ANNOTATION

of the dissertation work for the degree of Doctor of Philosophy (Ph.D.)
in specialty 6D070100 – "Biotechnology"

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on the topic: **"The study of antitumor activity of coordinated compound of iodine for new drug development"**

General characteristics of the study. The dissertation is devoted to the investigation of the pharmaceutical, biological, and pharmacological properties of a novel iodine coordination compound with potential antitumor activity, namely dilithium (I) (1→4)-L-D- polyglucopyran triiodide chloride, polypeptide (hereinafter referred to as KC-144).

Relevance of the study. Cancer remains one of the leading causes of mortality worldwide and represents one of the most significant challenges in modern medicine. Despite substantial advances in chemotherapy, targeted therapy, and immunotherapy, the treatment efficacy of many malignant tumors remains limited by systemic toxicity, pronounced tumor heterogeneity, and the development of drug resistance. Therefore, there is a continuing need to discover novel anticancer compounds with alternative mechanisms of action that can enhance tumor cell sensitivity to therapy and overcome resistance to existing anticancer drugs.

One promising area of modern pharmaceutical chemistry is the development of low-toxicity anticancer agents that can increase or restore the sensitivity of drug-resistant tumor cells to existing therapies. One such approach may involve reactivation of apoptotic death in cancer cells. Iodine and its derivatives can be considered among the compounds capable of activating apoptosis.

In recent years, accumulating evidence has demonstrated the antitumor potential of various chemical forms of iodine, including their antiproliferative and pro-apoptotic effects. It has been shown that iodine and iodine-containing systems may be associated with the modulation of mitochondrial processes, oxidative stress, and cell death signaling pathways, making them promising candidates for anticancer therapy. However, the therapeutic application of free molecular iodine is limited by its high chemical reactivity, instability, and challenges associated with controlled delivery. These limitations justify the development of more stable and pharmaceutically manageable iodine-containing systems. A particularly promising approach involves the design of iodine coordination compounds with polymeric and bioorganic ligands capable of stabilizing the active component and modifying its physicochemical and, consequently, biological properties. Previous studies have demonstrated that complex formation in polymeric iodophores and other iodine-containing systems facilitates iodine retention, modulates its release profile, and improves stability compared with free iodine. Moreover, such compounds may exhibit mechanisms of action distinct from those of conventional cytotoxic agents, including the modulation of redox homeostasis, mitochondrial function, cell-cycle regulation, and programmed cell death pathways. Therefore, the discovery and

development of novel iodine-based coordination systems represent an important and timely objective in both pharmaceutical chemistry and experimental oncology.

In the present dissertation, a novel iodine coordination compound, KC-144, was investigated as a potential antitumor agent. The aim of the study was to investigate its physicochemical, biological, and pharmacological properties, evaluate its antitumor activity under *in vitro* and *in vivo* conditions, and elucidate its molecular and cellular mechanisms of action. Particular attention was devoted to examining the effects of KC-144 on tumor cell viability, cell-cycle progression, mitochondrial membrane potential, oxidative status, and apoptosis induction. Furthermore, the study examined the combined effects of KC-144 with known anticancer drugs, which is of great importance for modern strategies aimed at improving therapeutic efficacy and overcoming drug resistance.

Thus, the development of a novel iodine coordination compound with antitumor activity, as well as a comprehensive study of its physicochemical, pharmaceutical, and biological properties, constitute a scientifically justified and highly relevant research endeavor.

The obtained results may serve as a basis for the further preclinical development of a novel domestic antitumor agent with a potentially new mechanism of action.

Objects of the study: An iodine coordination compound with biopolymers possessing antitumor activity; cultures of normal and tumor cells obtained from the American Type Culture Collection (ATCC, Manassas, USA); a transplantable affinity rat ovarian tumor (carcinoma) (Kazakh Research Institute of Oncology and Radiology); ATCC and NBRC microbial test strains; and Wistar laboratory rats.

The aim of the study: To develop a novel iodine coordination compound, dilithium (I) (1→4)-L-D-polyglucopyran triiodide chloride, polypeptide, investigate its physicochemical properties and biological activity, and evaluate its antitumor potential.

Research objectives:

1. To obtain an iodine coordination compound and investigate its physicochemical properties.
2. To investigate the biological activity of the iodine coordination compound in various tumor cell lines.
3. To investigate the combined effects of the iodine coordination compound with established anticancer drugs in various tumor cell lines.
4. To elucidate the molecular and cellular mechanisms underlying the antitumor activity of the iodine coordination compound.
5. To evaluate the *in vitro* genotoxicity of the iodine coordination compound.
6. To investigate the *in vivo* efficacy of the combined application of the iodine coordination compound with an anticancer drug in an experimental transplantable tumor model.

Research methods. A comprehensive set of modern physicochemical, pharmaceutical, biological, microbiological, and pharmacological methods was employed in this study.

Characterization of the iodine coordination compound included *in silico* quantum-chemical modeling, synthesis procedures, pharmaceutical quality assessment, ultraviolet (UV), infrared (IR), and Raman spectroscopy, as well as stability studies.

The *in vitro* biological activity of KC-144 was evaluated using cell culture methods, cell viability, antiproliferative, and cytotoxicity assays, apoptosis analysis, clonogenic assays, cell-cycle analysis, the TUNEL assay for DNA fragmentation, assessment of mitochondrial membrane potential, oxidative stress analysis, micronucleus assay, Western blotting, flow cytometry, electrophoresis, fluorescence microscopy, and light microscopy.

Genotoxicity studies included bacterial mutagenicity testing, cell subculturing, reactivation, and cryopreservation techniques.

In vivo studies comprised acute toxicity assessment and evaluation of antitumor activity in experimental rat tumor models, animal survival was analyzed using the Kaplan–Meier method, with intergroup differences assessed using the log-rank test.

Scientific novelty. The present doctoral research generated comprehensive experimental data characterizing the physicochemical, pharmaceutical, biological, and pharmacological properties of a novel iodine coordination compound, KC-144, with potential antitumor activity.

For the first time, a novel biologically active compound, dilithium (I) (1→4)-L-D-polyglucopyran triiodide chloride, polypeptide (KC-144), was synthesized and its physicochemical and selected pharmaceutical properties were characterized.

For the first time, the antiproliferative and cytotoxic activities of KC-144 were demonstrated against a variety of tumor cell lines under *in vitro* conditions, identifying this compound as a promising candidate for further pharmacological and preclinical investigations.

For the first time, KC-144 was shown to exhibit biological activity against both drug-sensitive and drug-resistant tumor cell lines, including the cisplatin-resistant mesothelioma cell line P31res1.2, highlighting its potential for overcoming tumor drug resistance.

For the first time, KC-144 was found to potentiate the effects of established anticancer agents, including doxorubicin and cisplatin, in both sensitive and resistant tumor cell lines, supporting its potential application in combination anticancer therapy.

For the first time, the antitumor activity of KC-144 was shown to involve multiple intracellular and molecular mechanisms, including disruption of mitochondrial membrane potential, increased production of reactive oxygen species, cell-cycle arrest, and induction of apoptotic cell death.

A preliminary evaluation of the efficacy of KC-144 in combination with doxorubicin was performed using an experimental transplantable rat carcinoma

model. In addition, acute toxicity and genotoxicity assessments demonstrated the potential of KC-144 for further preclinical development.

Based on the obtained results, a patent of the Republic of Kazakhstan was granted (Patent No. 35927, dated October 28, 2022) entitled: *"Dilithium (I) (1→4)-L-D-polyglucopyran triiodide chloride, polypeptide coordination compound possessing antitumor activity."*

Practical significance of the study. The practical significance of this research lies in the development of a technological process for the production of the KC-144 pharmaceutical substance, which may serve as a basis for the establishment of a pilot-scale manufacturing process.

Standardization of the KC-144 substance and determination of its key quality attributes provide a foundation for the further pharmaceutical development of dosage forms based on KC-144.

The research findings have been implemented in the pilot production facility of the Scientific Center for Anti-Infectious Drugs, as confirmed by an official implementation certificate.

The obtained data on the pharmacological efficacy and toxicity of KC-144 may be used in planning subsequent preclinical studies. Furthermore, the results provide an experimental basis for the further pharmaceutical development of KC-144 as a drug substance potentially applicable as a component of combination antitumor therapy.

Theoretical significance of the study. lies in expanding the understanding of the relationship between the chemical structure of the iodine coordination compound and its physicochemical properties, stability, biological activity, and mechanisms of antitumor action. The obtained results complement current scientific knowledge on the biological activity of polyiodide coordination compounds and substantiate the prospects for further investigation of KC-144 as a potential component of combination antitumor therapy.

Main provisions of the dissertation submitted for defense:

1. A novel iodine coordination compound, KC-144, containing polypeptides and dextrin, was developed and synthesized. Its main physicochemical properties and spectral characteristics were determined; the obtained data are consistent with the presence of iodide, triiodide, and polyiodide species and their stabilization by the polysaccharide–peptide matrix.
2. KC-144 is a brown-violet powder with the odor of iodine, readily soluble in water and stable under storage conditions of 25 ± 2°C and 60 ± 5% relative humidity, making it suitable for the development of novel pharmaceutical dosage forms.
3. Mesothelioma cell lines P31 and P31res1.2 exhibited greater sensitivity to KC-144 (IC_{50} values of 0.306 and 0.465 mg/mL, respectively) compared with normal mesothelial cells MeT-5A (IC_{50} = 1.30 mg/mL), indicating moderate selectivity toward malignant mesothelial cells.
4. KC-144 demonstrated potentiating effects when combined with cisplatin and doxorubicin. Synergistic interactions were observed between KC-144 and

doxorubicin in K562 cells, as well as between KC-144 and cisplatin in P31 and P31res1.2 mesothelioma cells. The absence of antagonistic interactions confirms the safety of co-administration of KC-144 with the investigated drugs. Clonogenic analysis confirmed long-term suppression of tumor cell proliferative capacity without evidence of resistance development.

5. The antitumor activity of KC-144 at concentrations of 0.125–0.25 mg/mL is associated with three major mechanisms: loss of mitochondrial membrane potential (Δψm), increased production of reactive oxygen species (ROS), and apoptosis induction accompanied by cell-cycle arrest during the presynthetic phase of interphase with transition to the G_0/G_1 phase.

6. KC-144 showed no mutagenic potential in the Ames test or in the *in vitro* micronucleus assay using L5178Y mouse lymphoma cells.

7. Acute toxicity studies in rats following oral administration demonstrated that the median lethal dose (LD_{50}) of KC-144 exceeds 4000 mg/kg under the conditions of the study. following oral administration

8. Combination therapy with KC-144 at a dose of 400 mg/kg and doxorubicin in a rat affinity ovarian carcinoma model increased the survival time of the rats by 64%.

All research objectives set forth in the dissertation were successfully accomplished.

Relationship with the main scientific research program. This dissertation research was conducted within the framework of the objectives of the Scientific and Technical Program *"Development of New Anti-Infective Drugs for 2015–2017 and 2018–2020"* of the Ministry of Industry and Infrastructure Development of the Republic of Kazakhstan (Program Registration No. O.0777).

Personal contribution of the doctoral candidate. The doctoral candidate's contribution to each publication included a review of the scientific literature, study design, experimental work, data acquisition, statistical analysis, interpretation of results, and preparation of manuscripts for publication. The candidate also contributed to the presentation and discussion of the research findings.

Approbation of the research work. The materials of the dissertation were presented and discussed at international scientific conferences, congresses, forums, and round tables, including: the IV International Farabi Readings, Kazakhstan (2017); the Astana Biotech International Symposium (2018); the VI International Farabi Readings (04/2019); *International Multidisciplinary Scientific GeoConference: SGEM* (2020); the International Conference of Students and Young Scientists *"FARABI ALEMI"* (2023); the *International Scientific Conference of Young Scientists dedicated to the 40th anniversary of the founding of the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry* (2023); and *BIO Web of Conferences* 100, 03001 (2024).

Publications. Based on the results of the dissertation research, 16 scientific works have been published and 1 patent for an invention of the Republic of Kazakhstan has been obtained, including: 2 articles in international peer-reviewed scientific journals indexed in the Scopus database; 3 articles in journals recommended by the Committee for Quality Assurance in Science and Higher

Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 1 article in a foreign peer-reviewed journal; 1 chapter in an international collective monograph; and 9 publications in the proceedings of international scientific conferences.