

UDC 615.281

IODINE COORDINATION COMPOUNDS OF ANTI-INFLAMMATORY DRUGS: NEW SYSTEMS OF INTEREST FOR ANTI-INFECTIVE RESEARCH

Shepilov D.A.^{1*}, Turganbay S.^{2,3}, Jumagazyeva A.B.¹, Iskakbayeva Zh. A.¹, Jumabayeva S.M.¹, Seysembekova A.B.¹, Jingcheng Hao⁴, Askarova D.¹, Azembayev A.A.¹¹JSC Scientific Center for Anti-Infectious Drugs, Almaty, 050060, Kazakhstan²Faculty of Natural Sciences, Kazakh National Women's Teacher Training University, Almaty, 050000, Kazakhstan³Kazakh-British Technical University, School of Petroleum Engineering, "One Belt, One Road" Research Center, Almaty, 050000, Kazakhstan⁴Key Laboratory of Colloid and Interface Chemistry, Shandong University, Jinan, 250100, China

*Corresponding author: shepilov2002@gmail.com

ABSTRACT

Given the increasing threat of antimicrobial resistance and multidrug-resistant pathogens, the development of combined therapeutic systems with preserved anti-infective activity and supportive anti-inflammatory properties is highly relevant. This study aimed to synthesize and evaluate novel iodine-containing coordination compounds based on flunixin meglumine and dexamethasone sodium phosphate. The synthesized compounds were characterized using capillary electrophoresis and UV-spectroscopy. Their cytotoxicity was assessed in PBMC culture, while antimicrobial and antiviral activities were evaluated against bacterial, fungal, and viral pathogens. Two stable compounds, KC-247 and KC-249, were successfully obtained. Both demonstrated antimicrobial activity against *Staphylococcaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Enterococcaceae*, and yeast-like fungi. MBC values ranged from 0.801–0.200 µg/mL for KC-247 and 2.093–0.523 µg/mL for KC-249. In PBMC culture, CC₅₀ values were 0.072 mg/mL and 0.225 mg/mL, respectively. KC-249 showed pronounced antiviral activity against influenza A (H1N1), while KC-247 demonstrated moderate effects. Neither compound was active against herpes simplex virus type 1. KC-247 and KC-249 exhibited promising antimicrobial and selective antiviral properties with acceptable cytotoxicity, highlighting their potential as multifunctional candidates for novel anti-infective therapeutics.

Keywords: iodine-containing compounds; active pharmaceutical substances; pharmaceutical innovation; novel antimicrobial adjuvants; antimicrobial resistance

INTRODUCTION

Antimicrobial resistance has transformed bacterial infection from a largely controllable condition into a major therapeutic problem characterized by increasing rates of treatment failure, prolonged hospitalization, and excess mortality. Clinical outcome data [1] illustrate this shift particularly clearly: in a recent cohort of multidrug-resistant bloodstream infections, the overall 30-day mortality reached 23.0%, whereas carbapenem-resistant *Enterobacteriaceae* bloodstream infections [2] were associated with a markedly higher mortality of 42.9%. These observations underscore that the central challenge is no longer limited to identifying intrinsically active antibacterial agents, but increasingly concerns the restoration of antimicrobial efficacy against pathogens that have already acquired multidrug-resistant phenotypes.

In this context, antibiotic potentiators have attracted considerable interest as pharmacologically auxiliary systems capable of enhancing the activity of established antimicrobials and overcoming resistance-associated bacterial adaptations. However, the practical relevance of such potentiators cannot be adequately judged within a simplified binary "potentiator–antibiotic" model alone. In real therapeutic settings, anti-infective regimens are frequently accompanied by supportive agents administered to modulate inflammation [3], pyrexia, pain, and tissue damage. In other words, the key question is not whether a non-steroidal anti-inflammatory drug or a glucocorticoid exerts pronounced direct antimicrobial activity, but whether the activity of the potentiator is preserved in their presence. This issue is methodologically important because concomitantly administered agents may alter the apparent an-

tibacterial, fungicidal, cytotoxic, or broader anti-infective profile of the studied system, thereby affecting its translational value.

This consideration is especially relevant for anti-inflammatory agents that already have a defined place in the management of infectious diseases. In human medicine, dexamethasone sodium phosphate is used as adjunctive therapy in severe infectious conditions in which host inflammatory injury significantly contributes to disease progression. Current guidance and recent clinical practice recommendations support corticosteroid administration in tuberculous meningitis [4], and WHO meningitis guidance indicates that intravenous corticosteroids may be initiated with the first dose of antibiotics in suspected acute bacterial meningitis under appropriate clinical conditions. Thus, dexamethasone is relevant in this setting not as an antimicrobial compound *per se*, but as a clinically meaningful co-administered anti-inflammatory agent.

A comparable rationale applies in veterinary medicine, where flunixin meglumine is widely used as a supportive non-steroidal anti-inflammatory drug during the treatment of infectious and inflammatory disorders in animals. Official veterinary labeling identifies flunixin meglumine as a supportive anti-inflammatory agent for the management of pyrexia associated with bovine respiratory disease [5, 6], endotoxemia, acute bovine mastitis, and swine respiratory disease, thereby emphasizing its therapeutic relevance as a co-administered component in anti-infective veterinary practice, including in formulations combined with florfenicol (Flr&Flx). Accordingly, investigation of potentiators only in combination with antibiotics provides an incomplete representation of their ap-

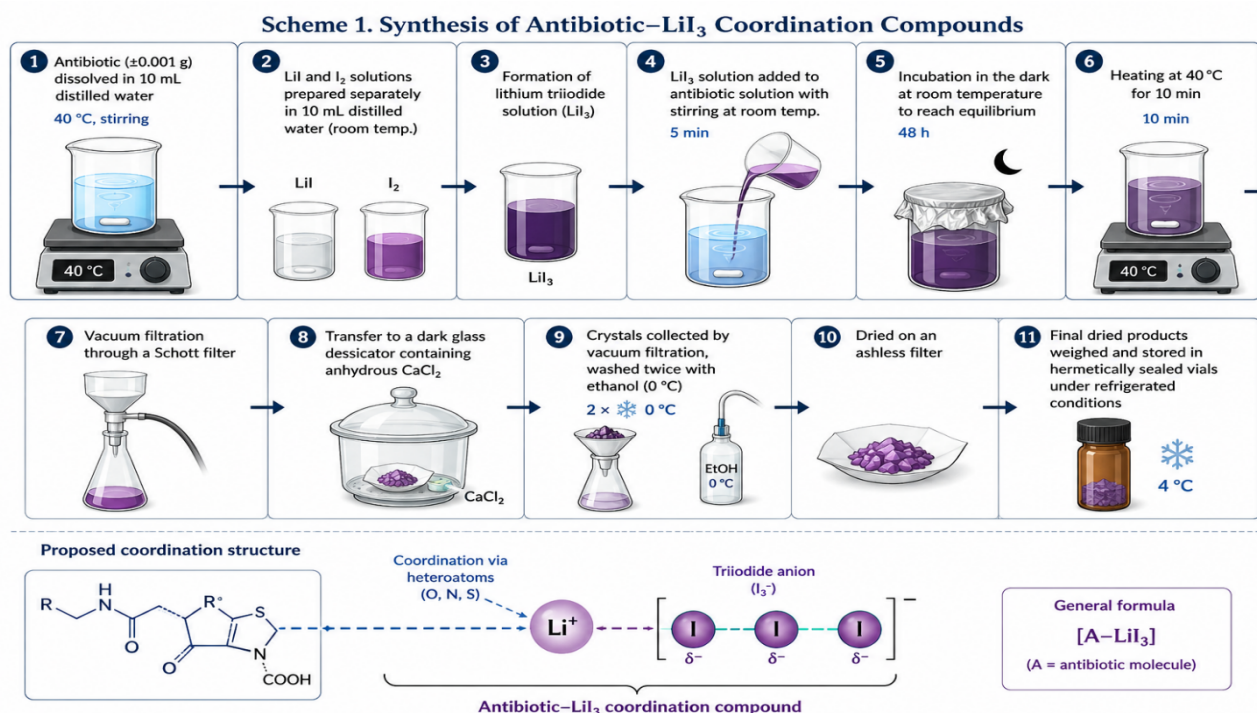


Figure 1. Scheme of synthesis of antibiotic–LiI₃ coordination compounds

plied potential. A more informative approach is to determine whether their antibacterial, fungicidal, cytotoxic, and, where relevant, virus-inhibitory properties are retained in the presence of therapeutically relevant supportive agents.

MATERIALS AND METHODS

Reagents

Glass laboratoryware was used throughout the study, including beakers of 50 and 100 mL, a 100 mL volumetric flask, and graduated cylinders of 10 and 25 mL (Duran, Czech Republic). Filter paper “Red Ribbon” (Hahnemühle FineArt GmbH, Dassel, Germany) and a Schott filter (Simax, Czech Republic) were employed for filtration. Analytical grade reagents were obtained from commercial suppliers and used without further purification: lithium iodide (Sigma-Aldrich, Burlington, MA, USA, ≥99%), iodine (Labpharm, JSC “Troitsky Iodine Plant”, Krasnodar, Russia, ≥98%), flunixin meglumine, and dexamethasone sodium phosphate (Sigma-Aldrich, Burlington, MA, USA, ≥99%).

Test-systems

The bacterial and fungal test strains were obtained from the American Type Culture Collection (ATCC) through the official distributor for Central Asia LGC Standards (Poland). Both reference (museum) and clinical strains, including sensitive and multidrug-resistant variants, were employed as follows: *Staphylococcus aureus* ATCC 6538-P (museum, sensitive strain), *Staphylococcus aureus* ATCC 333591 (museum, multidrug-resistant strain), *Escherichia coli* ATCC 8739 (museum, sensitive strain), *Escherichia coli* ATCC BAA-2523 (museum, multidrug-resistant strain), *Pseudomonas aeruginosa* ATCC 9027 (museum, sensitive strain), *Pseudomonas aeruginosa* TA2 (clinical, multidrug-resistant strain), *Klebsiella pneumoniae* ATCC 10031 (museum, sensitive strain), *Klebsiella pneumoniae* ATCC 2524 (museum, multidrug-resistant strain), *Salmonella enterica* ATCC 14028 (museum,

sensitive strain), *Salmonella enterica* ATCC 35988 (museum, multidrug-resistant strain), *Enterococcus hirae* ATCC 10541 (museum, sensitive strain), *Enterococcus faecalis* ATCC 51575 (museum, multidrug-resistant strain), *Candida albicans* ATCC 10231 (museum, sensitive strain), *Candida albicans* SCID PHRX 1-2019 (clinical, multidrug-resistant strain)

Virus strains – Influenza A virus A/Swine/Iowa/15/30 (H1N1) and Herpes Simplex Virus type 1 (strain «Victory»), obtained from the Laboratory of Viral Ecology, Institute of Virology and Microbiology, Committee of Science, MES RK.

Cell cultures – MDCK cells (Madin-Darby Canine Kidney), obtained from the Laboratory of Cellular Biotechnology, Research Institute for Biological Safety Problems, National Center for Biotechnology, MES RK, and RD cells (human embryonal rhabdomyosarcoma), obtained from the cell culture collection of the National Center of Infectious and Parasitic Diseases, Sofia, Republic of Bulgaria.

Human peripheral blood mononuclear cells (PBMCs) – isolated from healthy donors (without acute or severe chronic diseases) of both sexes.

Preparation of the APSs

For the synthesis of new active pharmaceutical substances (APSs) and the investigation of their physicochemical properties, the following procedure was employed. Accurately weighed portions of the antibiotics (±0.001 g) were dissolved in 10 mL of distilled water under continuous stirring in a water bath at 40 °C until complete dissolution. Lithium iodide (LiI) and crystalline iodine (I₂) solutions were prepared separately in 10 mL of distilled water at room temperature. The resulting lithium triiodide solution was then added to the antibiotic solution with thorough stirring at room temperature for 5 min. The reaction mixture was incubated in the dark at room temperature for 48 h to achieve equilibrium. After incubation, the mixture was heated to 40 °C for 10 min, vacu-

um-filtered through a Schott filter, and transferred to a dark glass desiccator containing anhydrous calcium chloride for bulk water removal. The formed crystals were collected by vacuum filtration, washed twice with ethanol pre-cooled to 0 °C, and dried on an ashless filter. The final dried products were weighed and stored in hermetically sealed glass vials under refrigerated conditions (Figure 1).

FT-IR (Fourier-transform infrared spectroscopy) spectroscopy

The samples were processed without prior preparation via placing directly onto the ATR (attenuated total reflectance) diamond crystal and pressing to ensure optimal contact, their FT-IR spectra were collected using an FT-IR spectrometer (Bruker Alpha II FT-IR spectrometer, Bruker Optics GmbH, Germany). The test conditions were as follows: wavenumber range, 4000–400 cm⁻¹; scan number, 32; and resolution, 4 cm⁻¹.

UV-vis spectroscopy

Ultraviolet–visible (UV–Vis) absorption spectra of the investigated systems were obtained using a double-beam Lambda 35 spectrophotometer (PerkinElmer), equipped with a monochromator operating over the 190–1100 nm spectral range. Spectral acquisition was carried out in the 190–500 nm wavelength interval under continuous scanning (SCAN mode). Measurements were performed in 1.0 cm path length quartz cuvettes at room temperature.

Capillary electrophoresis (CE)

The analyses were conducted using an Agilent 1600 capillary electrophoresis system (Agilent Technologies, USA) equipped with a diode-array detector (DAD). Analytical solutions were prepared at analyte concentrations ranging from 5 to 200 mg/L and filtered through 0.2 µm membrane filters prior to injection.

Qualitative identification was performed by comparing the migration times of the analyte ions with those of reference standards. Quantitative analysis was carried out by comparison of peak areas with those obtained for certified reference standards (CRS).

Cation determination was performed according to a validated internal analytical protocol using an uncoated fused-silica capillary (50 µm internal diameter, total length 56 cm). The background electrolyte (BGE) was adjusted to pH 3.2. Electrophoretic separation was conducted at 25 °C under normal polarity with an applied voltage of +30 kV. UV detection was performed at 310 and 215 nm.

Iodide ions were analyzed using a separate validated internal protocol under the following conditions: uncoated fused-silica capillary (50 µm *i.d.*, 56 cm total length), BGE pH 9.3, reversed polarity (-30 kV), and UV detection at 226 nm. Both analytical procedures were validated in compliance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan and relevant literature sources [7-9].

Quantum-chemical calculations

Quantum-chemical calculations were performed using the DFT/B3PW91/6-31G** method, which combines the hybrid B3PW91 functional with a polarized 6-31G** basis set to provide reliable prediction of electronic structure, charge distribution, and intermolecular interactions. This approach is particularly suitable for studying lithium iodide complexes with

antibiotics, as it effectively describes ion-dipole interactions, charge delocalization, and hydrogen bonding. All calculations were conducted on a Fujitsu PRIMERGY BX920 S1 super-computer with a peak performance of 10.9 TFLOPS.

Quantitative analysis of iodine and iodide

The quantitative determination of molecular iodine in the obtained complexes was performed by iodometric titration with sodium thiosulfate in the presence of starch indicator.

An accurately weighed sample (0.1–0.3 g) was transferred into a 50 mL beaker, dissolved in 20 mL of purified water, and titrated with 0.05 M sodium thiosulfate solution until the brown coloration disappeared. Subsequently, 1–2 drops of starch solution were added, and titration was continued until complete decolorization of the solution (endpoint).

The iodine content (C_{I_2}) per 1 kg of the preparation, expressed in grams, was calculated using Equation (1):

$$C_{I_2} = V_1 \times K_1 \times 12.69/m, \quad (1)$$

where:

V_1 – volume of sodium thiosulfate solution consumed for titration, mL;
 K_1 – correction factor for the molar concentration of 0.05 M sodium thiosulfate solution ($K_1 = 0.5$);
 m – sample mass, g.

According to the State Pharmacopoeia of the Republic of Kazakhstan, 1 mL of 0.1 M sodium thiosulfate corresponds to 12.69 g of iodine.

Potassium iodide in the obtained complexes was quantified by an electrochemical method according to an in-house validated procedure. After completion of iodine titration, 5 mL of 0.1 N nitric acid was added to the resulting solution, followed by potentiometric titration with 0.05 M silver nitrate solution.

Potentiometric measurements were performed using a combined platinum electrode with an integrated silver/silver chloride reference electrode. The equivalence point was determined from the titration curve (electrode potential versus volume of AgNO₃ solution) as the midpoint of the potential jump.

The potassium iodide content (C_{KI}) per 1 kg of the preparation, expressed in grams, was calculated using Equation (2):

$$C_{KI} = (V_2 \times K_2 - V_1 \times K_1) \times 16.60/m, \quad (2)$$

where:

V_2 – volume of silver nitrate solution consumed for sample titration, mL;
 K_2 – correction factor for the molar concentration of 0.05 M silver nitrate solution ($K_2 = 0.5$);
 V_1 – volume of sodium thiosulfate solution used for iodine titration, mL;
 K_1 – correction factor for the molar concentration of 0.05 M sodium thiosulfate solution ($K_1 = 0.5$);
 m – sample mass, g.

According to the State Pharmacopoeia of the Republic of Kazakhstan, 1 mL of 0.1 M silver nitrate corresponds to 16.60 g of potassium iodide.

Serial dilution method

The testing procedure and evaluation of antimicrobial activity were performed using the broth serial dilution method in accordance with the guidelines of the Clinical and Labora-

tory Standards Institute (CLSI) [10–14]. All experiments were carried out in triplicate to ensure reproducibility of the results.

Endpoint dilution assay for infectious titer determination

The infectious titer of influenza A virus was determined by the limiting dilution assay in MDCK cell culture (MDCK). The presence of virus was confirmed by the hemagglutination assay performed according to the standard protocol using a 0.75% suspension of human erythrocytes (blood group I, O) [15, 16].

The infectious titer of herpes simplex virus was determined by an analogous limiting dilution method in RD cell culture (RD). Virus-induced cytopathic effect (CPE) was assessed visually by examination of microplates under an inverted microscope. Viral infectious titers were calculated according to the Reed and Muench method (Reed and Muench) [17].

MTT cytotoxicity assay

The cytotoxicity of the synthesized coordination compounds was evaluated in human peripheral blood mononuclear cells (PBMCs; referred to as MNC), as well as in MDCK (Madin–Darby canine kidney) and RD lines.

Cell viability was assessed using the MTT assay [18, 19], based on the ability of metabolically active cells to reduce the soluble tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into insoluble intracellular formazan crystals.

Cell passaging, seeding, and cytotoxicity evaluation were performed according to internal laboratory protocols. Cells were seeded at a density of 2.5×10^5 cells/mL and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂. After removal of the growth medium, 200 µL of DMEM containing various concentrations of the test compounds were added. Cells cultured in incomplete DMEM served as the negative control.

After 72 h, the medium was replaced, 50 µL of MTT working solution was added, and incubation was continued for 4 h. Subsequently, the supernatant was removed and the formazan crystals were dissolved in 100 µL of DMSO. Optical density (OD) was measured using a Tecan Sunrise RC.4 (Austria) at 540/620 nm.

The arithmetic mean OD value ($\bar{Y}$) for the negative control was calculated as:

$$Y = \frac{y_1 + \dots + y_n}{n} = \frac{1}{n} \sum_{i=1}^n y_i, \quad (3)$$

where y_i is the OD value for each replicate and n is the number of replicates.

The percentage of viable cells for each concentration was calculated as:

$$\text{Cell viability (\%)} = \frac{Y_i}{\bar{Y}_{NC}} \times 100\%, \quad (4)$$

where Y_i is the OD value for the test sample and $\bar{Y}_{NC}$ is the mean OD of the negative control.

The half-maximal cytotoxic concentration (CC_{50}), defined as the concentration causing 50% cell death, was calculated

using linear interpolation:

$$CC_{50} = \left[\frac{X_1 - 50}{X_1 - X_2} \times (M_{x2} - M_{x1}) \right] + M_{x1}, \quad (5)$$

where X_1 and X_2 represent percentages of viable cells above and below 50%, respectively; M_{x1} and M_{x2} correspond to the concentrations associated with X_1 and X_2 .

Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Histopaque (Sigma-Aldrich, USA; $\rho = 1.077$ g/mL) at 3000 rpm for 20 min at 4 °C. Cells were resuspended in RPMI-1640 medium (Sigma-Aldrich, USA). Cell viability was assessed by trypan blue exclusion; only cell preparations with viability >90% were used in experiments.

PBMCs were cultured in complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2% L-glutamine, and 1% antibiotic–antimycotic solution in a CO₂ incubator (37 °C, 5% CO₂, 95% humidity).

Cell counting was performed using a Goryaev hemocytometer with trypan blue staining. The percentage of viable cells was calculated as:

$$\text{Viable cells (\%)} = \frac{N_1 \times 100\%}{N}, \quad (6)$$

Where N is the total number of cells counted in five large squares and N_1 is the number of unstained (viable) cells.

Cell concentration was calculated according to the standard Goryaev chamber method using the following equation:

$$C = \frac{N}{N_1} \times 225 \times 1111 \times 2, \quad (7)$$

where C is the cell concentration (cells/mL); 225 is a dimensionless scaling factor reflecting the ratio between the counted grid area and the total chamber grid area used for extrapolation; 1111 is a volumetric scaling factor with units of mL⁻¹, corresponding to the inverse chamber volume (1/0.0009 mL); and 2 is the dilution factor. The method was performed according to standard hemocytometer-based cell counting protocols [20]. Count cells using a hemocytometer (Gibco cell culture basics).

For compounds KC-247 and KC-249, the tested concentration range was 0.02–5.0 mg/mL. Incubation was carried out for 48 h, after which cytotoxicity was determined using the MTT assay as described above.

RESULTS

Physicochemical characterization

As a result of the synthesis, the iodine coordination compounds KC-247, based on flunixin meglumine, and KC-249, based on dexamethasone sodium phosphate, were successfully obtained. The physicochemical characterization results are presented in Table 1, while the iodide ion concentrations and quantification of lithium cations determined by capillary electrophoresis (CE) are presented in Tables 2 and 3.

Table 1. Physicochemical characterization

Compound name	Solubility	pH	Melting point, °C	Iodine, g/kg	Iodide, g/kg
				titration	
KC-247	Water 1g/10ml (soluble)	6.64 ± 0.05	78-80 ± 1.00	102.58 ± 2.26	279.76 ± 1.29
KC-249	Water 1g/5ml (freely soluble)	6.59 ± 0.05	106-108 ± 1.15	133.98 ± 4.17	221.19 ± 3.66

Table 2. Quantification of iodide ions using CE

Indicator	KC-247	KC-249
Iodide concentration, mg/L	393.20 ± 0.62	322.34 ± 0.20
Peak area, S (mEA×s)	1035.86	849.21

Table 3. Quantification of lithium cations using CE

Indicator	KC-247	KC-249
Cation concentration, mg/L	12.135 ± 0.04	10.16 ± 0.11
Peak area, S (mEA×s)	14.87	12.45

Table 4. Physicochemical characterization after 3-month storage.

Compound name	Solubility	pH	Melting point, °C	Iodine, g/kg	Iodide, g/kg
				Titration	
KC-247	Water 1g/10mL (soluble)	6.43 ± 0.04	77-78 ± 0.74	100.58 ± 1.84	282.86 ± 1.06
KC-249	Water 1g/5mL (freely soluble)	6.16 ± 0.03	108-109 ± 1.05	130.56 ± 2.54	220.09 ± 3.21

The stability of the obtained compounds over a period of 3 months was evaluated according to the following parameters: pH, melting point, iodine content, and iodide content. Since the synthesized coordination compounds are considered as potential medicinal agents, their stability is a critical factor for further pharmaceutical investigations.

The solubility of samples KC-247 and KC-249 was examined; the results are presented in Table 4. Both samples demonstrated high solubility in water at a ratio of 1g/5 mL to 1 g/10 mL. The high solubility may be attributed to changes in the molecular structure facilitating solvent interactions and reducing intermolecular interactions within the polymeric matrix.

To confirm stability, the solubility of the samples was reassessed after 3 months. The results showed that the solubility of KC-247 and KC-249 remained unchanged: both samples retained good solubility in water at a ratio of 1g/5mL to 1 g/10 mL. These data confirm the stability of the chemical structure and the absence of significant degradative changes in the sample characteristics during the investigated period.

FT-IR spectroscopy

The FT-IR spectrum of KC-247, presented on Figure 2, retains the characteristic absorption profile of flunixin meglumine,

confirming preservation of the parent flunixin structural framework. The intense bands in the 1600-1500 cm⁻¹ region are as-signed to aromatic C=C stretching, N–H bending, and carboxylate and carbonyl-related vibrations. Absorptions in the 1450-1300 cm⁻¹ region correspond to aromatic ring vibrations and C–N stretching, while the complex fingerprint region at 1250-1000 cm⁻¹ is associated with C–F, C–O, and C–N stretching vibrations, consistent with the trifluoromethyl-substituted aromatic system and meglumine fragment of flunixin meglumine.

The bands in the 900-700 cm⁻¹ region correspond to out-of-plane aromatic C–H bending and skeletal vibrations of the substituted aromatic rings. Importantly, additional bands observed near 1102 cm⁻¹ and 739 cm⁻¹ are assigned to Li–O stretching vibrations, confirming the formation of the coordination compound KC-247.

The FT-IR spectrum of KC-249, presented together in Figure 2 retains the characteristic vibrational features of dexamethasone sodium, confirming preservation of the steroidal framework of the parent compound. The broad absorption in the 3400-3200 cm⁻¹ region is assigned to O–H stretching vibrations, while bands in the 3000–2850 cm⁻¹ region correspond to aliphatic C–H stretching of the steroid skeleton. The intense bands in the 1700–1600 cm⁻¹ region are attributed to

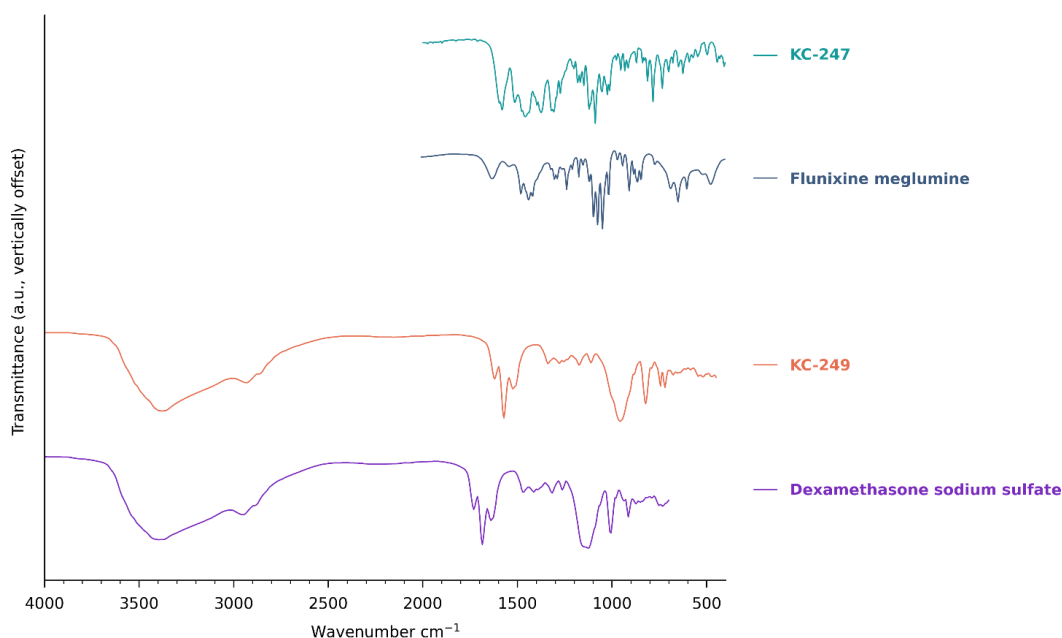


Figure 2. FT-IR spectra of KC-247 and KC-249 compared with those of their respective starting materials, flunixin meglumine and dexamethasone sodium phosphate.

C=O stretching and C=C vibrations, which are characteristic for the conjugated carbonyl-containing structure of dexamethasone.

The absorptions in the 1250-1000 cm^{-1} region correspond mainly to C–O stretching vibrations of hydroxyl and ether-related fragments, consistent with the original dexamethasone structure. Additional bands observed at approximately 1107 cm^{-1} and 746 cm^{-1} are assigned to Li–O stretching vibrations, indicating successful formation of the coordination compound KC-249.

UV spectroscopic methods of analysis

The obtained spectral characteristics, with indication of the transitions, are presented in Table 5. In Figure 3, the UV spectrum of the 0.00125% KC-247 solution is shown. The presence of an intense peak at $\lambda_{\text{max}} = 225.13 \text{ nm}$ ($A = 0.91$), despite the low concentration, may be explained by the contribution of iodide-containing complexes. Iodine and its complexes can produce absorption in this region, as can other simple compounds exhibiting signals in the short-wavelength UV region ($<240 \text{ nm}$). The absorption intensity also depends on the iodide concentration, which may enhance the overall absorbance.

The peak at 280.74 nm represents characteristic absorption of aromatic or conjugated structural fragments present in the flunixin molecule. This may indicate the presence of chromophores undergoing $\pi \rightarrow \pi^*$ transitions or the existence of

a conjugated system within the molecule, which is typical of biologically active compounds. KC-247 exhibits a peak at 225.13 nm associated with the effect of iodides in solution. This peak differs from that of pure flunixin at 280.74 nm, confirming spectral alteration due to interactions between the components of the mixture. The absence of the 280.74 nm peak in the mixture indicates possible shielding of flunixin or its interaction with other components, which may reduce or shift the intensity of its intrinsic absorption.

Figure 3 also presents the UV spectrum of the 0.0025% KC-249 solution. The peak in the region around 194 nm is associated with ionic components such as lithium iodide and iodides. Absorption in this range, corresponding to the deep UV region, indicates $\sigma \rightarrow \sigma^*$ electronic transitions characteristic of simple compounds, including halides. The high intensity of this band ($A = 1.93$) indicates that the peak is predominantly governed by the contribution of lithium iodide and iodine, which dominate the spectrum.

The peak at 227.06 nm ($A = 1.56$) may indicate an interaction between dexamethasone and iodide ions or the influence of iodine-containing species in the mixture. In this region, $\pi \rightarrow \pi^*$ transitions associated with conjugated structures or ionic complexes may also occur. The peak at 241.66 nm ($A = 0.73$) in the individual dexamethasone solution demonstrates that dexamethasone sodium phosphate itself possesses a chromophoric system responsible for absorption in the UV region. The absorption at 241.66 nm is associated with its conjugated

Table 5. Spectral characteristics of the synthesized complex compounds (UV spectra).

Compound name	$\lambda_{\text{max}}, \text{ nm}$	A_{max}	Transitions
KC-247	225.13	0.91	$\pi \rightarrow \pi^*$
Flunixin meglumine	280.74	0.43	$n \rightarrow \pi^*$
KC-249	193.99	1.93	$\sigma \rightarrow \sigma^*$
	227.06	1.56	$\pi \rightarrow \pi^*$
Dexamethasone sodium phosphate	241.66	0.73	$n \rightarrow \pi^*$

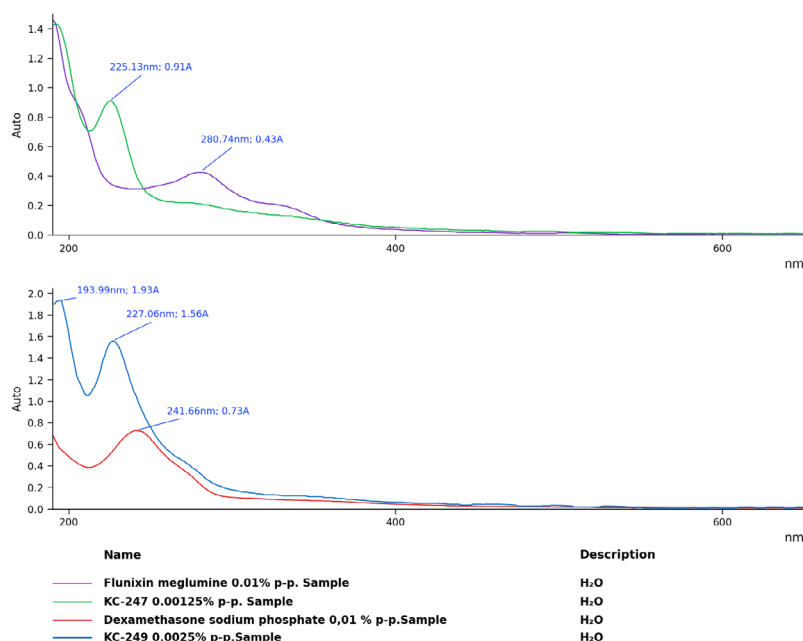


Figure 3. UV spectra of KC-247, KC-249, and their respective starting materials solutions.

Thus, the spectral profiles of KC-247 and KC-249 are likely governed by iodine-containing species in solution. KC-247 exhibits an intense absorption band at 225.13 nm, whereas KC-249 shows dominant peaks at approximately 194 and 227.06 nm.

system involving the phosphate group and other structural moieties of the dexamethasone molecule. In the KC-249 mixture, absorption arising from iodides and lithium iodide predominates, with intense peaks at 194 and 227 nm. Dexamethasone sodium phosphate in the mixture exhibits weakened or masked absorption, and its characteristic peak at 241.66 nm is not clearly expressed, which may be associated with interaction with iodine ions.

Considering the obtained data on the stability of the compounds over 3 months, it can be assumed that samples with a higher melting point and lower mass loss upon drying have a higher probability of stability.

***In vitro* safety testing**

The cytotoxicity of the synthesized APSs KC-247 and KC-249 was investigated on human peripheral blood mononuclear cell (PBMC) cultures, as well as on MDCK and RD cells under *in vitro* conditions. The CC_{50} values were determined by the nonlinear regression method using GraphPad Prism 6 software based on the full concentration range.

To determine the cytotoxic concentration of KC-247 and KC-249, PBMCs (1×10^5 cells/well) were seeded into 96-well plates with the addition of the substance at concentrations of 0.02–5.0 mg/mL, where 5.0 mg/mL was used as the maximum dose. The control group consisted of cells with the addition of RPMI-1640 medium. Each concentration was added in triplicate.

Incubation of PBMCs was carried out for 48 h at 37 °C, 5% CO₂, and 95% humidity, after which the cytotoxic effect was assessed by the MTT assay based on cell viability.

For KC-247, 48-hour exposure at concentrations of 0.31–5.0 mg/mL caused a cytotoxic effect exceeding the permissible limit of (55 ± 5) %. The calculated CC_{50} value was 0.072 mg/mL (95% confidence interval: 0.061–0.086). The compound KC-249 also exhibited cytotoxicity at concentrations

of 0.31–5.0 mg/mL. The CC_{50} value was 0.225 mg/mL (95% confidence interval: 0.149–0.341) (Table 6) (Figure 6).

Table 6. Percentage of viable PBMCs after 48-hour exposure to KC-247 and KC-249 substances.

Concentrations, mg/mL	KC-247 M ± SD (n = 4)	KC-249 M ± SD (n = 4)
5.00	18.0 ± 4.2	65.0 ± 3.7
2.50	18.7 ± 1.3	36.2 ± 2.2
1.25	22.7 ± 4.5	29.5 ± 2.1
0.62	21.8 ± 1.9	28.2 ± 2.2
0.31	22.2 ± 1.6	52.1 ± 3.8
0.15	56.7 ± 7.7	64.6 ± 7.5
0.08	63.9 ± 2.8	77.8 ± 5.6
0.04	78.9 ± 8.5	73.6 ± 5.5
0.02	122.5 ± 15.1	83.7 ± 5.8
CC_{50} , mg/mL	0.072	0.225

The maximum non-toxic concentration for KC-247 is 0.018 mg/mL, and for KC-249 it is 0.056 mg/mL.

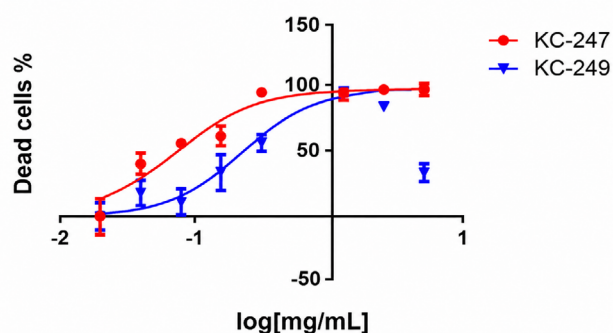
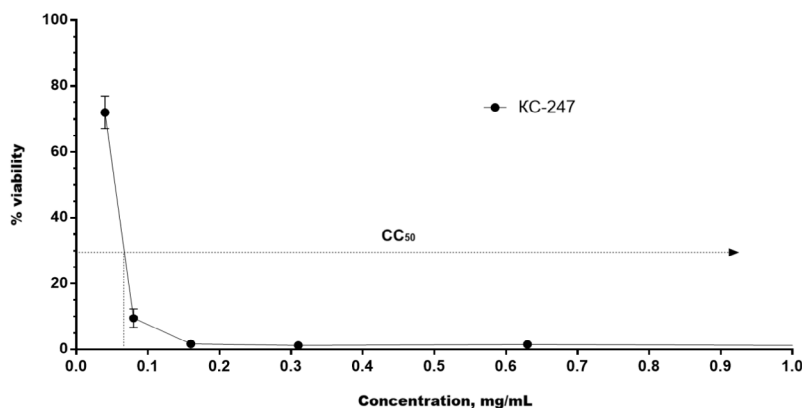
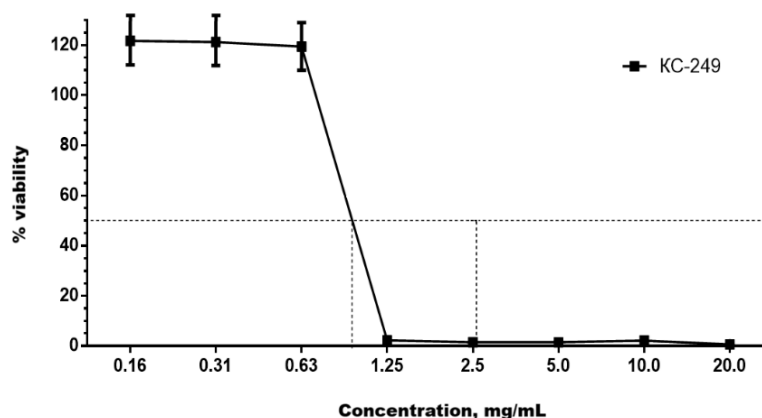


Figure 6. Dose-response curve for compound KC-247 and KC-249 on PBMC culture.

Table 7. Assessment of the cytotoxic effect of the new compounds KC-247 and KC-249 in MDCK and RD cell culture models after 72 h of incubation.

Compound name	CC ₅₀ , on MDCK cell culture, (mg/mL)	CC ₅₀ , on RD cell culture, (mg/mL)
KC-247	0.054 ± 0.09	0.057 ± 0.07
KC-249	1.000 ± 0.8	0.530 ± 0.18

**Figure 7.** Cytotoxic effect of compound KC-247 on MDCK cell culture.**Figure 8.** Cytotoxic effect of compound KC-249 on MDCK cell culture.

MDCK and RD cell cultures were seeded into 96-well plates at a concentration of 2×10^5 cells/mL. Cultivation was carried out at 37 °C and 5% CO₂.

To assess the cytotoxicity of KC-247 and KC-249, the test substance was administered once at the following concentrations: 20.0 mg/mL, 10.0 mg/mL, 5.0 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.063 mg/mL, 0.031 mg/mL, 0.016 mg/mL.

The exposure lasted 72 h. Upon completion of incubation, the optical density in the wells was measured using a Tecan Sunrise RC.4 plate reader (Austria) at wavelengths of 540 nm (main filter) and 620 nm (reference filter).

The study results (Table 7) confirmed the CC₅₀ values for KC-247 and KC-249 in MDCK (Figures 7, 8) and RD (Figures 9, 10) cell cultures after 72 h of incubation, which indicates the cytotoxic effect of these newly synthesized potentiators.

Antimicrobial activity screening

The antimicrobial activity of KC-247 and KC-249 sub-

stances was investigated against reference and clinical strains. Testing was performed by the method of two-fold serial dilutions in physiological saline.

During the studies, the minimum inhibitory concentration (MICs) and minimum bactericidal concentrations (MBCs) of the samples were determined. The MIC was defined as the lowest concentration that inhibited visible microbial growth, whereas the MBC was defined as the lowest concentration of the sample in dilution that completely suppressed the growth of the test microorganism on a Petri dish after inoculation onto the corresponding medium. The obtained study data are presented in Table 8.

From the data presented in Table 8, it was established that KC-247 possesses antimicrobial activity against all test strains. The coordination compound KC-247 exhibited identical activity against *S. aureus* ATCC 33591, *E. coli* ATCC 8739, *E. coli* ATCC 2523, *S. enterica* ATCC 14028, *S. enterica* ATCC 35988, *Kl. pneumoniae* ATCC 10031, *P. aeruginosa* ATCC 9027, and *Ent. faecalis* ATCC 51575; the MBC

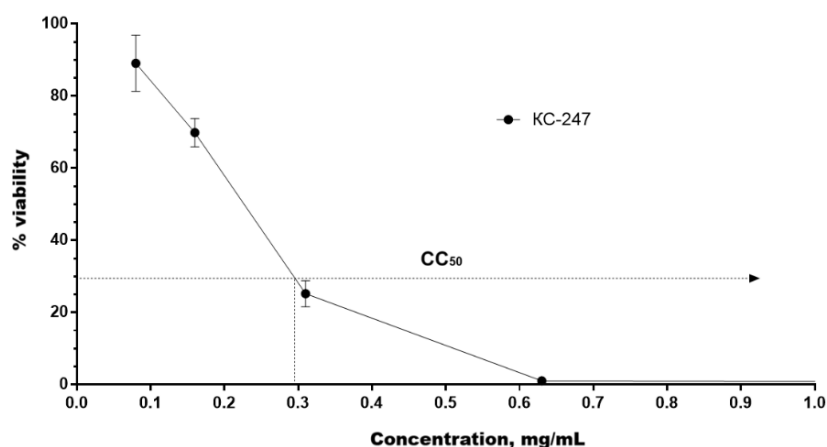


Figure 9 – Cytotoxic effect of compound KC-247 on RD cell culture.

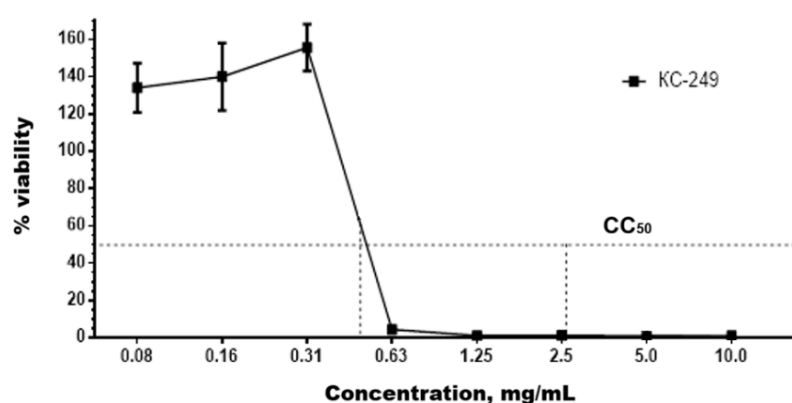


Figure 10. Cytotoxic effect of compound KC-249 on RD cell culture.

Table 8. Results of the antimicrobial activity of KC-247 and KC-249 substances.

Test strains	Compound name	
	KC-247	KC-249
	Minimum bactericidal/inhibitory concentration (MBC/MIC) values, µg/mL, expressed as I ₂	
<i>S.aureus</i> ATCC 6538	0.801/0.401	0.523/0.262
<i>S. aureus</i> ATCC 33591	0.401/0.200	0.523/0.262
<i>E. coli</i> ATCC 8739	0.401/0.200	1.047/0.523
<i>E. coli</i> ATCC 2523	0.401/0.200	0.523/0.262
<i>S. enterica</i> ATCC 14028	0.401/0.200	1.047/0.523
<i>S. enterica</i> ATCC 35988	0.401/0.200	1.047/0.523
<i>Kl. pneumoniae</i> ATCC 10031	0.401/0.200	0.523/0.262
<i>Kl. pneumoniae</i> ATCC 2524	0.200/0.100	0.523/0.262
<i>P. aeruginosa</i> ATCC 9027	0.401/0.200	1.047/0.523
<i>P. aeruginosa</i> TA2	0.801/0.401	2.093/1.047
<i>Ent.hirae</i> ATCC 10541	0.801/0.401	1.047/0.523
<i>Ent.faecalis</i> ATCC 51575	0.401/0.200	1.047/0.523
<i>C.albicans</i> ATCC 10231	0.801/0.401	2.093/1.047
<i>C.albicans</i> SCAID PHPX 1-2019	0.801/0.401	2.093/1.047

was 0.401 µg/mL, while the MIC was 0.200 µg/mL. The minimum bactericidal concentration against *Kl. pneumoniae* ATCC 2524 was 0.200 µg/mL, and the MIC was 0.100 µg/mL. Against *S. aureus* ATCC 6538, *P. aeruginosa* TA2, *Ent. hirae* ATCC 10541, as well as the yeast-like fungi *C. albi-*

cans ATCC 10231 and *C. albicans* SCAID PHPX 1-2019, the MBC was 0.801 µg/mL, whereas the MIC was 0.401 µg/mL.

The coordination compound KC-249 also possessed antimicrobial activity against all tested microorganisms. It was established that against *S. aureus* ATCC 6538, *S. aureus* ATCC

33591, *E. coli* ATCC 2523, and *Kl. pneumoniae* ATCC 10031, the minimum bactericidal concentration was 0.523 µg/mL, while the MIC was 0.262 µg/mL. Against *E. coli* ATCC 8739, *S. enterica* ATCC 14028, *S. enterica* ATCC 35988, *P. aeruginosa* ATCC 9027, *Ent. hirae* ATCC 10541, and *Ent. faecalis* ATCC 51575, the MBC was 1.047 µg/mL and the MIC was 0.523 µg/mL. The minimum bactericidal concentration against *P. aeruginosa* TA2, as well as against the yeast-like fungi *C. albicans* ATCC 10231 and *C. albicans* SCAID PHPX 1-2019, was 2.093 µg/mL, while the MIC was 1.047 µg/mL. Against *Kl. pneumoniae* ATCC 2524, the MBC was 0.523 µg/mL and the MIC was 0.262 µg/mL.

Thus, the coordination compounds KC-247 and KC-249 were shown to possess antimicrobial activity against Gram-positive and Gram-negative microorganisms, as well as yeast-like fungi. Both compounds demonstrated low MIC and MBC values, indicating pronounced inhibitory and bactericidal effects; however, the magnitude of this activity depended on the test strain, as reflected by the differences in MIC and MBC values.

Virus-inhibitory activity screening

The antiviral activity of KC-247 and KC-249 substances was investigated on MDCK and RD cell cultures, based on the calculated CC_{50} values. The study was carried out *in vitro* to assess the effect on influenza A/Swine/Iowa/15/30 (H1N1) virus and herpes simplex virus type 1 (HSV-1), strain "Victory", using a virus-inhibitory scheme of substance administration.

The virus was used at a dose of 100 ID/0.2 mL, and untreated virus was used as the control. The efficacy of the substances was assessed by comparing the levels of inhibition of viral activity in the experimental and control groups. The study results are presented in Tables 9 and 10.

Table 9. Assessment of the virus-inhibitory activity of KC-247 and KC-249 substances on the influenza A/Swine/Iowa/15/30 (H1N1) virus model.

Compound name	Concentration, mg/mL	K_u , %
KC-247	0.027	50.0
	0.014	13.6
	0.007	0
KC-249	0.500	100
	0.250	26.1
	0.125	26.1

Table 10. Assessment of the virus-inhibitory activity of KC-247 and KC-249 substances on the herpes simplex virus type 1, strain "Victory" model.

Compound name	Concentration, mg/mL	K_u , %
KC-247	0.029	28.6
	0.015	19.0
	0.007	19.0
KC-249	0.265	0
	0.133	0
	0.066	0

The study of the antiviral activity of the coordination compounds against influenza A/Swine/Iowa/15/30 (H1N1) virus showed that the substance KC-247, at a concentration of 0.027 mg/mL, reduces viral replication by 50%. Meanwhile, the substance KC-249 demonstrates a 100% reduction in influenza A virus replication at a concentration of 0.5 mg/mL.

In the study of antiviral activity against herpes simplex virus type 1, strain "Victory", it was established that KC-247 and KC-249 do not exhibit antiviral activity against this virus.

Thus, KC-249 demonstrated pronounced virus-inhibitory activity against influenza A/Swine/Iowa/15/30 (H1N1), whereas KC-247 showed only moderate antiviral activity against influenza A virus and no antiviral effect against herpes simplex virus type 1, strain "Victory".

DISCUSSION

The present study reports the synthesis and comprehensive biological characterization of two novel lithium triiodide coordination compounds – KC-247, based on the anti-inflammatory NSAID flunixin meglumine, and KC-249, based on the corticosteroid dexamethasone sodium phosphate. The results demonstrate that both compounds retain broad-spectrum bactericidal and fungicidal activity across sensitive and multi-drug-resistant (MDR) clinical strains, exhibit acceptable cytotoxicity profiles, and display selective virus-inhibitory activity against influenza A (H1N1) virus. These findings support the conceptual premise of the study: that coordination with io-dine-containing species preserves anti-infective potential even when the ligand scaffold originates from a clinically established anti-inflammatory agent.

Physicochemical Stability and Pharmaceutical Relevance

Both KC-247 and KC-249 maintained their key quality parameters – pH, melting point, iodine content, and iodide content – without statistically significant changes over the three-month observation period. This physicochemical stability is consistent with prior reports of lithium triiodide coordination compounds from the same research center, in which analogous systems based on amino acid scaffolds demonstrated comparable shelf-life under refrigerated storage conditions. The stability of iodine content, in particular, is notable, as molecular iodine is known to be susceptible to loss through sublimation and oxidative degradation under ambient conditions; the coordination environment provided by the organic ligand and the presence of lithium iodide appear to effectively suppress this degradation, consistent with the stabilizing role of LiI described in earlier iodine formulation literature. The observed melting points of 78–80 °C for KC-247 and 106–108 °C for KC-249 further suggest the formation of distinct crystalline phases relative to the starting materials (flunixin meglumine and dexamethasone sodium phosphate), corroborating the coordination interaction confirmed by UV-Vis spectroscopy. The higher thermal stability of KC-249 relative to KC-247 may reflect a more compact and thermodynamically favorable coordination geometry, as supported by the higher complexation energy calculated for the LiI_3 –dexamethasone model ($\Delta E = -19.31$ kcal/mol) compared to the LiI_3 –flunixin structure ($\Delta E = -12.57$ kcal/mol).

Mechanistic Interpretation of DFT Findings

Quantum-chemical analysis using the DFT/B3P-W91/6-31G** method revealed that LiI forms highly stable coordination complexes with both flunixin meglumine ($\Delta E = -68.70$ kcal/mol; Me–N bond length 1.85 Å) and dexamethasone sodium phosphate ($\Delta E = -63.21$ kcal/mol; Me–N 1.84 Å). These large complexation energies confirm strong ion–dipole coordination interactions with the heteroatom donor sites of both ligands, consistent with the spectral evidence of ligand-induced peak shifts observed in UV-Vis analysis. For the corresponding triiodide structures (structures c and d), the I_2 bond dissociation energies ($\Delta E_1 = 10.88$ and 21.73 kcal/mol, respectively) significantly exceed the calculated interaction energy of free I_2 with a model protein binding site ($\Delta E = -2.23$ kcal/mol). This disparity supports a key mechanistic hypothesis: within the coordination framework, molecular iodine is not released indiscriminately but rather delivered in a directed manner to high-affinity intracellular targets – most plausibly nucleotide triplets in bacterial DNA. This interpretation is consistent with earlier work by Turganbay et al. (2021), in which radioisotope labeling of a tryptophan–iodine co-ordination compound demonstrated targeted DNA halogenation upon intracellular complex breakdown, resulting in the formation of N–iodinated nucleobases that disrupted essential biosynthetic processes. The organic ligand scaffold thus plays a dual functional role: stabilizing the triiodide species in the coordination complex and modulating the intracellular delivery kinetics of active iodine. Differences in the I_2 bond dissociation energies between structures c (10.88 kcal/mol) and d (21.73 kcal/mol) suggest that KC-247 releases molecular iodine more readily than KC-249 under equivalent conditions, which is consistent with the lower MBC values observed for KC-247 across the majority of tested strains.

Antibacterial and Antifungal Activity

KC-247 demonstrated potent bactericidal activity with MBC values ranging from 0.200 to 0.801 µg/mL, while KC-249 was active within the range of 0.523–2.093 µg/mL against Gram-positive cocci (*S. aureus*, *E. faecalis*, *E. hirae*), Gram-negative rods (*E. coli*, *K. pneumoniae*, *S. enterica*, *P. aeruginosa*), and yeast-like fungi (*C. albicans*), including multidrug-resistant clinical isolates. These findings are comparable to previously reported structurally related iodine coordination compounds. Turganbay et al. (2024) reported that diaminopropionic acid hydrogen triiodide exhibited pronounced bactericidal activity against MDR Gram-positive and Gram-negative pathogens, while Gendron et al. (2023) demonstrated that iodine–amino acid complexes exert antimicrobial effects through membrane destabilization, oxidative stress induction, and disruption of nucleic acid integrity [21,22].

Importantly, the biological activity of KC-247 and KC-249 is likely mediated through a multifactorial mechanism involving controlled iodine release, direct iodination of microbial proteins and nucleotides, oxidative membrane damage, and disruption of essential metabolic pathways [21–24]. Iodine species can induce halogenation of cellular macromolecules, alter membrane permeability, inhibit respiratory enzymes, and promote oxidative stress, ultimately leading to irreversible microbial cell death [22–25]. Additionally, coordination with flunixin meglumine or dexamethasone sodium phosphate may

enhance compound stability, optimize lipophilicity, and improve membrane penetration, thereby preserving both anti-infective and anti-inflammatory properties [21,23].

Notably, KC-247 demonstrated the highest activity against *K. pneumoniae* ATCC 2524, with an MBC of 0.200 µg/mL, while KC-249 showed similarly strong activity (0.523 µg/mL). The superior efficacy of KC-247 may be associated with its lower I_2 bond dissociation energy and the physicochemical properties of flunixin meglumine, whose moderate lipophilicity may facilitate improved membrane penetration relative to the more hydrophilic dexamethasone sodium phosphate ligand.

Both compounds also demonstrated significant antifungal activity against sensitive and MDR *Candida albicans* strains. This broad-spectrum antimicrobial and anti-fungal efficacy further support the concept that iodine coordination compounds act through multiple simultaneous cellular targets, thereby reducing the probability of resistance development while maintaining therapeutic versatility [22–24]. Overall, the preservation of anti-infective potency in coordination systems incorporating anti-inflammatory agents confirms the feasibility of developing multifunctional therapeutic compounds with combined antimicrobial, antifungal, antiviral, and supportive anti-inflammatory properties.

Cytotoxicity Profile and Therapeutic Window

The CC_{50} of KC-247 in the PBMC model was 0.072 mg/mL (maximum non-toxic concentration, MNC: 0.018 mg/mL), while KC-249 showed a more favorable profile with $CC_{50} = 0.225$ mg/mL (MNC: 0.056 mg/mL). To contextualize the safety window of each compound relative to its bactericidal potency, a Selectivity Index ($SI = CC_{50}/MBC$) was calculated using PBMC CC_{50} values and the lowest MBC observed for each compound. For KC-247, $SI = 0.072$ mg/mL / 0.000200 mg/mL = 360; for KC-249, $SI = 0.225$ mg/mL / 0.000523 mg/mL $\approx$ 430. These SI values substantially exceed the threshold of $SI > 10$ conventionally considered the minimum criterion for further pharmaceutical development of anti-infective candidates, indicating that both compounds exert bactericidal effects at concentrations far below those cytotoxic to human immune cells. The higher CC_{50} and SI of KC-249, despite its higher MBC values, suggests that the dexamethasone scaffold confers a comparatively lower inherent cellular toxicity – a finding that aligns with dexamethasone's established clinical safety profile as an anti-inflammatory drug widely administered at pharmacological doses. In MDCK and RD cell lines, KC-249 likewise exhibited higher CC_{50} values than KC-247, reinforcing the pattern of differential cytotoxicity between the two compounds and warranting additional investigation to clarify whether this difference reflects intrinsic ligand toxicity, differential cellular uptake, or altered complex stability in cell culture media.

Virus-Inhibitory Activity

A/Swine/Iowa/15/30 (H1N1), achieving 100% inhibition at 0.500 mg/mL, while KC-247 showed only moderate activity (50% inhibition at 0.027 mg/mL). Neither compound exhibited antiviral activity against herpes simplex virus type 1, strain "Victory." The divergent antiviral profiles of KC-247 and KC-249, despite their shared triiodide framework, are mechanistically informative. The selective activity of KC-249

against influenza A likely reflects the modulatory effects of the dexamethasone ligand on the antiviral response. Dexamethasone has been studied as an immunomodulatory adjunct in severe influenza, and its incorporation into the coordination complex may contribute to antiviral potency through mechanisms beyond simple iodine delivery – potentially including interference with viral hemagglutinin function or suppression of host inflammatory amplification of viral cytopathic effect. This interpretation is consistent with recent clinical data showing that immunomodulatory corticosteroids, while controversial in mild influenza, may reduce viral damage in the context of severe infection by attenuating excessive host inflammatory responses.

The antiviral activity of iodine-containing coordination compounds more broadly may involve direct oxidative inactivation of viral surface glycoproteins, consistent with the known virucidal properties of molecular iodine. Molecular modeling studies by Turganbay et al. (ACS Omega, 2023) on structurally related systems (FS-1 and Armenicum) demonstrated that $\text{LiI}\cdot\text{I}_2$ active centers inhibit viral integrase catalytic domains through N–I bond formation in viral DNA, a mechanism that may partially explain the differential activity observed against RNA viruses (influenza A, H1N1) versus DNA viruses (HSV-1) in the present study. Iodine-mediated halogenation may be more disruptive to RNA replication machinery than to the more redundantly protected HSV-1 DNA genome, which is packaged within a protein capsid providing additional steric protection from electrophilic attack.

The selective activity against H1N1 and inactivity against HSV-1 observed for both compounds mirrors patterns reported for the related compound KC-246 (sodium sulfadimidine– LiI_3) from the same research center, where a similar virus-selectivity profile was observed, suggesting that this pattern reflects a class-level property of lithium triiodide coordination compounds rather than ligand-specific pharmacology.

Significance in the Context of Combined Anti-Infective/Anti-Inflammatory Therapy

A central rationale of this study was the recognition that, in real clinical and veterinary settings, anti-infective agents are frequently co-administered with anti-inflammatory drugs. The present findings demonstrate that coordination with iodine-containing species not only preserves the pharmaceutical integrity of flunixin meglumine and dexamethasone sodium phosphate but transforms these clinically established anti-inflammatory agents into hybrid compounds with intrinsic bactericidal, fungicidal, and selective antiviral activity. This approach has direct relevance to veterinary infectious disease management, where flunixin meglumine is authorized as a supportive anti-inflammatory agent in bovine respiratory disease, mastitis, and swine respiratory infections – conditions involving mixed bacterial and viral pathogens. The incorporation of flunixin meglumine into an iodine coordination compound that retains anti-infective activity against the very pathogens encountered in these conditions represents a potentially valuable pharmacological strategy for reducing the number of agents required in multidrug anti-infective regimens. Similarly, the anti-infective properties of KC-249 are relevant in the context of human medicine, where dexamethasone is recommended as adjunctive therapy in tuberculous meningitis and severe bacterial meningitis; a compound that combines

corticosteroid ligand functionality with independent bactericidal activity could, if further validated *in vivo*, reduce therapeutic complexity in these high-severity infections.

Limitations

Several limitations of the present study should be acknowledged. First, all biological evaluations were conducted exclusively under *in vitro* conditions; *in vivo* pharmacokinetic and pharmacodynamic validation, including assessment of tissue distribution, systemic toxicity, and therapeutic efficacy in infection models, remains to be performed. Second, antiviral screening was limited to two viral models; broader screening against additional RNA and DNA viruses would clarify the scope of antiviral activity and further test the HSV-1 selectivity hypothesis.

Third, the structural assignment of KC-247 and KC-249 is currently based on DFT calculations and spectroscopic evidence alone; confirmation by single-crystal X-ray diffraction would provide unambiguous structural proof and further strengthen the mechanistic interpretation. Finally, the study did not include synergy testing against conventional antibiotics, which would be relevant given the original motivation of this compound class as antibiotic potentiators.

CONCLUSION

Two novel iodine-containing active pharmaceutical substances, KC-247 and KC-249, were successfully synthesized and demonstrated satisfactory physicochemical stability over three months of storage. Spectroscopic and quantum-chemical analyses confirmed the structural features, stability, and iodine-associated electronic transitions of the synthesized coordination compounds. Both APSs exhibited pronounced broad-spectrum antimicrobial activity against Gram-positive, Gram-negative, and yeast-like microorganisms, with KC-247 showing greater potency. Cytotoxicity studies indicated acceptable safety profiles within biologically relevant concentrations. Antiviral evaluation revealed selective activity against influenza A (H1N1), particularly for KC-249, while neither compound was effective against herpes simplex virus type 1. Overall, these findings highlight the potential of KC-247 and KC-249 as promising multifunctional anti-infective agents combining antimicrobial and selective antiviral properties. Further studies on dosage form development and pharmaceutical optimization are ongoing.

AUTHOR CONTRIBUTIONS

Conceptualization – Sh.D.A. and T.S.; writing–original draft – Sh.D.A., I.Zh.A. and S.A.B.; writing–review and editing – J.A.B., J.H. and A.A.A.; visualization – Sh.D.A., J.S.M. and A.D.; investigation – T.S., I.Zh.A., J.S.M. and A.D.; supervision – S.A.B. and A.A.A.

FUNDING

This research was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. BR24992760).

REFERENCES

1. Pandey P., Singh H., Omar B. J., Kumari D., Panda P. K. Clinical outcomes of multidrug-resistant organism in-

fections in a tertiary care hospital in India // *World Journal of Clinical Cases*. – 2026. – Vol. 14, No. 5. – Art. 117981. – DOI: <https://doi.org/10.12998/wjcc.v14.i5.117981>

2. Deng S. M., Wei W. J., Duan T. J., Jiang Z. P. Risk factors and outcomes of bloodstream infection with multidrug-resistant bacteria in adult patients with acute leukemia // *Journal of Infection and Public Health*. – 2026. – Vol. 19. – Art. 103088. – DOI: <https://doi.org/10.1016/j.jiph.2025.103088>

3. Kuchar E., Karlikowska-Skwarnik M., Wawrzuta D. Anti-inflammatory therapy of infections // *Encyclopedia of Infection and Immunity*. – 2022. – P. 791–797. – DOI: <https://doi.org/10.1016/B978-0-12-818731-9.00181-6>

4. Donovan J., Cresswell F. V., Tucker E. W., Davis A. G., Rohlwick U. K., Huynh J. et al. A clinical practice guideline for tuberculous meningitis // *The Lancet Infectious Diseases*. – 2026. – Vol. 26. – P. e96–e111. – DOI: [https://doi.org/10.1016/S1473-3099\(25\)00364-0](https://doi.org/10.1016/S1473-3099(25)00364-0)

5. Martin M. S., Kleinhenz M. D., White B. J., Johnson B. T., Montgomery S. R., Curtis A. K. et al. Assessment of pain associated with bovine respiratory disease and its mitigation with flunixin meglumine in cattle with induced bacterial pneumonia // *Journal of Animal Science*. – 2022. – Vol. 100. – Art. skab373. – DOI: <https://doi.org/10.1093/jas/skab373>

6. Storoni C., Preziuso S., Attili A.-R., Li Y., Cuteri V. Bacterial bovine respiratory disease: A comprehensive review of etiology, pathogenesis and management strategies // *Microbiology Research*. – 2026. – Vol. 17, No. 1. – Art. 18. – DOI: <https://doi.org/10.3390/microbiolres17010018>

7. Altria K. D. Capillary Electrophoresis Guidebook: Principles, Operation, and Applications. – Berlin; Heidelberg : Springer Science & Business Media, 1996. – Vol. 52.

8. Agilent Technologies. Instruction Manual for the Capillary Electrophoresis System. – Waldbronn : Agilent Technologies, 2000. – P. 18–27.

9. European Pharmacopoeia. 7th ed. Vol. 1. – Strasbourg : Council of Europe, 2011. – P. 77–82.

10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI Supplement M100. – Wayne, PA : Clinical and Laboratory Standards Institute, 2019. – P. 1–320.

11. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 10th ed. CLSI Document M07-A10. – Wayne, PA : Clinical and Laboratory Standards Institute, 2015. – P. 1–88.

12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI Supplement M100. – Wayne, PA : Clinical and Laboratory Standards Institute, 2018. – P. 1–296.

13. Andrews J. M. Determination of minimum inhibitory concentrations // *Journal of Antimicrobial Chemotherapy*. – 2001. – Vol. 48, Suppl. 1. – P. 5–16. – DOI: https://doi.org/10.1093/jac/48.suppl_1.5

14. Balouiri M., Sadiki M., Ibensouda S. K. Methods for in vitro evaluating antimicrobial activity: A review // *Journal of Pharmaceutical Analysis*. – 2016. – Vol. 6. – P. 71–79. – DOI: <https://doi.org/10.1016/j.jpha.2015.11.005>

15. Freshney R. I. Culture of Animal Cells: A Manual of Basic Technique. 5th ed. – Hoboken, NJ : John Wiley & Sons, 2005.

16. Gibaldi M., Perrier D. Pharmacokinetics. 2nd ed. – New York, NY : Informa Healthcare, 2007. – DOI: <https://doi.org/10.1201/9780429137648>

17. Reed L., Muench H. A simple method of estimating fifty percent endpoints // *American Journal of Hygiene*. – 1938. – Vol. 27. – P. 493–497.

18. Ershov F. I., Tazulakhova E. B., Narovlyanskii A. N., Mironov A. N., Merkulov V. A., Leneva I. A. et al. Metodicheskie rekomendatsii po doklinicheskomu izucheniyu spetsificheskoi protivovirusnoi aktivnosti lekarstvennykh sredstv [Guidelines for the Preclinical Study of Specific Antiviral Activity of Medicinal Products] // *Rukovodstvo po provedeniyu doklinicheskikh issledovaniy lekarstvennykh sredstv* [Guidelines for Conducting Preclinical Studies of Medicinal Products]. – Moscow : Ministerstvo zdravookhraneniya RF, 2012. – P. 525–549.

19. Loginova S. Ya., Borisevich S. V., Semenova I. V., Maksimov V. A., Bondarev V. P., Nebolsin V. E. Izucheniye protivovirusnoi aktivnosti Ingavirina v otnoshenii vozbuditelya grippa A (H3N2) in vitro [Study of the Antiviral Activity of Ingavirin against Influenza A (H3N2) in Vitro] // *Antibiotiki i Khimioterapiya*. – 2009. – Vol. 54. – P. 19–22.

20. Kulagin A. A. To the method of counting blood cells in the chamber of I.T.M.O. with a Goryaev grid // *Kazan Medical Journal*. – 1938. – Vol. 34, No. 5–6. – P. 603–607. – DOI: <https://doi.org/10.17816/kazmj57400>

21. Turganbay S., Kenesheva S., Jumagazyeva A., Ilin A., Askarova D., Azembayev A., Kurmanaliyeva A. Synthesis, physicochemical properties and antimicrobial activity of a diaminopropionic acid hydrogen triiodide coordination compound // *BMC Research Notes*. – 2024. – Vol. 17. – Art. 384. – DOI: <https://doi.org/10.1186/s13104-024-07052-8>

22. Barwa P., Asija S., Deswal Y., Kirar J. S. Investigating the antimicrobial potential of diorganotin(IV) complexes incorporating hydrazone ligands: Synthesis, structural elucidation, X-ray crystallography, DFT, and antimicrobial activity // *Inorganica Chimica Acta*. – 2024. – Vol. 564. – Art. 121941. – DOI: <https://doi.org/10.1016/j.ica.2024.121941>

23. Shepilov D., Turganbay S., Jumagazyeva A., Bukeyeva T., Askarova D., Bolatova D. et al. The iodine–dextrin–lithium complex: Morphology, antibacterial activity, and cytotoxicity // *Molecules*. – 2025. – Vol. 30. – Art. 4822. – DOI: <https://doi.org/10.3390/molecules30244822>

24. Barwa P., Asija S., Lal K. et al. Integrated experimental and theoretical studies of organotin(IV)-hydrazone complexes: Synthesis, structural elucidation, computational insights, and antimicrobial activity // *Chemistry & Biodiversity*. – 2025. – Vol. 22, No. 9. – Art. e202500544. – DOI: <https://doi.org/10.1002/cbdv.202500544>

25. Doszhanov Y., Atamanov M., Jandosov J., Saurykova K., Bassygarayev Z., Orazbayev A., Turganbay S., Sabitov A. Preparation of granular organic iodine and selenium complex fertilizer based on biochar for biofortification of parsley // *Scientifica*. – 2024. – Vol. 2024. – Art. 6601899. – DOI: <https://doi.org/10.1155/2024/6601899>

ЙОДНЫЕ КООРДИНАЦИОННЫЕ СОЕДИНЕНИЯ ПРОТИВОВОСПАЛИТЕЛЬНЫХ ЛЕКАРСТВЕННЫХ СРЕДСТВ: НОВЫЕ СИСТЕМЫ, ПРЕДСТАВЛЯЮЩИЕ ИНТЕРЕС ДЛЯ ПРОТИВОИНФЕКЦИОННЫХ ИССЛЕДОВАНИЙ

Шепилов Д.А.¹, Турганбай С.^{2,3}, Джумагазиева А.Б.¹, Искакбаева Ж.А.¹, Джумабаева С.М.¹, Сейсембекова А.Б.¹, Цзинчэн Хао⁴, Аскарова Д.¹, Азембаев А.А.¹

¹АО «Научный центр противоинфекционных препаратов», Алматы, 050060, Казахстан

²Факультет естественных наук, Казахский национальный женский педагогический университет, Алматы, 050000, Казахстан

³Школа нефтяной инженерии, Исследовательский центр «Один пояс, один путь», Казахстанско-Британский технический университет, Алматы, 050000, Казахстан

⁴Ключевая лаборатория коллоидной и интерфейсной химии, Шаньдунский университет, Цзинань, 250100, Китай

*Автор для корреспонденции: shepilov2002@gmail.com

АННОТАЦИЯ

Учитывая возрастающую угрозу устойчивости к противомикробным препаратам и множественно лекарственно-устойчивых патогенов, разработка комбинированных терапевтических систем с сохраненной противоинфекционной активностью и поддерживающими противовоспалительными свойствами является весьма актуальной. Целью данного исследования являлись синтез и оценка новых йодсодержащих координационных соединений на основе флуниксина меглумина и дексаметазона натрия фосфата. Синтезированные соединения были охарактеризованы с использованием капиллярного электрофореза и УФ-спектроскопии. Их цитотоксичность оценивали в культуре РВМС, тогда как противомикробную и противовирусную активности определяли в отношении бактериальных, грибковых и вирусных патогенов. Были успешно получены два стабильных соединения – КС-247 и КС-249. Оба продемонстрировали противомикробную активность в отношении *Staphylococcaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Enterococcaceae* и дрожжеподобных грибов. Значения МБК варьировали от 0,801 до 0,200 мкг/мл для КС-247 и от 2,093 до 0,523 мкг/мл для КС-249. В культуре РВМС значения CC_{50} составили 0,072 мг/мл и 0,225 мг/мл соответственно. КС-249 проявил выраженную противовирусную активность в отношении гриппа А (H1N1), тогда как КС-247 продемонстрировал умеренные эффекты. Ни одно из соединений не было активно в отношении вируса простого герпеса 1-го типа. КС-247 и КС-249 продемонстрировали перспективные противомикробные и селективные противовирусные свойства при приемлемой цитотоксичности, что подчеркивает их потенциал в качестве многофункциональных кандидатов для новых противоинфекционных терапевтических средств.

Ключевые слова: йодсодержащие соединения; активные фармацевтические субстанции; фармацевтические инновации; новые противомикробные адъюванты; устойчивость к противомикробным препаратам.

ҚАБЫНУҒА ҚАРСЫ ДӘРІЛІК ЗАТТАРДЫҢ ЙОДТЫ КООРДИНАЦИЯЛЫҚ ҚОСЫЛЫСТАРЫ: ИНФЕКЦИЯҒА ҚАРСЫ ЗЕРТТЕУЛЕР ҮШІН ҚЫЗЫҒУШЫЛЫҚ ТУДЫРАТЫН ЖАҢА ЖҮЙЕЛЕР

Шепилов Д.А.¹, Тұрғанбай С.^{2,3}, Джумагазиева А.Б.¹, Искакбаева Ж.А.¹, Джумабаева С.М.¹, Сейсембекова А.Б.¹, Цзинчэн Хао⁴, Асқарова Д.¹, Азембаев А.А.¹

¹«Инфекцияға қарсы препараттар ғылыми орталығы» АҚ, Алматы, 050060, Қазақстан

²Жаратылыстану ғылымдары факультеті, Қазақ ұлттық қыздар педагогикалық университеті, Алматы, 050000, Қазақстан

³Мұнай-газ инженериясы мектебі, «Бір белдеу, бір жол» зерттеу орталығы, Қазақстан-Британ техникалық университеті, Алматы, 050000, Қазақстан

⁴Коллоидтық және интерфейстік химияның негізгі зертханасы, Шаньдун университеті, Цзинань, 250100, Қытай

*Хат-хабарларға жауапты автор: shepilov2002@gmail.com

АННОТАЦИЯ

Микробқа қарсы препараттарға төзімділіктің және көптеген дәрілік заттарға төзімді патогендердің артып келе жатқан қаупін ескере отырып, сақталған инфекцияға қарсы белсенділігі және қолдаушы қабынуға қарсы қасиеттері бар біріктірілген терапиялық жүйелерді әзірлеу аса өзекті болып табылады. Осы зерттеудің мақсаты флуниксин меглумині мен дексаметазон натрий фосфаты негізіндегі жаңа йодқұрамды координациялық қосылыстарды синтездеу және бағалау болды. Әдістері: Синтезделген қосылыстар капиллярлық электрофорез және УК-спектроскопияны қолдану арқылы сипатталды. Олардың цитоциттілігі РВМС дақылында бағаланды, ал микробқа қарсы және вирусқа қарсы белсенділіктері бактериялық, саңырауқұлақтық және вирустық патогендерге қатысты зерттелді. Екі тұрақты қосылыс – КС-247 және КС-249 – сәтті алынды. Екеуі де *Staphylococcaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Enterococcaceae* және ашытқы тәрізді саңырауқұлақтарға қарсы микробқа қарсы белсенділік көрсетті. МБК мәндері КС-247 үшін 0,801–0,200 мкг/мл, ал КС-249 үшін 2,093–0,523 мкг/мл аралығында болды. РВМС дақылында CC_{50} мәндері тиісінше 0,072 мг/мл және 0,225 мг/мл құрады. КС-249 тұмау А (H1N1) вирусына қарсы айқын вирусқа қарсы белсенділік

көрсетті, ал КС-247 орташа әсерлер танытты. Қосылыстардың ешқайсысы 1-типті қарапайым герпес вирусына қарсы белсенді болмады. КС-247 және КС-249 қолайлы цитоуыттылық жағдайында перспективалы микробқа қарсы және селективті вирусқа қарсы қасиеттер көрсетті, бұл олардың жаңа инфекцияға қарсы терапиялық құралдар үшін көпфункционалы кандидаттар ретіндегі әлеуетін айқындайды.

Түйінді сөздер: йодқұрамды қосылыстар; белсенді фармацевтикалық субстанциялар; фармацевтикалық инновациялар; жаңа микробқа қарсы адьюванттар; микробқа қарсы препараттарға төзімділік.