliability in creating biocompatible carrier systems for controlled release of active complexes with antitumor and antiviral action. In this context, the present dissertation work was developed, aiming to elucidation of a number of issues related to the spectrum of cytotoxicity and antiproliferative activity of these compounds, their potential mechanisms, including induction of apoptosis and changes in the expression of viral oncogenes (v-myc, v-src). In this respect, the subject of the present dissertation was logically determined, which are newly synthesized metal complexes of Schiff bases, and of salinomycin with ions of essential metals (Zn, Cu, Co, Ru, Ni, Mn) and ruthenium, as well as nanoscale modifications of chitosan. Their cytotoxic and potential

antitumor activity was studied in laboratory-cultured human and animal tumor cells transformed with human papillomavirus (HPV18), myelocytomatosis virus (Mc29, containing the v-myc gene) and Rous sarcoma virus (RSV, containing the v-src gene).

The dissertation is written on 213 pages and is structured in the usual sections with a predominance of own results and discussion. Hristo Hristov aims two goals for his work, the first of which directly corresponds to the subject of the dissertation. For its implementation, 4 tasks have been formulated, with tasks 1 and 2 having several sub-tasks. In a synthesized form, these tasks aim to determine the cytotoxic/potential antitumor activity of the newly synthesized metal complex compounds and 1,8-naphthalimide-modified glucosamines/chitosan (nanoparticles) on virus-transformed (avian retroviruses, human papillomaviruses) tumor cells and their influence on the replication of human herpes simplex virus (HSV). The second goal is methodological and is related to the development of an *in vitro* cell system for infection with the hepatitis E virus (HEV) and maintenance of viral replication.

To achieve the first goal and the corresponding tasks, extensive short-term (monolayer) and long-term (3D colonies) cell cultures of the virus-transformed tumor lines Hela (human papillomavirus HPV18), LSR-SF-SR (rat transformed with avian Rous sarcoma virus) and LSCC-SF-Mc29 (avian myelocytomatosis virus Mc29) were carried out. The cytotoxicity of the newly synthesized metal complexes, applied in different concentrations, was determined on the basis of cell survival using the MTT test, crystal violet and neutral red staining. The tested substances were arranged in hierarchical sequences reflecting their cytotoxic activity. The same methodological approach was applied to determine the cytotoxic activity of chitosan and its modifications, and in addition to this, their influence on the distribution of cells in the phases of the cell cycle, their ability to induce apoptosis and change the mitochondrial potential was also studied. In addition, the antiviral effect of chitosan on Herpes simplex virus -1 and 2 was investigated. The histopathological picture was visualized with double staining with acridine orange-propidium iodide.

In general, the metals investigated exhibit different cytotoxicity towards different cell lines and depending on the ligand used, which demonstrates some specificity to the type of oncoviral transformation. For example, among the metal complexes with the Schiff base SALTIAZ with the highest cytotoxic activity on 2D cultures, the complexes of Zn (for HeLa) and Cu (for LSR-SF-SR) stand out, which is also established in 3D cultures of HeLa, while in rat sarcoma cells of the LSR-SF-SR line the most toxic is the Ru complex. With Schiff base L, the complex with Mn is

most capable of suppressing 3D growth of tumor cells. The LSR-SF-SR cell line seems to be most sensitive to the metal complexes of Schiff base V, compared to HeLa cells, and the latter are most sensitive to the complexes of salinomycin with copper, zinc and cobalt.

A more modern approach in oncopharmacology is the development of substances capable of influencing mitochondrial function and the redox balance of cells, interacting with nucleic acids and proteins. In this aspect, Hristo Hristov logically focuses his interest to chitosan and its modifications. In the group of glucosamines modified with 1,8-naphthalimides, the highest cytotoxic and genotoxic (double-strand breaks in the DNA molecule) activity is exhibited by Ch-1, which completely suppresses the mitochondrial potential (mitotoxicity) in the treated cells. Their cytotoxic effect is weaker to that of the metal complexes with Schiff bases and the antitumor preparations used as controls. The chitosan and its modifications seems to not affect the replication of herpes viruses.

During the course of the dissertation work, Hristo Hristov has developed and introduced into the laboratory a cell model system for continuous cultivation of hepatitis E virus. Although it is not directly related to the fulfillment of the goal of the dissertation, this model system might be useful in future studies on the potential oncogenic effect of this virus and the testing of antiviral agents.

Based on the results obtained, 6 conclusions were formulated. Three original scientific contributions were formulated on the antitumor activity of the newly synthesized metal complexes with Schiff bases and salinomycin, as well as on the cytotoxic, genotoxic and mytotoxic potential of four modifications of chitosan with 1,8-naphthalimides. Two contributions are of a scientific-applied nature – optimization of a complex approach for studying the cytotoxic activity of 1,8-naphthalimide-modified glucosamines in virus-transformed tumor cells; development of a cell model system for continuous cultivation of hepatitis E virus.

At the end of the dissertation, Hristo Hristov presents results from studies on the content of metals in eggs from different production systems in Romania. As they do not correspond to the topic of the dissertation, I do not intend to analyze them.

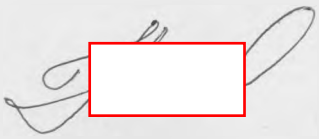
The data presented in the dissertation have been published in two articles, one of which is a review, and both articles already have citations. A third publication is in the process of reviewing.

As a remark, I would like to note the way the "Results and Discussion" section is presented. Despite the fact that the work is very well illustrated with 58 tables and 73 figures (graphs and

multiple micrographs), the results themselves are described as text below the figures, and the discussion is briefly written.

Conclusion: The topic of dissertation work of Hristo Hristov is highly relevant and modern The dissertation is an original study of the author with theoretical and practical importance for tumor biology, oncopharmacology and virology. A large volume of research work has been carried out, from which fundamental and applied contributions have been obtained. The antitumor and antiviral activity of a number of newly synthesized complex compounds, potential candidates for application in the clinic, has been characterized. I find that the volume and quality of the work meets the criteria of the ZRASRB and the relevant regulations for its application.

In connection with the above, I give a positive assessment of the dissertation work on the topic "Antitumor and antiviral activity of newly synthesized metal complexes of Schiff bases and salinomycin and of 1,8-naphthalimide-modified glucosamines" with author Hristo Kanchev Hristov and I recommend to the esteemed Scientific Jury to vote positively for awarding the educational and scientific degree "Doctor" (PhD) in "Virology" to Hristo Hristov in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", Professional direction 4.3. "Biological Sciences" at the Department of Pathology, IEMPAM-BAS, Sofia

16.02.2026 Reviewer: 

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